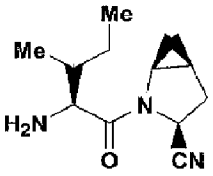
DP4 Chemistry Significant Events **November 4 ,1999**

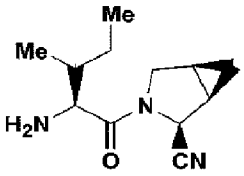
Competitive Update: Nothing to report

Current FTE's: 3

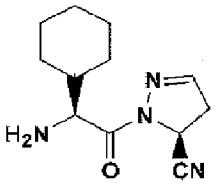
Monthly Summary: The goal of the program is to discover small molecule inhibitors of dipeptidyl peptidase IV (DP4) for use in the treatment and prevention of diabetes. Inhibition of DP4 should prevent the degradation of GLP-1 and potentiate its action in vivo. In a past report, BMS-356379 was identified as a potent and proprietary inhibitor of DP4. In vitro and MAP characterization has recently shown this compound to possess 53% oral biavailability in the rat (t1/2 = 4.4 hr) and low propensity for in vitro metabolism and P450 inhibition. Scale-up for in vivo testing is in progress. Recently, two unique lead compounds have been identified: I) the 3,4-methano cyanopyrollidide BMS-383680 (Ki = 15 nM), the most potent proprietary DP4 inhibitor identified to date and ii) BMS-382436 (Ki = 36 nM) which contains a cyano-substituted dihydropyrazole. In recent in vivo studies, the non-proprietary DP4 inhibitor BMS-328201 (Ile-Thia, Ki = 110 nM) exhibited glucose lowering I) acutely in the normal rat upon oral glucose loading and ii) in the fasting high fat fed mouse when dosed chronically. The in vivo responses are consistant with GLP-1(7-36) potentiation via DP4 inhibition.



BMS-356379
DP4 Ki = 28 nM



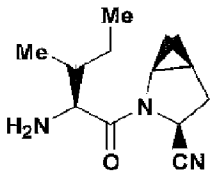
BMS-383680
DP4 Ki = 15 nM



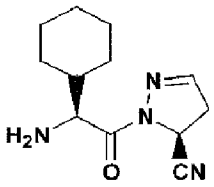
BMS-382436
DP4 Ki = 36 nM

Full Text: 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. BMS-356379 was previously identified as a novel and proprietary inhibitor with an inhibitory constant of 28 nM vs pig kindey DP4. Recent MAP data indicates this compound exhibits 53% oral systemic bioavailability in the rat with a t1/2 of 4.4 hr. In vitro, BMS-356379 was not metabolized in human liver microsomes after 10 min exposure. This data coupled with its low propensity for P450 inhibition (>10 uM for all isozymes) makes BMS-356379 an excellent lead for further development. Scale-up of this compound is nearing completion for evaluation in our in vivo assays. Work is progressing in determining the SAR about the amino terminus in order to optimize in vitro potency.

BMS-382436 has also recently been identified as a potent and proprietary DP4 inhibitor. This compound (Ki = 36 nM) possesses a unique cyano-substituted dihydropyrazole. Since this structural chemotype has not been reported in the literature, efforts will be made to determine the solution and metabolic stability of this compound prior to future work.

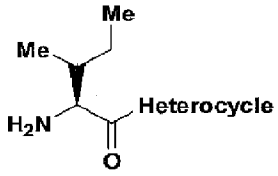


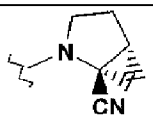
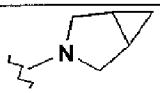
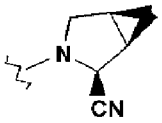
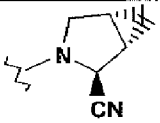
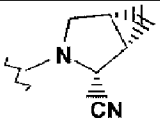
BMS-356379
DP4 Ki = 28 nM
rat oral bioavail. 53%
$t_{1/2}$ = 4.4 hr



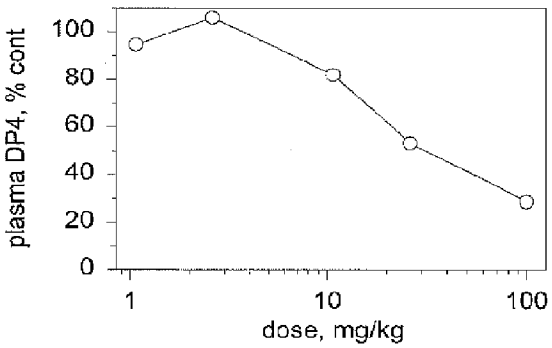
BMS-382436
DP4 Ki = 36 nM

In an attempt to expand the SAR around BMS-356379 and to identify additional proprietary chemotypes, several novel cyclopropanated cyanoproyl derivatives have been explored. Incorporation of the methano group at the 2,3-position, affording BMS-378736, resulted in a dramatic loss in potency (Ki = 76,400 nM). The des-cyano 3,4-methano derivative BMS-378738 exhibited modest activity vs DP4 (Ki = 1,400 nM) but activity was enhanced 100-fold by incorporation of the (S)-cyano functionality, affording BMS-383680 (Ki = 15 nM). This is the most potent proprietary DP4 inhibitor yet discovered in the program. Subsequent SAR has shown DP4 is exquisitely sensitive to the correct stereochemical configuration of the substituents on the five-membered ring. The related trans-methano isomer of BMS-383680, BMS-380845 (Ki = 1,300 nM), exhibited marked reduction in potency against DP4. Its stereoisomer BMS-384189 was essentially inactive (0% inhibition @ 10 μM). Stereoselective generation of the core cyano-cyclopropanated pyrrolide in BMS-383680 has proved difficult, requiring further efforts before SAR studies in this series can be pursued.

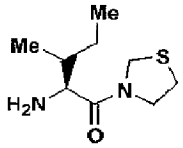


BMS#	378736	378738	383680	380845	384189
Heterocycle					
DP4 Ki (nM)	76,400	1,400	15	1,300	0% @ 10 μM

In vivo results: P32/98 (Ile-Thia, BMS-328201) is a weak and reportedly short-acting DP4 inhibitor being developed by Probiodrug for the treatment of diabetes. Due to its ease of large scale synthesis and previous reports of efficacy in animal models, this compound was used to refine our in vivo models in-house. A dose response versus plasma DP4 inhibition is depicted below. BMS-328201 demonstrates >70% inhibition @ 100 mpk and an ED50 of ~30 mpk in this assay. The simplicity of this assay should allow plasma DP4 inhibition to serve as a potential acute in vivo assay for the discrimination of future analogs, allowing a comparison based on ED50 values and potentially duration of action.



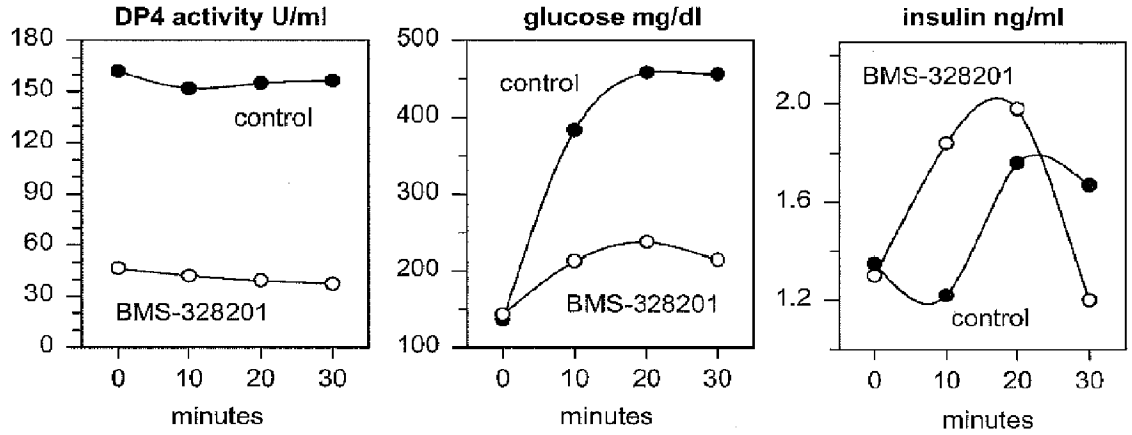
Oral dose-response in rats for inhibition of plasma DP-4 activity at 30 min



P32/98 (Ile-Thia)
BMS-328201
DP4 Ki = 110 nM

Based on these results, BMS-328201 (100 mpk) or vehicle were orally administered to normal rats (n = 4) and subject to a standard oral glucose challenge 30 min later. DP4 activity, plasma glucose, and insulin were measured at 10 minute intervals. During the course of the study, BMS-328201 treated animals reduced plasma DP4 activity to ~30% of control while plasma glucose concentrations were significantly attenuated. Plasma insulin exhibited a more rapid rise and fall in mean levels. All of these results are consistent with the expected biological responses of GLP-1(7-36) potentiation.

Acute effects of BMS-328201 (ile-thiazolidide) in normal rats given a glucose challenge



The effect of BMS-328201 on plasma glucose, food intake, and body weight upon chronic (14 days) dosing of BMS-328201 (0.1% in diet, ~ 80 mg/kg/d) in high fat fed (3 month feeding) mice was recently studied. At day 15, fasting plasma glucose levels were significantly and dramatically reduced versus control (49%). Minimal effect was observed on food intake but a trend towards lower body weight in the treated mice was observed. This study is still in progress and the effects of the DP4 inhibitor will be ascertained at later time points.

Compound	Plasma glucose (mg/mL)	Total food intake over 15 days (gms)	Avg. body weight gain Per mouse (gms)
Vehicle control	175 ± 13	387	1.3
BMS-328201 (0.1% in diet)	89 ± 2*	377	0

n = 10 per group, *p < 0.05