

Potent and Selective Thiamine Antagonists That Inhibit Transketolase

Allen A. Thomas¹, Josh Ballard¹, Bryan Bernat¹, Steven A. Boyd¹, Barb Brandhuber¹, Kevin Condroski¹, Jason De Meese¹, Walter DeWolf¹, Stephen S. Gonzales¹, Indrani Gunawardana¹, May Han², Susan Larson¹, Yvan Le Huerou¹, Patrice Lee¹, Tomas Kaplan¹, Christine Lemieux¹, Robin Pedersen², Jed Pheneger¹, Greg Poch¹, Todd Romoff¹, Darin Smith¹, Francis Sullivan¹, Solly Weiler², S. Kirk Wright², Guy Vigers¹, Carolina Vong¹ (1) Array BioPharma, 3200 Walnut St., Boulder, CO 80301; (2) AVEO Pharmaceuticals, 75 Sidney St., Cambridge, MA 02139

Transketolase (TK) as a cancer target

Activation of the nonoxidative branch of the PPP for ribose-5P synthesis (70-99% of pentose source from nonoxidative while 10-20% from oxidative). *Nutrition and cancer* 2000, 36(2), 150; *Cancer Res.* 2000, 60, 1183.

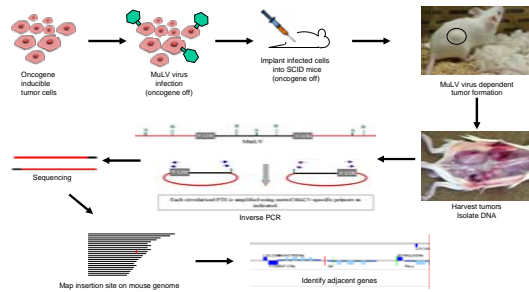
Mobilization of thiamine pool from normal cells leading to thiamine deficiency symptoms (severe cardiac failure) (*Cancer*, 1992, 69, 1710). Tumors are thiamine deficient and contain a high proportion of apo-TK

Biochemistry and Physiology of Thiamin Diphosphate Enzymes, 1996, 438, Blauberer, Germany: Heinrich-Fabri Inst. This reorganization of cell metabolism explains how cancerous cells can maintain a continuous proliferating rate despite decreased glucose oxidation and hypoxia in weakening host.

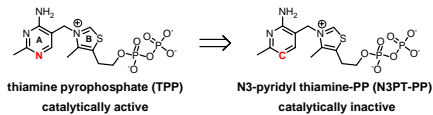
Inhibition of the thiamine-utilizing enzyme transketolase (TK) has been linked with diminished tumor cell proliferation (Boros, et al. *Cancer Res.* 1997, 57(19), 4242)

The high control coefficient of TK in ribose production makes it a target for anti-cancer therapy

In vivo genetic screen identified TK as a possible oncogene

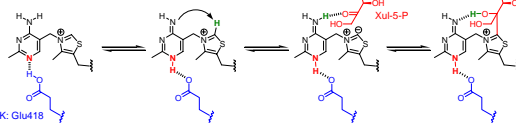


N³-Pyridyl thiamine (N3PT) is a thiamine antagonist



König, S. et al. *J. Biol. Chem.* 1994, 269(14), 10879.

N3PT can not act as a cofactor in this process

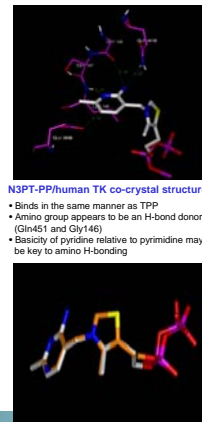


Ch418 binds N₃ and orientates TPP as a cofactor; the 4-NH₂ group is in the vicinity of the C2 carbon of the thiazolium ring.

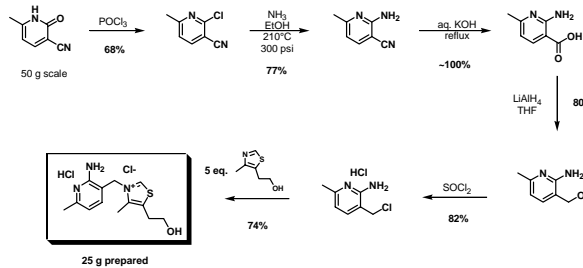
N₃ is protonated generating the 4-imino group.

The 4-imino group abstracts the C2 proton of the thiazolium ring.

Covalent bond formation with the new Ox-S-P.



Scaleable synthesis



25.4% Overall yield, >98% purity (LC/MS/NMR)

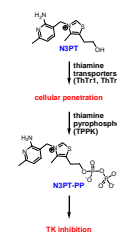
In vitro assays for thiamine "pro-drug" mimetics

Apo-TK (binding to thiamine-free transketolase)
Requires pyrophosphate or pyrophosphate mimic

TPPK (substrate for thiamine pyrophosphokinase)
Indirect; measures disappearance of NADH

Coupled Apo-TK/TPPK
Eliminates need to make pyrophosphate
Sufficient time allowed for complete PP formation

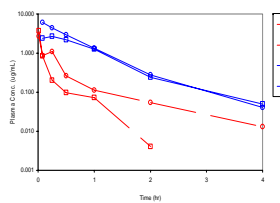
Cellular (inhibition of TK activity in HCT116 cells)
Indirect: measures disappearance of NADH



Data summary for N3PT

TPPK substrate: k_{cat} 5.77 min⁻¹, K_M 3.55 μ M
Apo-TK IC_{50} = 22 nM (as the pyrophosphate)
Cellular TK EC_{50} = 26 nM
Cellular KGDH EC_{50} = 86 nM
HT1080 cell proliferation IC_{50} = 6 nM

Plasma Pharmacokinetics of N3PT in the Rat



20-65% of dose eliminated unchanged in urine by 8 hr

IV		IP	
Dose (mg/kg)	2.00	Dose	20.00
$C_{max, obs}$ (ng/mL)	3.20	$C_{max, obs}$	4.39
$t_{max, obs}$ (hr)	0.02	$t_{max, obs}$	0.17
C_0 (ng/mL)	4.51	C_0	0.00
$t_{1/2, obs}$ (hr)	1.11	$t_{1/2, obs}$	0.64
k_{el} (hr ⁻¹)	0.64	k_{el}	1.09
$AUC_{0-\infty}$ (ng-hr/mL)	0.62	$AUC_{0-\infty}$	3.83
AUC_{0-t} (ng-hr/mL)	0.62	AUC_{0-t}	3.83
Cl_{ss} (mL/min/kg)	58.56	$Cl_{ss, F}$	88.97
V_z (mL/kg)	5.862	V_z, F	4.938
V_{ss} (mL/kg)	1.716	V_{ss}	4.874
MRT (hr)	0.56	MRT	0.90
X^2	0.00	X^2	0.07
ER	72.3%	F_z	87.3%

IV: intravenous
IP: intraperitoneal

Efficacy Studies

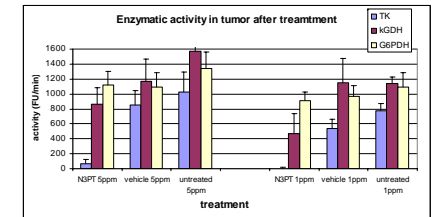
HCT-116 Xenograft Model
Cell Injection: 1 week after switching from normal to a low-thiamine diet (LTD)
1x10⁶ cells/mouse with Matrigel
Tumor take = 100%, tumors selected 70%
Treatment started when tumors were 50mm³

Mice
Balb/c nu/nu, male, 6-7 wks

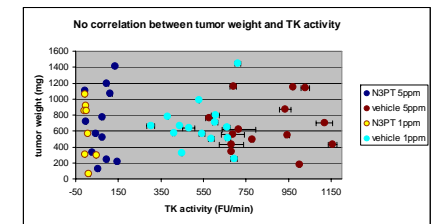
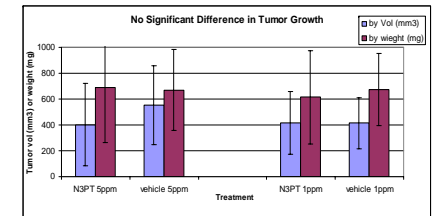
Diet
Normal: Teklad Global 18% Protein Rodent Diet 2918, thiamine content 16.5 mg/kg
5ppm LTD: Teklad custom order, thiamine content 5mg/kg
1ppm LTD: Teklad custom order, thiamine content 1mg/kg

Compounds
N3PT in D-PBS (w/o Mg²⁺ & Ca²⁺)

Treatment Groups
1. N3PT @ 100mg/kg bid ip 5ppm LTD for 2 weeks
2. Vehicle bid ip 5ppm LTD for 2 weeks
3. N3PT @ 100mg/kg bid ip 1ppm LTD
4. Vehicle bid ip 1ppm LTD



TK: 0-8% of vehicle
KGDH: 50% of vehicle in 1ppm
G6PDH: no change



Conclusions

N3PT is a potent and selective TK inhibitor both *in vitro* and *in vivo*

Lack of correlation between TK activity and proliferation argues against the importance of TK in cancer growth