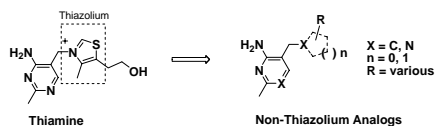


Synthesis of Non-Charged Thiamine Mimetics as Inhibitors of Transketolase

¹Tomas Kaplan, Bryan Bernat, Steven A. Boyd, Jason De Meese, Walter E. DeWolf, Jr., Stephen S. Gonzales, Indrani Gunawardana, Yvan Le Huerou, Christine Lemieux, Todd T. Romoff, Francis Sullivan, Allen A. Thomas; ²May Han, S. Kirk Wright; ¹Array BioPharma, 3200 Walnut St., Boulder, CO 80301, ²AVEO Pharmaceuticals, 75 Sidney St., Cambridge, MA



Thiamine antagonists have potential as anti-cancer agents. We sought to prepare thiamine antagonists that did not require phosphorylation by thiamine pyrophosphokinase (TPPK) or recognition by thiamine transporters (ThTr1, ThTr2) to penetrate cells and inhibit thiamine-utilizing enzymes. We studied transketolase (TK) inhibition because it has been suggested that TK inhibitors would suppress tumor growth. There have been few examples of non-thiazolium TK inhibitors and no examples of potent, non-pyrophosphate inhibitors. We describe the synthesis and selected SAR for potent, non-thiazolium TK inhibitors based on various heterocyclic cores. Low μM inhibitors of Apo-TK that do not require a pyrophosphate moiety for binding are presented herein.

Thiamine exists in four possible forms; free -OH, -O-mono-phosphate, -O-pyrophosphate, and -O-tri-phosphate.

Thiamine phosphates are hydrolyzed in the gastrointestinal tract by phosphatases and then actively transported by specific transporters ThTr1 and ThTr2 ($K_m = 2.6 \mu\text{M}$).

ThTr1 and ThTr2 are ubiquitously distributed and expressed on cell surfaces.

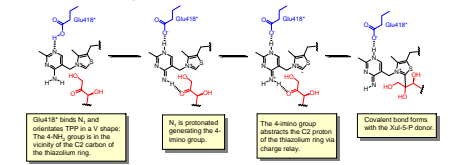
Thiamine is pyrophosphorylated in the cytoplasm by thiamine pyrophosphokinase or by other kinases not yet characterized.

TPP is essential for enzymes involved in carbohydrate metabolism including transketolase, pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, and branched chain α -ketoacid dehydrogenase.

Thiamine as a Cofactor

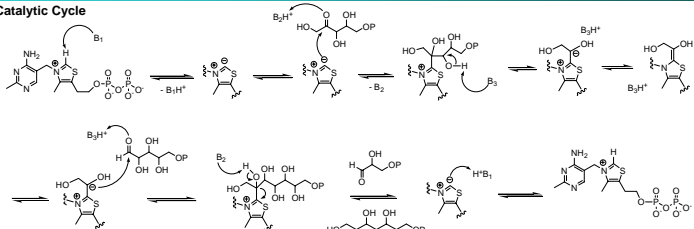
RHF/6-31G* Optimized Geometry and ChelpG Charges

Non-charged mimetics can not act as cofactors in this process.



Enamine form predominates as seen by pyramidalization of thiazolium nitrogen and presence of negative charge on thiazolium nitrogen.

Full Catalytic Cycle



Precedents for Transketolase as a Target for Cancer

Activation of the non-oxidative branch of the PPP for ribose-5P synthesis (70-99% of pentose source from non-oxidative while 10-20% for oxidative). *Nutrition and Cancer*; 2000, 36(2), 150; *Cancer Research*; 2000, 60, 183.

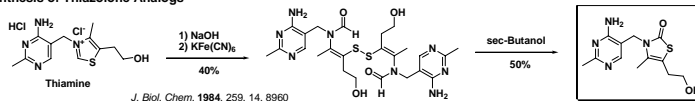
Mobilization of thiamine pool from normal cells leading to thiamine deficiency symptoms (severe cardiac failure) (*Cancer*, 1992, 69, 1710). Tumors are thiamine deficient and contain a high proportion of apo-TK Biochemistry and Physiology of Thiamine Diphosphate Enzymes, 1996, 438, Blauberger, Germany: Heinrich-Fabriz Institute.

This reorganization of cell metabolism explains how cancerous cells can maintain a continuous proliferating rate despite decreased glucose oxidation and hypoxia in weakening host.

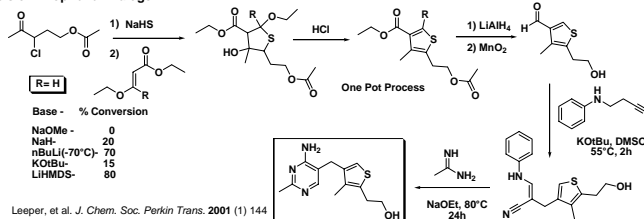
Inhibition of the thiamine-utilizing enzyme transketolase (TK) has been linked with diminished tumor cell proliferation. Boros, et al. *Cancer Research*; 1997, 57(19), 4242.

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

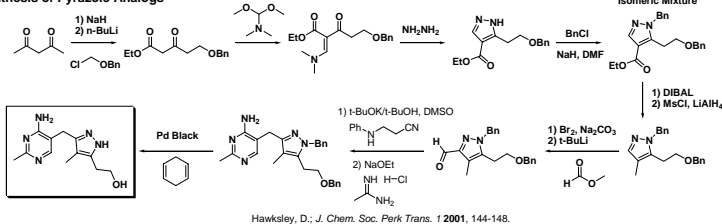
Synthesis of Thiazolone Analogs



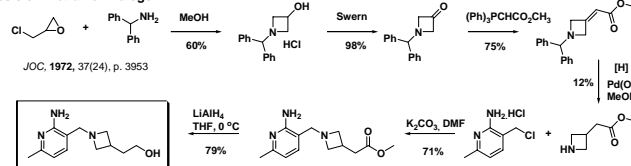
Synthesis of Thiophene Analogs



Synthesis of Pyrazole Analogs



Synthesis of Azetidine Analogs



Selected SAR of Non-Charged Analogs

Structure	AR00340220	AR00344083	AR00344860	AR00344859
Thiamine				
Apo-TK / TPPK K_d :	165 nM	-	-	-
EC ₅₀ (HCT116):	-	16.8 μM cell	4.7 μM cell	1.1 μM cell
TPPK k_{cat}/K_m :	1.18×10^{-5}	-	-	1.86×10^{-5}
Apo-TK K_d :	-	304 μM Apo TK Enz	126 μM Apo TK Enz	>500 μM Apo TK Enz
Structure	AR00329032	AR00340369	AR00340513	AR00365207
Apo-TK / TPPK K_d :	7.39 nM	-	2.9 nM	-
EC ₅₀ (HCT116):	1.7 μM cell	3.2 μM cell	1.2 μM cell	30.2 μM cell
TPPK k_{cat}/K_m :	1.20×10^{-5}	-	3.01×10^{-5}	-
Apo-TK K_d :	34.7 μM Apo TK Enz	>500 μM Apo TK Enz	18.1 μM Apo TK Enz	-