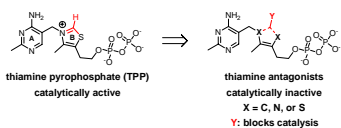


Non-Charged Thiamine Analogues as Inhibitors of Enzyme Transketolase

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, Tomas Kaplan¹, Yvan Le Huerou¹, Christine Lemieux¹, Todd Romoff¹, Darin Smith¹, Francis Sullivan¹, Solly Weiler², S. Kirk Wright², Guy Vigers¹ (1) Array BioPharma, 3200 Walnut St., Boulder, CO 80301; (2) AVEO Pharmaceuticals, 75 Sidney St., Cambridge, MA 02139

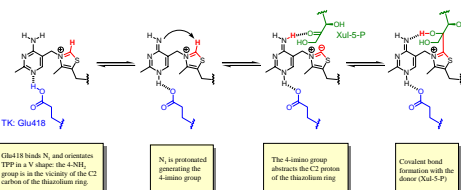
Transketolase (TK) as a cancer target

- 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).
- Most thiamine antagonists except thiamine thiazolone have a permanent positive charge on the B-ring, and it has been suggested that this charge is required for diphosphorylation by thiamine pyrophosphokinase (TPPK) to enable binding to TK.
- We sought neutral thiazolium replacements that would be substrates for TPPK, while not necessarily needing thiamine transporters (ThTr1 and ThTr2) for cell penetration.

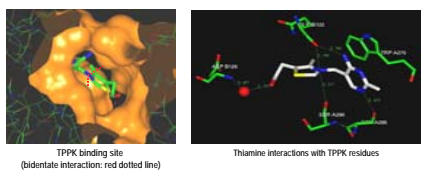


Catalysis by thiamine requires thiazolium deprotonation

Non-charged B-ring analogs can not act as a cofactor in this process



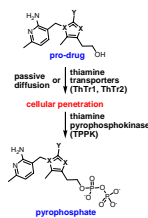
Is positive charge necessary for TPPK activity?



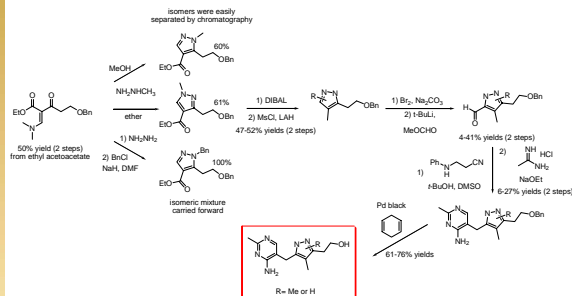
- Mouse TPPK crystal structure is known (Timm, et al. *J. Mol. Biol.* 2001, 310(1), 195)
- Two backbone amide carbonyls point toward the thiazolium cation suggesting a bidentate interaction
- Evidence for thiazolium requirement to be a substrate for TPPK (Voskbojev, et al. *Ann. N.Y. Acad. Sci.* 1982, 378, 161)
- Evidence against thiazolium requirement to be a substrate for TPPK (Kandiko, et al. *Biochem. Pharm.* 1988, 37, 4375)

In vitro assays for thiamine "pro-drug" mimetics

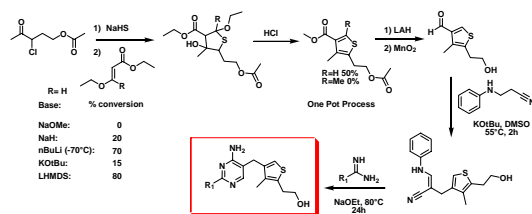
- Apo-TK** (binding to thiamine-free transketolase)
Requires pyrophosphate or pyrophosphate mimic
- TPPK** (substrate for thiamine pyrophosphokinase)
Indirect; measures disappearance of NADH
- Coupled Apo-TK/TPPK**
Eliminates need to make pyrophosphate
Sufficient time allowed for complete PP formation
- Cellular** (inhibition of TK activity in HCT116 cells)
Indirect: measures disappearance of NADH



Pyrazole Synthesis



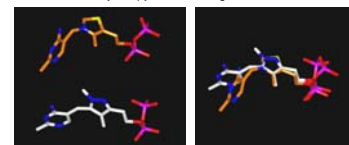
Thiophene Synthesis



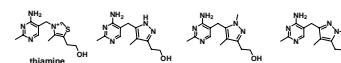
References for deazathiamine and thiophene synthesis:
Leeper, et al. *Org. Biomol. Chem.* 2004, (2), 1732
Leeper, et al. *J. Chem. Soc. Perkin Trans.* 2001, (1) 144
Fevig, et al. *J. Org. Chem.* 2001, 66, 2493

Pyrazole Modeling and SAR

Overlays of pyrazole analogs with thiamine



Pyrazole isomers docked into TK structure

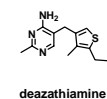
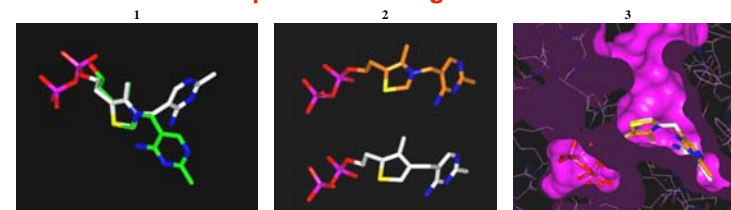


EC₅₀ (HCT116):
TPPK K_{cat}/K_m:
(nM⁻¹ s⁻¹)

thiamine 17 μM
1.1 μM
1.9 x 10⁵
4.7 μM

A permanent charge is not required for TPPK activity but cell potency against TK is poor

Thiophene Modeling and SAR



EC₅₀ (HCT116): 1.2 μM
Apo-TK K_d (alcohol): 18 μM
TPPK/Apo-TK K_d: 12 nM
TPPK k_{cat}/K_m: 3.4 x 10⁻⁶ (nM⁻¹ s⁻¹)

6.3 μM
1.1 μM
3 nM
1.1 x 10⁻⁶

8.5 μM
2.2 μM
-
-

52 μM
>100 μM
-
-

Conclusions

- Non-permanently charged thiamine mimetics can be substrates for TPPK
- Pyrophosphates of non-charged mimetics antagonize the activity of TK *in vitro*
- Low micromolar non-pyrophosphate TK inhibitors were identified in a thiophene series
- Cellular potencies were modest to poor
- Future: improve cell potency by modifying properties and incorporating functionalities to enable recognition by thiamine transporters.