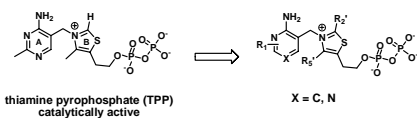


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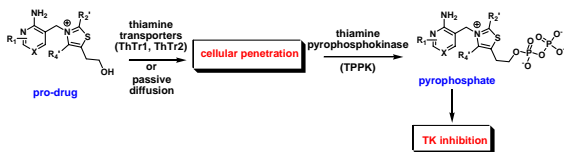
Abstract

Thiamine antagonists have been implicated as anti-tumor agents. In particular, 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). Using structure-aided design, we synthesized thiamine mimetics which are highly potent TK inhibitors both *in vitro* and *in vivo*. In addition to optimizing for TK inhibition, we desired thiamine mimetics which could achieve cell penetration via passive diffusion or thiamine transporters (ThTr1 and ThTr2). Once inside the cell, such mimetics should be diphosphorylated by thiamine pyrophosphokinase (TPPK) to enable binding interactions with TK. To achieve these goals, optimization for multiple protein targets (i.e. TK, TPPK, and ThTr1/ThTr2) was required. Synthesis and SAR of thiamine mimetics with modifications of both the pyrimidine and thiazole rings will be described.

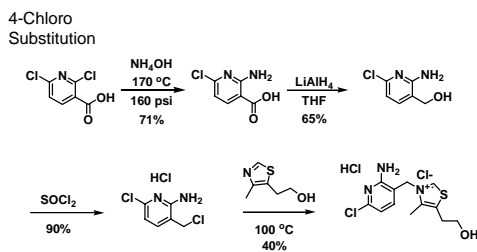


In vitro assays for thiamine "pro-drug" mimetics

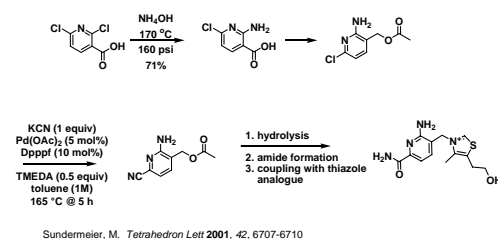
- ❖ **APO-TK** (binding to thiamine-free transketolase)
Requires pyrophosphate or pyrophosphate mimic
- ❖ **TPPK** (substrate for thiamine pyrophosphokinase)
Indirect; measures disappearance of NADH
- ❖ **Coupled APO-TK/TPPK**
Eliminates need to make pyrophosphate
Sufficient time allowed for complete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



Synthesis: A-Ring variations

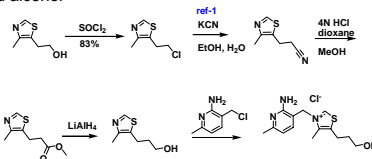


4-Carboxamide Substitution

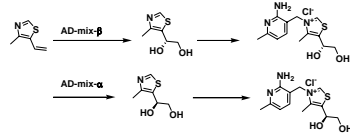


Synthesis: B-Ring variations

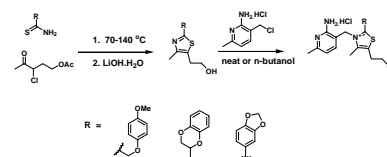
Homologated alcohol



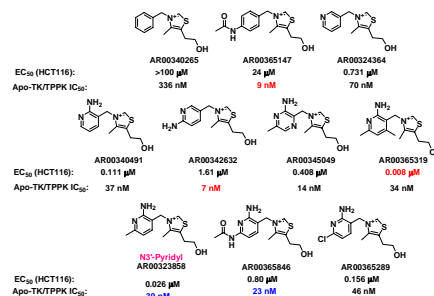
1,2-Diol synthesis



Synthesis: C-2'-Modifications

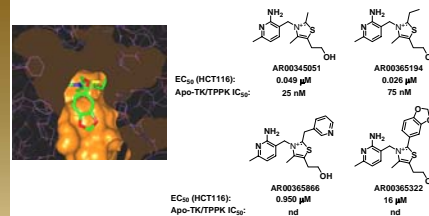


A-Ring SAR



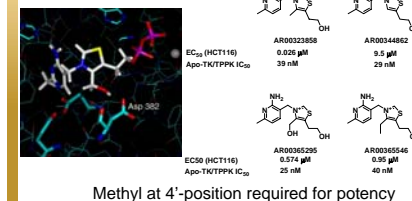
- ❖ "4-amino-N3-pyridyl" A-ring important for cell potency, less critical for TK binding
- ❖ Amino at C2 of N3PT may offer opportunity for improved TK binding and cell potency

C-2' Modeling and SAR

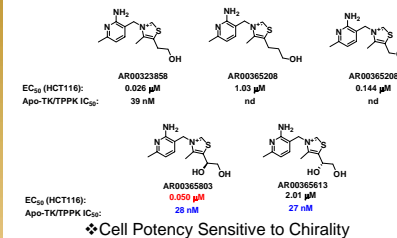


- ❖ Small groups at C-2' tolerated

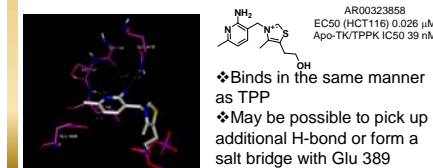
C-4' Modeling and SAR



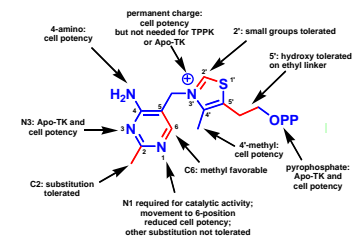
C-5' SAR



N3'-Pyridyl PP/Human TK Co-Crystal Structure



Summary of SAR



- ❖ Future: An understanding of the SAR of thiamine mimetics will allow more potent inhibitors in the future.