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Non-charged thiamine analogs as inhibitors of enzyme transketolase

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Abstract—Inhibition of the thiamine-utilizing enzyme transketolase (TK) has been linked with diminished tumor cell proliferation. Most thiamine antagonists 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) and binding to TK. We sought to make neutral thiazolium replacements that would be substrates for TPPK, while not necessarily needing thiamine transporters (ThTr1 and ThTr2) for cell penetration. The synthesis, SAR, and structure-based rationale for highly potent non-thiazolium TK antagonists are presented.

Activation of the non-oxidative branch of the pentose phosphate pathway for ribose-5 phosphate synthesis has been demonstrated in tumor cells.[1] As described in the preceding paper, the enzyme transketolase (TK) has a high control coefficient toward ribose synthesis in the pathway, suggesting a potential role in the treatment of cancer.

Since thiamine is a cofactor of transketolase, and since the thiamine B-ring is an essential component of catalysis (see Fig. 1, for mechanistic rationale), thiamine analogs possessing B-rings unable to participate in the cycle have potential to be TK antagonists. In order to test the hypothesis that a TK inhibitor would result in diminished tumor cell proliferation, we synthesized various non-permanently charged thiamine analogs based on literature compounds (i.e., thiamine thiazolone, deazathiamine; details below). In addition, we utilized the human crystal structure of TK (to be published elsewhere) to rationally design novel, potent inhibitors.

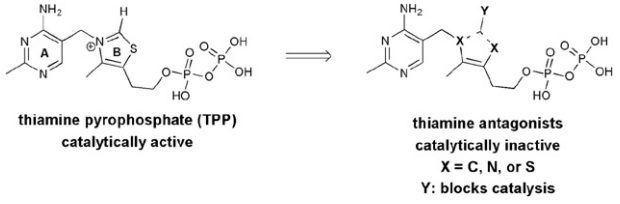

Figure 1. Catalysis by thiamine requires thiazolium deprotonation.

We sought non-charged thiamine analogs based on a hypothesis that they would have cell penetration independent of active transport via thiamine transporters, and that they would have improved pharmacokinetic properties compared with permanently charged thiamine mimetics (see preceding article).

Although there have been previous reports of TK inhibitors in the literature,[2] the majority of these have a permanently charged B-ring and require pyrophosphate to effectively compete with TPP. It has been stated that the permanent charge is necessary for recognition by TPPK.[3] In contrast, others have reported that a neutral thiamine analog, thiamine thiazolone (Table 1, compound **2**), may be pyrophosphorylated in the cytosol.[4] Considering the critical role of the pyrophosphate group for binding to TK, this question was one for which we sought resolution.

Keywords: Transketolase; Thiamine; Pyrophosphokinase; Deazathiamine; Pyrophosphate; Thiazolone; Pyrazolothiamine.
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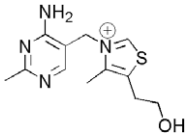
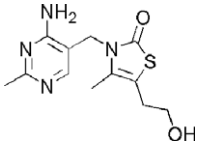
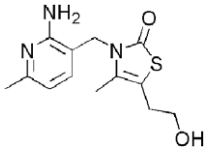
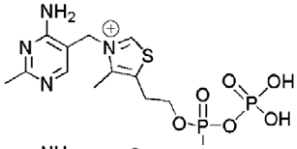
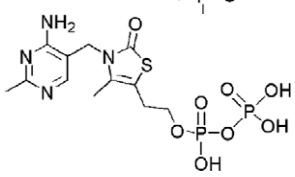
Table 1. SAR for thiamine and thiazolone analogs **1**–**5**

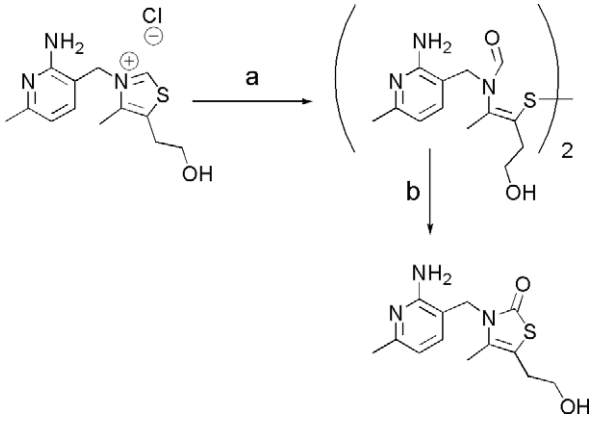
Compound	Structure	Apo-TK enzymatic K_d[a] (nM)	HCT-116 Cell TK EC_{50}[b] (μM)	TPPK k_{cat}/K_m[c] ($nM^{-1}\ s^{-1}$)
1: Thiamine		6.8×10^6	—	1.2×10^{-5}
2: Thiamine thiazolone		3.4×10^4	2.71	1.0×10^{-5}
3: N3P-TT		$>5.0 \times 10^5$	3.24	—
4: Thiamine pyrophosphate (TPP)		170	—	—
5: Thiamine thiazolone pyrophosphate (TTPP)		16	—	—

[a] Average K_d, nM, for enzymatic assay performed in triplicate. Data were within 2-fold of the mean.
[b] Average EC_{50}, μM, for cellular assay performed in triplicate. Data were within 4-fold of the mean.
[c] Average k_{cat}/K_m, $nM^{-1}\ s^{-1}$, for enzymatic assay performed in triplicate. Data were within 2-fold of the mean.

To rationally design thiamine mimetics which might retain TPPK activity, we evaluated the published mouse TPPK crystal structure.[5] In the crystal structure, there are two backbone amide carbonyls pointing in the direction of the thiazolium cation. One might hypothesize that this apparent bi-dentate interaction plays an important role in conversion of the primary alcohol to the pyrophosphate. To test this, we explored replacement of the thiazolium ring with various heterocycles, including thiazolone, pyrazole, and thiophene.

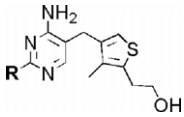
Previously it was reported that replacement of thiamine's B-ring with a neutral thiazolone resulted in dramatically greater binding to thiamine-utilizing enzymes.[6] One explanation for this increased potency was a reduction in desolvation energy associated with binding of a neutral, hydrophobic species (thiazolone) versus a cationic one (thiazolium).[7]

From examination of the human crystal structure of holo-TK (to be published elsewhere), we hypothesized that removal of thiamine's positive charge should not diminish analog binding since contribution of the cation to enzyme interaction appears to be negligible. Consistent with the literature, we found that thiamine thiazolone was a significantly better binder to TK than thiamine, both as the free alcohol **2** (>100×; Table 1) and as the pyrophosphate **5** (>10×; Table 1). Also consistent with the observations by Kandiko et al.,[4] thiamine thiazolone had TPPK activity comparable to thiamine. Furthermore, we discovered that thiazolone analogs, including a novel aminopyridine **3** (N3P-TT), synthesized according to Scheme 1, possessed low



Scheme 1. Synthesis of N3P-TT (**3**).[9] Reagents and conditions: (a) $K_3Fe(CN)_6$, NaOH; (b) *s*-butanol, reflux.

Table 2. SAR for deazathiamine and related analogs **6–13**



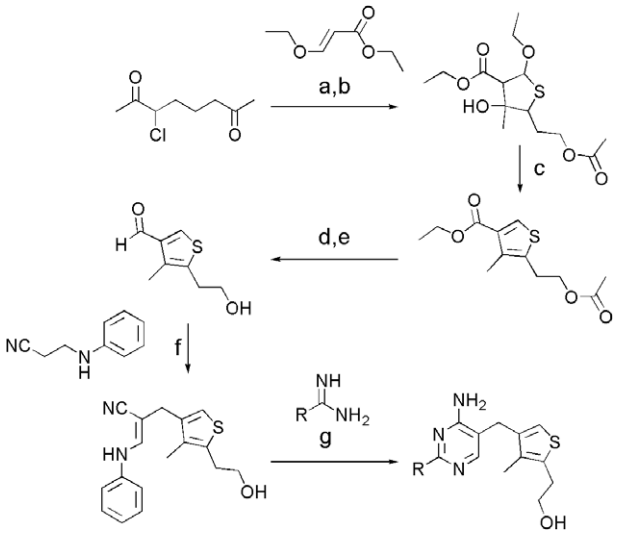
Compound	R	Apo-TK enzymatic K_d[a] (μM)	TPPK/Apo-TK coupled assay K_d[b] (nM)	HCT-116 Cell TK EC_{50}[c] (μM)	TPPK k_{cat}/K_m[d] (nM^{-1} s^{-1})
6	H	>100	—	52	1.2×10^{-5}
7 Deazathiamine	CH_3	18	12	1.2	3.4×10^{-5}
8	*i*-Propyl	>500	64	2.6	2.4×10^{-5}
9	*c*-Propyl	>500	60	0.66	2.7×10^{-5}
10	CF_3	—	63	25	8.5×10^{-4}
11	Ph	>500	230	>10	1.0×10^{-6}
12	NH_2	2.2	—	8.5	—
13	$NHCOCH_3$	1.1	3.0	6.3	1.1×10^{-6}

[a] Average K_d, μM, for enzymatic assay performed in triplicate. Data were within 2-fold of the mean.
[b] Average K_d, nM, for coupled assay from at least three experiments. Data were within 2-fold of the mean.
[c] Average EC_{50}, μM, for cellular assay performed in triplicate. Data were within 4-fold of the mean.
[d] Average k_{cat}/K_m, nM^{-1} s^{-1}, for enzymatic assay performed in triplicate. Data were within 2-fold of the mean.

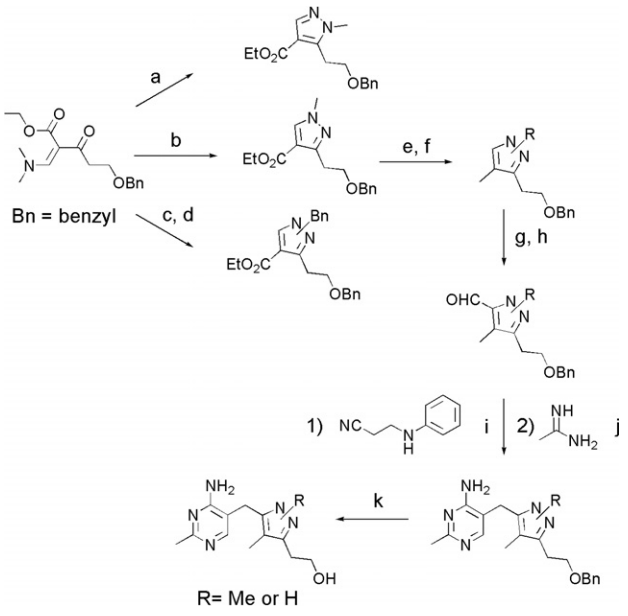
micromolar cellular potency against TK in a human colon cancer cell line (HCT-116).[8]

A synthesis for another neutral thiamine mimetic, deazathiamine (Table 2, compound **7**), was previously described in the literature,[10] however activity against TK was not reported. Using a shorter, published route toward trisubstituted thiophenes,[11] we were able to conveniently synthesize (Scheme 2) several deazathiamine analogs that had unprecedented in vitro activity as the free alcohols (Table 2: Apo-TK K_d of 1.1 μM for **13**). The increased hydrophobicity of the thiophene ring versus the thiazolone ring (e.g. compounds **2** and **3**) may be a contributing factor in the tighter TK binding of analogs **7**, **12**, and **13**.[12] In order to assess their potential as pyrophosphate prodrugs, a coupled TPPK/Apo-TK enzymatic assay was developed.[13] As with thiamine thiazolone, the deazathiamines retained TPPK activity. And in the coupled assay they demonstrated remarkable potency (Table 2: TPPK/Apo-TK K_d of 3 nM for **13**). Despite improvements in binding to TK in enzymatic assays, cell potency relative to the thiazolones and charged thiamine mimetics (published in the preceding article) decreased.

We hypothesized that replacement of the thiamine thiazolium ring with a reversibly charged pyrazole ring, synthesized according to Scheme 3, would maintain TPPK substrate recognition and might allow recognition by

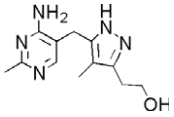
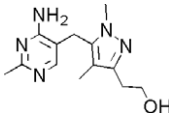
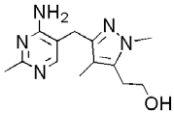


Scheme 2. Synthesis of deazathiamines. Reagents and conditions: (a) NaHS, 3:1 toluene/water, 10 °C, 1 h; (b) ethyl 3-ethoxyacrylate, LiHMDS, rt, 18 h, 80%; (c) concd HCl, 2 h, 50%; (d) $LiAlH_4$, THF, reflux, 18 h, 85%; (e) MnO_2, $CHCl_3$, 77%; (f) DMSO, KOtBu, *t*-BuOH, 55 °C, 77%; (g) NaOEt, 65 °C, 3 d, 10–50%.



Scheme 3. Synthesis of pyrazolothiamines.[14] Reagents and conditions: (a) methyl hydrazine, MeOH, 60%; (b) methyl hydrazine, diethyl ether, 61%[15]; (c) hydrazine, MeOH, diethyl ether; (d) BnCl, NaH, DMF, 100% for steps c and d; (e) DIBAL, THF; (f) MsCl, DIEA, THF, then $LiAlH_4$, 0 °C, 47–52% for steps e and f; (g) Br_2, Na_2CO_3, CH_2Cl_2, −10 °C; (h) *t*-BuLi, MeOCHO, diethyl ether, −78 °C, 4–41% for steps g and h; (i) DMSO, KOtBu, *t*-BuOH, 55 °C; (j) NaOEt, EtOH, reflux, 6–27% for steps i and j; (k) Pd black (1 equiv), 1,4-cyclohexadiene (100 equiv), EtOH, reflux, 61–76%.

Table 3. SAR for pyrazolothiamine analogs **14–16**

Compound	Structure	HCT-116 CellTK EC_{50}[a] (μM)	TPPK k_{cat}/K_m[b] ($nM^{-1}\ s^{-1}$)
14		17	—
15		1.1	1.9×10^{-5}
16		4.7	—

[a] Average EC_{50}, μM, for cellular assay performed in triplicate. Data were within 4-fold of the mean.

[b] Average k_{cat}/K_m, $nM^{-1}\ s^{-1}$, for enzymatic assay performed in triplicate. Data were within 2-fold of the mean.

thiamine transporters leading to improved cell potency. Also, pyrazole analogs would be amenable to further substitutions on the B-ring including extension of functionalities into the substrate-binding region of TK, which might allow for improved potency. As with the thiazolones and deazathiamines, the pyrazoles possessed TPPK activity (Table 3). Unfortunately, they demonstrated only modest activity in the tumor cell assay (HCT-116).

Taken together, these results support our hypothesis that non-permanently charged thiamine mimetics are competent substrates for TPPK, and that the resulting pyrophosphates antagonize the activity of TK in vitro. For the first time, low micromolar non-pyrophosphate TK inhibitors were identified in a deazathiamine series. Despite remarkable potencies in enzymatic assays, cellular potencies were modest to poor. An explanation for the discrepancy between the two assays is not obvious, although one might surmise that these analogs do not achieve sufficient intracellular concentrations to compete with endogenous thiamine pyrophosphate for TK. Future work in this area will attempt to improve cell potency by modifying their physiochemical properties and incorporating functionalities which might enable recognition by thiamine transporters.

Supplementary data

Procedures and analytical data are available for compounds **2**, **3**, **5**, and **6–16**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.098.

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8. Though it is possible that the cellular activity of non-pyrophosphates such as compound **3**, N3P-TT, is a result of their direct interaction with TK, we believe it is much more likely that they are first converted by TPPK inside the cell into their pyrophosphate forms. The pyrophosphate binding to TK is greater by several orders of magnitude than the corresponding non-pyrophosphate analogs (Table 1).
9. Synthesis of starting material N3PT is presented in the preceding publication, Thomas, et al.
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13. Prior to performing the TPPK/Apo-TK assay, each compound was evaluated for its ability to be pyrophosphorylated by TPPK using thiamine as a control substrate. The extent of substrate pyrophosphorylation relative to thiamine pyrophosphorylation was determined prior to competition with TPP in inhibiting TK activity according to methods described in WO2005/095391.
14. Starting material (*Z*)-ethyl 5-(benzyloxy)-2-[(dimethylamino)methylene]-3-oxopentanoate was prepared in two steps from ethyl acetoacetate (50% yield). *J. Org. Chem.* **1986**, *51*, 106.
15. Either *N*-methylated pyrazole regioisomer can be prepared in a 3:1 regioselectivity by choice of methanol or diethyl ether as solvent Menozi, G. et al. *J. Heterocycl. Chem.* **1987**, *24*, 1669.