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Synthesis, in vitro and in vivo activity of thiamine antagonist transketolase inhibitors

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Abstract—Tumor cells extensively utilize the pentose phosphate pathway for the synthesis of ribose. Transketolase is a key enzyme in this pathway and has been suggested as a target for inhibition in the treatment of cancer. In a pharmacodynamic study, nude mice with xenografted HCT-116 tumors were dosed with **1** ('N3′-pyridyl thiamine'; 3-(6-methyl-2-amino-pyridin-3-ylmethyl)-5-(2-hydroxy-ethyl)-4-methyl-thiazol-3-ium chloride hydrochloride), an analog of thiamine, the co-factor of transketolase. Transketolase activity was almost completely suppressed in blood, spleen, and tumor cells, but there was little effect on the activity of the other thiamine-utilizing enzymes α-ketoglutarate dehydrogenase or glucose-6-phosphate dehydrogenase. Synthesis and SAR of transketolase inhibitors is described.
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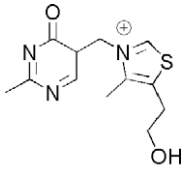
Activation of the nonoxidative branch of the pentose phosphate pathway for ribose-5 phosphate synthesis has been demonstrated in tumor cells.[1] The enzyme transketolase (TK) has a high control coefficient toward ribose synthesis in the pathway, suggesting a potential role in cancer. Mobilization of the transketolase co-factor thiamine from normal cells to tumors, leading to thiamine deficiency symptoms in cancer patients (e.g., severe cardiac failure), has been described.[2] This reorganization of cell metabolism offers one explanation of how cancerous cells maintain a continuous proliferation rate in the presence of decreased glucose metabolism and hypoxic conditions in the weakening host. Further support for the importance of TK in tumor cell maintenance was provided by Rais et al.[3] They demonstrated that a TK inhibitor oxythiamine induces G_1 arrest in Ehrlich's ascites tumor cells implanted in mice.



Oxythiamine

Thiamine exists in four possible forms: free alcohol (Vitamin B1), thiamine monophosphate (TP), diphosphate also called pyrophosphate (TPP), and triphosphate (TPPP). Upon ingestion, thiamine phosphates are hydrolyzed in the gastrointestinal tract by phosphatases and then the free alcohol is actively transported by specific transporters ThTr1 and ThTr2 (K_m = 2.6 μM).[4] Thiamine is pyrophosphorylated in the cytoplasm by thiamine pyrophosphokinase (TPPK) or other less well-characterized kinases.[5] Because binding to thia-

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mine-utilizing enzymes depends heavily upon the pyrophosphate group, pyrophosphorylation of the alcohol is an essential step in obtaining active TPP. In light of the importance of the pyrophosphate group, synthetic thiamine analogs which maintain TPPK substrate activity would be desirable. Antagonists of thiamine have been previously described.[6] In this report, we summarize our initial efforts to identify a potent inhibitor of human transketolase as a novel anticancer agent (Fig. 1).

The substitution of carbon for the N1 nitrogen of thiamine results in a potent in vitro thiamine antagonist, the 'N3′-pyridyl thiamine' **1**, originally described by Matsukawa.[7] We developed an improved synthesis of this material (Scheme 1), in six steps starting from the commercially available 3-cyano-6-methyl-2(1*H*)-pyridinone, with an overall yield of 25%. The key step was SNAr of **3** with ammonia, in a pressurized steel bomb at 210 °C, to provide aminopyridine **4** in good yield.

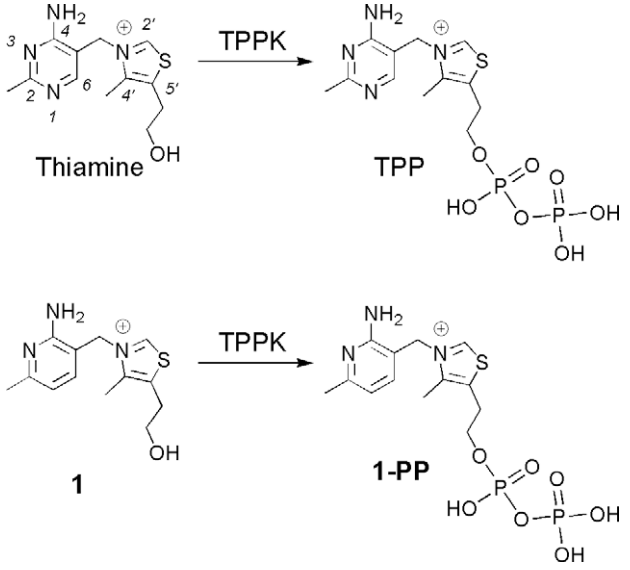
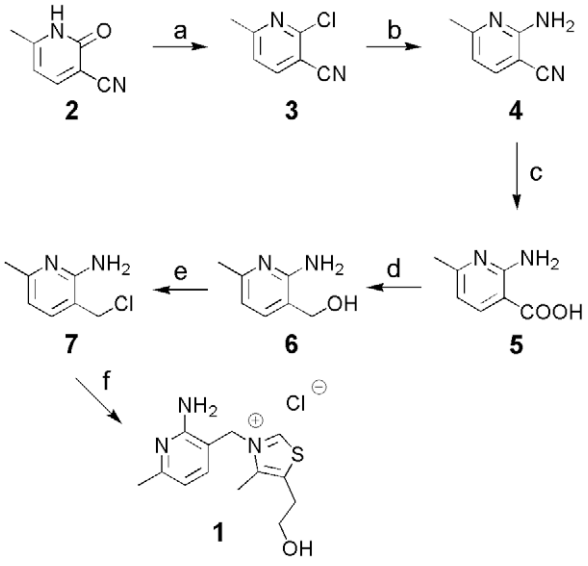


Figure 1. Structures of thiamine, thiamine pyrophosphate (TPP), and the transketolase inhibitors **1** and **1-PP**.



Scheme 1. Synthesis of **1**. Reagents and conditions: (a) $POCl_3$, 68%; (b) NH_3, EtOH, 210 °C, 300 psi, 77%; (c) aq KOH, reflux, 100%; (d) $LiAlH_4$, THF, 80%; (e) $SOCl_2$, THF, 82%; (f) 2-(4-methylthiazol-5-yl)ethanol (5 equiv), neat, 100 °C, 74%.

We carried out numerous in vitro and in vivo studies on **1** and a variety of novel analogs. **1** has an average EC_{50} value of 26 nM in a functional assay of human transketolase inhibition utilizing HCT-116 cells, a human colon cancer cell line.[8] Pyrophosphorylation of **1** is necessary for binding to transketolase, and this apparently takes place intracellularly. The average K_d of **1-PP**[9] for binding to apo-TK (transketolase lacking bound thiamine) is 22 nM, while the free alcohol shows no significant binding. Synthesis of pyrophosphates is exceedingly tedious, so we developed a coupled TPPK/apo-TK assay, in which compounds are exposed to TPPK for an extended period of time prior to testing against apo-TK.[10] In this assay, **1** has a K_d of 33 nM, in good agreement with the pyrophosphate binding result. We extensively explored the SAR around **1** and thiamine in our search for potent and selective transketolase inhibitors.

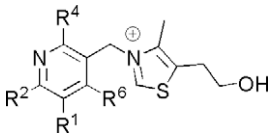
With the thiazolium B-ring of thiamine held constant, the SAR of the four substitutable positions of the N3-pyridine A-ring was explored (Table 1). Potent cellular activity was seen only when the R^4-amino group was present as well as small substitution (Me, Et, Cl) at the R^2-position.

With the N3-pyridine A-ring held constant, the SAR of the three substitutable positions of the thiazolium B-ring was explored (Table 2). There is a requirement for methyl substitution at the 4′-position in order to maintain cellular potency, although substitutions by ethyl, hydroxymethyl, or hydrogen are still active in the coupled assay. The 5′-position appears to have a size restriction. Substitution of a hydroxypropyl group for the native hydroxyethyl group results in a 50-fold loss in cellular potency, while substitution of a hydroxymethyl group only results in a 6-fold loss. Substitution of a hydroxyl group at the α-position of the hydroxyethyl group is tolerated, and interestingly, the *S*-enantiomer is 40-fold more potent than the *R*-enantiomer. Protection of the free alcohol of **1** as an acetate did not cause any significant change in cellular potency; it is very likely that the acetyl group is hydrolyzed under the conditions of the cellular assay.

The ability to substitute at the 2′-position is crucial, because such compounds are unable to effect catalysis. Moreover, appropriate groups at this position may be able to extend into the substrate-binding site of TK and perhaps make additional binding interactions. The simple 2′ substitutions of methyl, ethyl, and α-hydroxyethyl all showed cellular potency essentially identical to **1**. Substitution of larger groups, however, significantly decreased cellular potency.

Figure 2 shows inhibitor **34** from Table 2 docked into the thiamine pyrophosphate binding site of transketolase with a 2′-ethyl substituent extending toward the substrate binding region. The protein crystallographic X-ray structure of compound **1-PP** bound in a transketol-

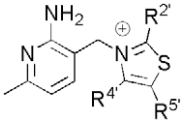
Table 1. SAR for substituted N3-pyridine A-rings

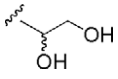
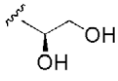
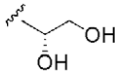


Compound	R^1	R^2	R^4	R^6	TPPK/apo-TK[a]	HCT-116[b]
1	H	Me	NH_2	H	33	26
8	H	CH_3CONH	NH_2	H	23	800
9	H	CF_3	NH_2	CF_3	1460	—
10	H	CF_3	NH_2	H	104	160
11	I	H	NH_2	H	109	1000
12	H	CN	NH_2	H	57	360
13	H	Et	NH_2	H	10	8.7
14	H	Me	NH_2	Me	34	8.3
15	H	Cl	NH_2	H	46	9.0
16	H	CF_3	H	H	310	470
17	H	H	H	NH_2	64	24,000
18	Me	H	H	H	220	38,000
19	H	NH_2	H	H	7.0	1600
20	H	H	NH_2	H	36	110
21	H	Phthaloyl	H	H	140	3100

[a] Average K_d, nM, for coupled assay from at least three experiments. Data were within 2-fold of the mean.
[b] Average EC_{50}, nM, for cellular assay performed in triplicate. Data were within 4-fold of the mean.

Table 2. SAR for substituted thiazolium B-rings



Compound	$R^{2'}$	$R^{4'}$	$R^{5'}$	TPPK/apo-TK[a]	HCT-116[b]
1	H	Me	CH_2CH_2OH	33	26
22	H	Me		53	300
23	H	Me		28	50
24	H	Me		27	2000
25	H	Et	CH_2CH_2OH	40	950
26	H	Me	$CH_2CH_2OCOCH_3$	ND	4.7
27	H	Me	$CH_2CH_2CO_2CH_3$	ND	2300
28	H	CH_2OH	CH_2CH_2OH	25	570
29	Me	H	CH_2CH_2OH	36	490
30		Me	CH_2CH_2OH	ND	840
31		Me	CH_2CH_2OH	ND	25,000
32	H	Me	CH_2CH_2CN	ND	2200
33	H	Me	$CH_2CH_2CH_2OH$	ND	1300
34	Et	Me	CH_2CH_2OH	75	21
35	$CH(CH_3)OH$	Me	CH_2CH_2OH	120	30
36	Me	Me	CH_2CH_2OH	25	56
37	H	H	CH_2CH_2OH	29	9500

[a] Average K_d, nM, for coupled assay from at least three experiments. Data were within 2-fold of the mean. ND, no data.
[b] Average EC_{50}, nM, for cellular assay performed in triplicate. Data were within 4-fold of the mean.

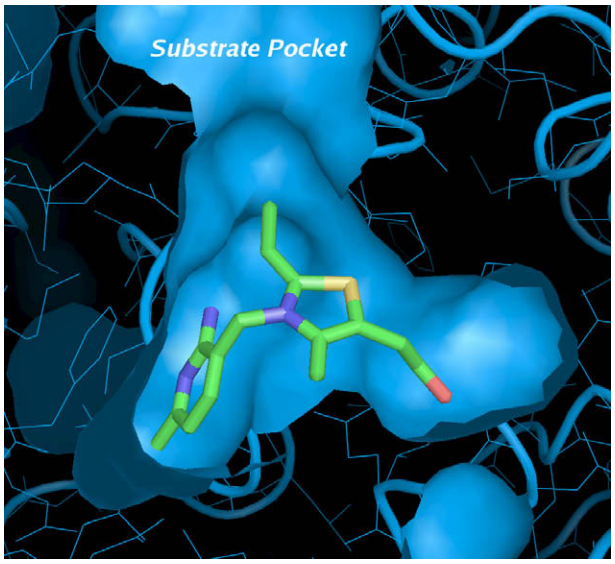


Figure 2. Inhibitor **34** docked into human TK active site.

ase construct was determined (in preparation), and inhibitor **34** was then docked with Schrodinger's *Glide* program using extended precision parameters. Prior to docking, the **1-PP** ligand was removed from the transketolase X-ray structure coordinates, and the protein was prepared by optimizing all asparagine, glutamine, histidine, and side chain hydroxyl groups and then running a short constrained refinement of the resulting structure. Indeed, inhibitor **34** was 75 nM in the coupled TPPK/apo-TK assay (Table 2), supporting the structural observations of the TK binding site that substitution at the 2′ position should be tolerated. A xenograft model in which human colon cancer HCT-116 cells were implanted in nude mice was employed to test the efficacy of our transketolase inhibitors in vivo.[11] In order to boost chances of success, the mice were put on one of two low thiamine diets (5 or 1 ppm, vs a normal diet of 16.5 ppm). The tumors were allowed to grow to 50 mm^2 before treatment was initiated. The mice were treated with compound 100 mg/kg iv bid for two weeks, after which time the tumors were assessed for size and enzymatic activity. Control animals were dosed with the vehicle.

Figure 3 shows the pharmacodynamic effects of **1**[12] in these HCT-116 tumor-bearing nude mice, with a preferential reduction of transketolase activity in tumor over that of another thiamine-utilizing enzyme, α-ketoglutarate dehydrogenase (α-KGDH), and no effect on the NADPH-containing glucose-6-phosphate dehydrogenase (G6PD). Figure 4 shows that despite the reduction of transketolase activity, there was no apparent effect on tumor size in either cohort.

Thus, although we did prepare potent and selective thiamine antagonists that inhibited transketolase both in vitro and in vivo, there was no apparent effect on tumor cell growth, at least in this particular xenograft model and cell line. This result is in contradiction to earlier reports that inhibition of TK prevents tumor growth.[2] As for why this is the case, we can only speculate that there are alternative pathways to generate ribose for DNA synthesis that are operating in these tumor cell lines. To gain a full understanding of this is beyond the scope of the current work, but worthy of future investigation.

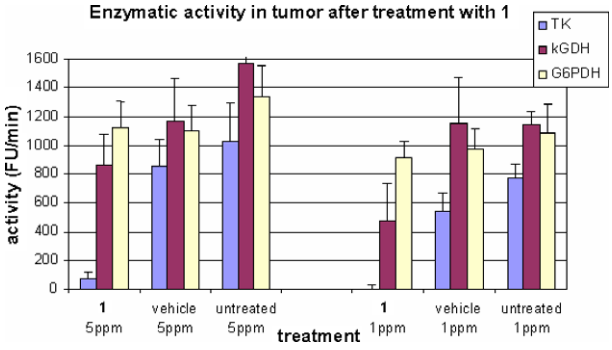


Figure 3. Comparison of selectivity of thiamine-utilizing enzymes of treated and control animals after treatment with **1**. Error bars represent standard deviation between mice (3–5/group).

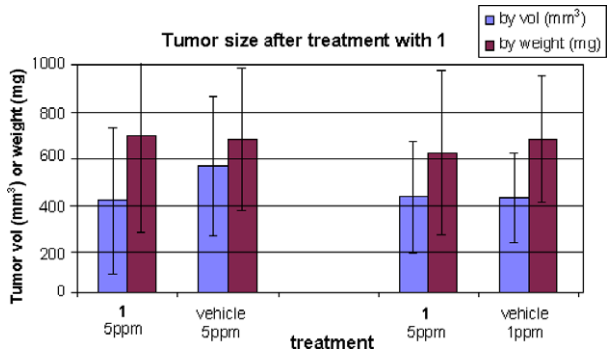


Figure 4. Comparison of tumor sizes of treated and control animals after treatment with **1**. Error bars represent standard deviation between mice (3–5/group).

Supplementary data

Procedures and analytical data are available for compounds **1** and **8**–**37**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.101.

References and notes

1. Boros, L. G.; Torday, J. S.; Lim, S.; Bassilian, S.; Cascante, M.; Lee, W.-N. P. *Cancer Res.* **2000**, *60*, 1183.
2. Cascante, M.; Centelles, J. J.; Veech, R. L.; Lee, W.-N. P.; Boros, L. G. *Nutr. Cancer* **2000**, *36*, 150.
3. Rais, B.; Comin, B.; Puigjaner, J.; Brandes, J. L.; Creppy, E.; Saboureau, D.; Ennamany, R.; Lee, W. N.; Boros, L. G.; Cascante, M. *FEBS Lett.* **1999**, *456*, 113.
4. Subramanian, V. S.; Marchant, J. S.; Parker, I.; Said, H. M. *J. Biol. Chem.* **2003**, *278*, 3976.
5. Baker, L.-J.; Dorocke, J. A.; Harris, R. A.; Timm, D. E. *Structure* **2001**, *9*, 539.
6. Du, M. X.; Sim, J.; Fang, L.; Zheng, Y.; Koh, S.; Stratton, J.; Pons, J.; Wang, J. J.-X.; Carte, B. *J. Biomol. Screen*

2004, *9*, 427; Solovjeva, O. N.; Kochetov, G. A. *FEBS Lett.* **1999**, *462*, 246; Boros, L. G. *Cancer Res.* **1998**, *58*, 3188; Boros, L. G.; Puigjaner, J.; Cascante, M.; Lee, W.-N. P.; Brandes, J. L.; Bassilian, S.; Yusuf, F. I.; Williams, R. D.; Muscarella, P.; Melvin, W. S.; Schirmer, W. J. *Cancer Res.* **1997**, *57*, 4242; Kochetov, G. A.; Izotova, A. E.; Meshalkina, L. E. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1198.

7. This compound has been previously reported (a) Matsukawa, T.; Matsuno, T. *Yakugaku Zasshi* **1944**, *64*, 145; (b) Dornow, A.; Hargesheimer, A. *Chem. Ber.* **1953**, *86*, 461; (c) Ostrovskii, Y. M.; Chernikevich, I. P.; Voskoboev, A. I.; Shellenberger, A. *Bioorg. Khim.* **1977**, *3*, 1083; (d) Meshalkina, L. E.; Golbik, R.; Usmanov, R. A.; Neef, H.; Schellenberger, A.; Kochetov, G. A. *Dokl. Akad. Nauk SSSR* **1989**, *307*, 486.
8. Boyd, S. A. WO2005095391.
9. Gutowski, J. A. *Methods Enzymol.* **1979**, *62*, 120.
10. 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.
11. Gyuris, J.; O'Hagan, R. C.; Han, M.; Robinson, M.; Weiler, S., WO2005094803.
12. Though other compounds (e.g., **13** and **26**) demonstrated comparable or better cellular potency to compound **1**, the latter was tested in the xenograft model due to its synthesis being amenable to scaleup at the time the xenograft experiments were performed.