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Prodrug thiamine analogs as inhibitors of the enzyme transketolase

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Abstract—Transketolase, a key enzyme in the pentose phosphate pathway, has been suggested as a target for inhibition in the treatment of cancer. Compound **5a** ('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 the transketolase cofactor thiamine, is a potent transketolase inhibitor but suffers from poor pharmacokinetics due to high clearance and $C_{\max}$ linked toxicity. An efficient way of improving the pharmacokinetic profile of **5a** is to prepare oxidized prodrugs which are slowly reduced in vivo yielding longer, sustained blood levels of the drug. The synthesis of such prodrugs and their evaluation in rodent models is reported.
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Thiamine (Vitamin B1) is a key nutrient involved in carbohydrate metabolism.[1] Thiamine deficiency caused by poor diet, alcoholism or other dysfunctions has been linked to severe disorders.[2] An obvious way to alleviate the symptoms of thiamine deficiency is to supplement the dietary uptake of thiamine. Unfortunately, oral absorption of thiamine is poor due to the nature of its active transporter[3] (low capacity) in the intestine, and it is rapidly cleared through the kidneys.[4] As a result, high, long-lasting blood levels of thiamine are difficult to achieve. Many thiamine prodrugs have been developed to improve the oral absorption and the pharmacokinetic profile of thiamine. The majority of these are derivatives of the thiamine disulfides discovered by Fujiwara and Watanabe in 1952.[5] Since then, about a dozen thiamine analogs have been commercialized as food additives or thiamine supplements for chronic and acute diseases, including thiamine disulfide **1**,[6] thiamine propyl disulfide **2**,[7] thiamine dibenzoyl sulfide **3**,[8] and benfotiamine **4** (Fig. 1).[9]

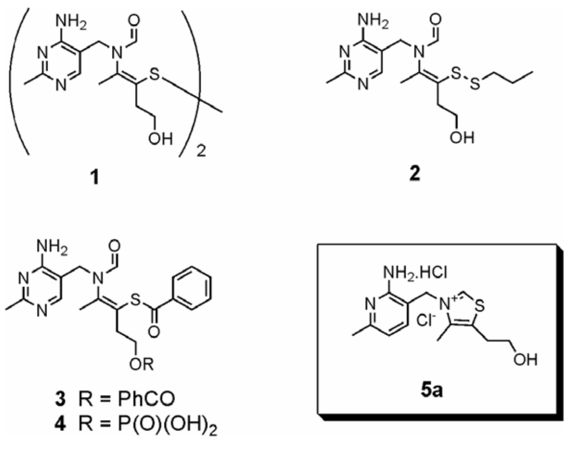

Figure 1.

These analogs are passively absorbed through the intestine due to their increased lipophilicity and then reduced in the blood, liver or kidneys to thiamine by protein thiols or glutathione.[10]

As mentioned in a previous report,[11] we were interested in developing small molecule inhibitors of transketolase, an enzyme involved in the non-oxidative branch of the pentose-phosphate pathway.[12] The most promising mol-

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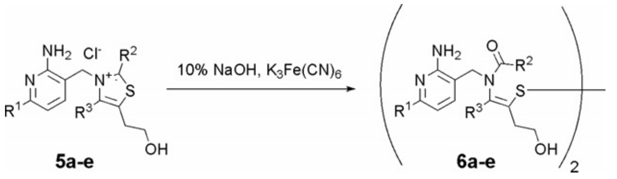
ecule, thiamine analog **5a**,[13] possessed the desired inhibitory profile but also the same pharmacokinetic liabilities. Since this compound was intended for iv administration, intestinal absorption was not an issue. However, when **5a** was dosed at 100 mg/kg iv in mice, it was rapidly cleared and showed a C_{max} linked toxicity similar to that observed with high doses of iv thiamine.[14] We report analogs of **5a** with improved pharmacokinetic profiles, leading to lower toxicity and extended transketolase inhibition in mice. Symmetric disulfide derivatives of **5a**–**e** were synthesized[15] by thiazolium ring opening under basic conditions followed by oxidation in the presence of potassium ferricyanide. This method proved to be general and was applied to a series of pyridine-ring analogs **6a**–**e** (Table 1). Since the symmetric disulfides were as potent as their parent thiazoliums in the cellular TK (transketolase) inhibition assay,[17] we then prepared mixed thiol esters/ester of **5a** in an effort to improve the physical properties of the compounds. Although acylation of thiamine is typically performed under aqueous conditions with sodium hydroxide and an acylating reagent,[18] this sequence failed with **5a** due to the higher nucleophilicity of the aminopyridine. This problem was solved by using phase transfer conditions and anhydrides as acylating agents. The bis-benzyl **7b** and bis-acetyl **7a** analogs of **5a** were prepared in good yields with no degradation (amide formation) (Scheme 1). The cyclic thiocarbonate analog **8** was prepared using triphosgene as the electrophile. This protocol was also applied to the synthesis of thioethers **9a**–**g**, alkyl sulfides **10a** and **10b**, and thiocarbonate **11**.

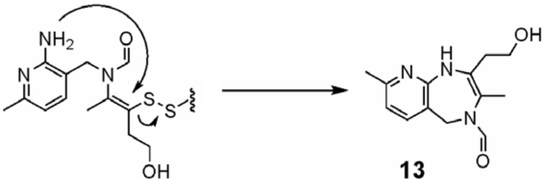
Table 1. Cell (HCT-116) activity correlation between thiazolium (**5a**–**e**) salts and disulfides (**6a**–**e**)[16]



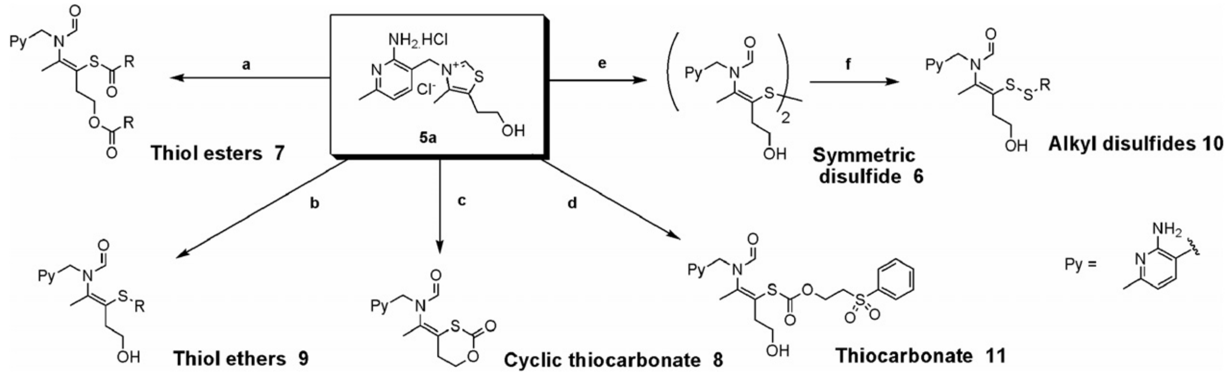
Entry	R^1	R^2	R^3	Thiazolium 5 HCT-116 TK EC_{50} (nM)	Disulfides 6 HCT-116 TK EC_{50} (nM)
a	Me	H	Me	18	12
b	Me	Me	Me	28	32
c	H	H	Me	110	479
d	Me	H	H	9541	101,870
e	Cl	H	Me	9	8

We attempted to prepare alkyl disulfides by treating the symmetric disulfide of **5a** with dialkyl disulfides in the presence of base.[19] Most of the alkyl disulfides prepared decomposed readily leaving a strong sulfury smell. The decomposition product was identified as 2-(2-hydroxyethyl)-3,8-dimethyl-1,5-dihydro-pyrido[2,3-*e*][1,4]diazepine-4-carbaldehyde **13** which resulted from the intramolecular nucleophilic attack of the pyridyl amino group on the disulfide (Scheme 2). This phenomenon has been described for thiamine but required the presence of a base.[20] Again, the reactivity of the amino group of **5a** proved to be different from that of thiamine, leading to synthetic and stability issues. Nevertheless, a few stable alkyl disulfides **10a** and **10b** were prepared. Another means of reducing the reactivity of the amino group consists in deactivating it by introducing electron-withdrawing groups. This strategy was successfully applied by replacing the pyridyl methyl group with a chlorine **5e**. In that case, the propyl disulfide **6e** was stable.

As observed with the symmetric disufides **6a**–**e**, most of the thiazolium-ring opened analogs of **5a** proved to be active in the cellular TK assay (Table 2). It is interesting to note that the inhibitory effect, indicating the presence of **5a**, could be tuned by using different moieties on the sulfur. While disulfides, thioesters, esters, and cyclic thiocar-

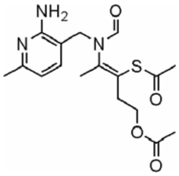
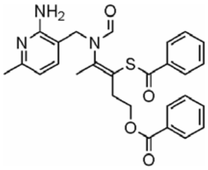
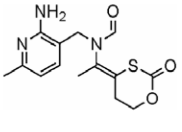
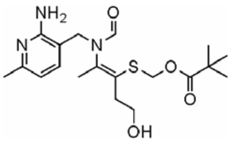
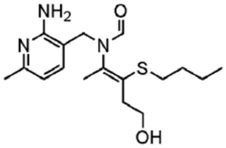
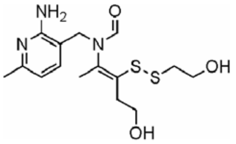
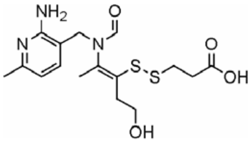


Scheme 2. Decomposition of the disulfides.



Scheme 1. Reagents and conditions: (a) LiOH, H_2O/DCM, *n*-Bu_4NBr, RCOCl, 1 h, rt, 54–87%. (b) LiOH, H_2O/DCM, *n*-Bu_4NBr, RCl, 1 h, rt, 32–76%. (c) LiOH, H_2O/DCM, *n*-Bu_4NBr, triphosgene, 1 h, rt, 42%. (d) LiOH, H_2O/DCM, *n*-Bu_4NBr, $Cl(CO)O(CH_2)_2SO_2Ph$, 1 h, rt, 42%. (e) 10% NaOH, H_2O, $K_3Fe(CN)_6$, 10 min, rt, 40–94%. (f) NaOH, RSSR, rt.

Table 2. Cell (HCT-116) activity of prodrugs of **5a**[16]

Compound	Structure	HCT-116 TK EC_{50} (nM)
7a		8
7b		28
8		15
9a		116
9b		6000
10a		13
10b		52

bonates seem to be readily transformed to the active inhibitor **5a**, sulfides **9a** and **9b** seemed to be poorly or not converted to **5a** under the assay conditions. Even if these analogs did not seem to be cleaved in the HCT-116 cells, these compounds may be cleaved by blood or liver enzymes. Indeed, the analogs **6a–e**, **7a**, **7b**, **8**, **9a**, **10a**, and **10b** were rapidly transformed in whole blood to the active inhibitor **5a** (data not shown).

Table 3. Rat PK of prodrugs (iv, 3 mg/kg)

Compound	C_{max} (μg/mL)	AUC[a] (μg h/mL)	CL_{obs} (mL/min/kg)	MRT[b] (h)	V_{ss} (mL/kg)
5a	6.61	1.25	41.3	0.72	1978
6a	1.12	1.59	32.5	12.7	19,289
9a	3.02	79.16	0.63	48.3	1832

[a] Area under the curve.

[b] Mean residence time.

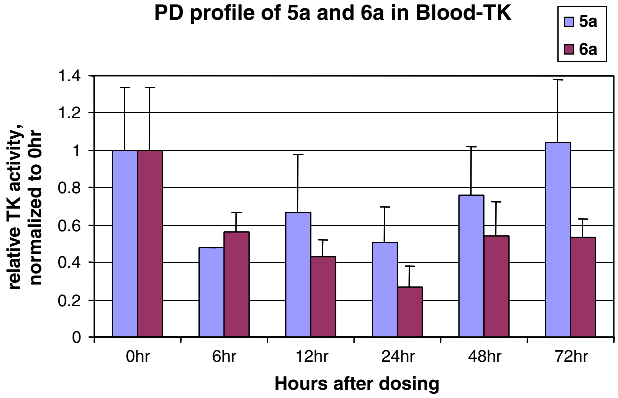


Figure 2. In vivo inhibition of blood transketolase by **5a** and **6a**.[22]

A rat pharmacokinetics assay was used to determine whether the prodrugs were reduced in vivo to the parent **5a**. In the experiment, the prodrugs were administered iv (3 mg/kg) in rats (three animals per group) in saline as vehicle. The results summarized in Table 3 indicate a clear improvement of the exposure relative to **5a** as well as a diminution of the C_{max}, thereby curtailing the C_{max} associated toxicity observed with **5a**. The high V_{ss} of **6a** (19,289 mL/kg) is consistent with the hypothesis made by Watanabe et al. which states that disulfide analogs of thiamine are readily absorbed in erythrocytes and slowly released as thiamine in the plasma.[21] The reduction occurs in the cell membrane (reaction with glutathione) and the increase of the mean residence time (MRT) is a consequence of the slow passive release of the reduced quaternary amine through the red blood cell membrane.

Disulfide **6a** was chosen to be evaluated in a mouse pharmacodynamic study. Balb-c mice were administered 100 mg/kg **6a** ip (vehicle: 10% ethanol/0.1% DMSO/90% D-PBS) and the levels of blood transketolase activity recorded over 72 h. The results presented in Figure 2 indicate that the increased exposure of **6a** correlated in vivo with a stronger and longer inhibition of blood transketolase.

We showed that ring-opened analogs of the transketolase inhibitor **5a** were effectively transformed in vivo to the active thiazoliums. The effectiveness of this transformation is dependent on the sulfur moiety. Furthermore, the pharmacokinetic properties were improved. The C_{max} was reduced, thus avoiding toxicity, and higher distribution and exposure correlated in vivo with a stronger and longer inhibition of blood transketolase.

Supplementary data

Procedures and analytical data are available for compounds **6b**, **6c**, **6e**, **7a**, **7b**, **8**, **9a**, **9b**, **10a**, and **10b**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.100.

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16. Average EC_{50}, nM, for cellular assay performed in triplicate. Data were within 4-fold of the mean.
17. Though it is possible that the cellular activity of disulfides, such as compound **6a**, is a result of their direct interaction with TK, we believe it is much more likely that they are first enzymatically reduced inside the cell to their thiazolium forms (see, Ref. 11). The thiazolium analog is then pyrophosphorylated by TPPK inside the cell to provide the corresponding pyrophosphate. Pyrophosphate binding to TK is greater by several orders of magnitude than the corresponding non-pyrophosphate analogs (see previous articles, Thomas et al.).
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22. Error bars represent standard deviations between mice (3–5/group).