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Short Communication

COMPETITIVE INHIBITION OF *TRYPANOSOMA BRUCEI* PHOSPHOGLUCOSE ISOMERASE BY D-ARABINOSE-5-PHOSPHATE DERIVATIVES

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We report four new strong high energy intermediate analog competitive inhibitors of fructose-6-phosphate isomerization catalyzed by purified *Trypanosoma brucei* phosphoglucose isomerase: D-arabinonhydroxamic acid-5-phosphate, D-arabinonate-5-phosphate, D-arabinonamide-5-phosphate and D-arabinonhydrazide-5-phosphate. For comparison, the inhibitory properties of the corresponding non-phosphorylated analogues D-arabinonhydroxamic acid, D-arabinonate, D-arabinonamide and D-arabinonhydrazide were aslo evaluated. D-Arabinonhydroxamic acid-5-phosphate appears as the most potent competitive inhibitor ever evaluated on a phosphoglucose isomerase with an inhibition constant value of 50 nM and a Michaelis constant over inhibition constant ratio of about 2000. Our results show that anionic high energy intermediate analogues, and more particularly D-arabinonhydroxamic acid-5-phosphate, display a weak but significant specificity for *Trypanosoma brucei* phosphoglucose isomerase versus yeast phosphoglucose isomerase, while neutral high energy intermediate analogues are not selective at all. This would indicate the presence of more positively charged residues in the active site for *Trypanosoma brucei* phosphoglucose isomerase as compared to that of yeast phosphoglucose isomerase.

Keywords: 5.3.1.9 Phosphoglucose isomerase; Carbohydrates; Competitive inhibitors; Hydroxamic acids; *Trypanosoma brucei*

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Abbreviations: 6PGA, 6-phospho-D-gluconate; BsPGI, *Bacillus stearothermophilus* phosphoglucose isomerase; F6P, fructose-6-phosphate; HEI, high energy intermediate; PGI, phosphoglucose isomerases (EC 5.3.1.9); RmPGI, rabbit-muscle phosphoglucose isomerase; TbPGI, *Trypanosoma brucei* phosphoglucose isomerase; Tris–HCl, tris(hydroxymethyl)aminomethane hydrochloride; YPGI, yeast phosphoglucose isomerase

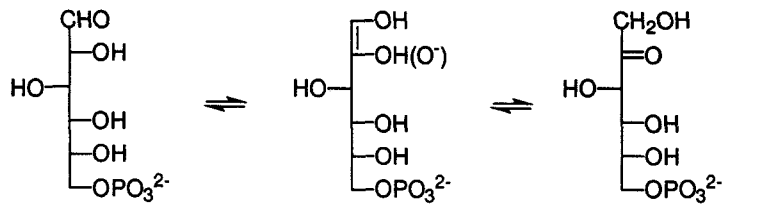
INTRODUCTION

The parasitic protozoan *Trypanosoma brucei* is the causative agent of sleeping sickness in tropical Africa. Trypanosomes have drawn much attention because of their microbody-like organelle, the glycosome, which contains most of their glycolytic enzymes. Owing to the absence of a functional mitochondrion, the bloodstream form of *T. brucei* is entirely dependant on glycolysis for its production of energy, with exogeneous glucose as its preferred source of carbohydrate.[1] Inhibition of glycolysis was shown to result in immediate death of the parasite and its elimination from the bloodstream of the infected organism.[2,3] Therefore, trypanosomal glycolytic enzymes would serve as excellent targets for drug design, provided that sufficient parasite versus host selectivity can be obtained.[4] A recent study on glyceraldehyde-3-phosphate-dehydrogenase using structure-based drug design confirmed that energy production can be blocked in trypanosomatids with a specific tight-binding competitive inhibitor of an enzyme in the glycolytic pathway.[5] Computer modeling[7] of glycolysis in *T. brucei*[6–8] shows that competitive inhibitors of the rate-limiting enzymatic steps significantly reduce glycolytic flux when $[I]/K_i$ ratios are in the range 10–100. These studies pointed out that there is no single-rate limiting step in a metabolic pathway, but control can be shared among several steps.[8] Hence, a combination of several potent competitive inhibitors may be very effective. Therefore, a detailed knowledge of all glycosomal enzymes involved in glycolysis is required for the design of effective and specific antitrypanosomal molecules.

Most glycolytic enzymes of *T. brucei* have been purified and characterized extensively. However, *T. brucei* phosphoglucose isomerase (TbPGI)[9,10] is undoubtedly the least studied of all seven glycosomal enzymes, with no high-resolution three-dimensional structure yet reported (the first high-resolution three-dimensional structure of phosphoglucose isomerases (PGI), namely *Bacillus stearothermophilus* PGI (BsPGI),[11] appeared only very recently in the Protein Data Bank). The main reason is that TbPGI is generally not considered as a suitable target for antitrypanosomal drug design. The enzyme, which interconverts D-glucose-6-phosphate and D-fructose-6-phosphate (F6P) (Scheme 1) is assumed to operate at equilibrium, and therefore exerts little or no control on the glycolytic flux.[6] However, any

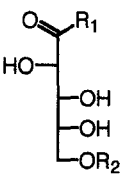
enzyme of a pathway may be considered a potential target for drug development, particularly when transition state or high-energy intermediate (HEI) analog inhibitors in the submicromolar range are considered. To date, this type of inhibitor has never been evaluated on TbPGI. Although substrate analog competitive inhibitors are easy to design, it is very difficult to deliver and maintain such compounds to the target enzyme at concentrations that greatly exceed their inhibition constant.[12] Uncompetitive inhibitors (or mixed inhibitors)[13,14] may be very effective, but they are much less easy to find or design.[12] To date, the only competitive inhibitor of TbPGI reported is the substrate analog D-gluconate-6-phosphate (6PGA, $K_i = 140\,\mu M$ versus F6P).[10] 6PGA did not display any significant specificity for TbPGI as compared to yeast PGI (YPGI), RmPGI and BsPGI.

As part of an ongoing project on the study of aldose–ketose isomerases, we recently reported the synthesis of the new D-arabinonhydroxamic acid-5-phosphate **1a** and its kinetic evaluation versus the known D-arabinonate-5-phosphate **2a**[15] (Scheme 2) on YPGI, RmPGI and BsPGI.[16] New types



D-glucose-6-phosphate | 1,2-cis-enediol(ate) high energy intermediate | D-fructose-6-phosphate

SCHEME 1 Isomerization reaction catalyzed by phosphoglucose isomerases (PGI).



R_1	NHOH	O⁻	NH_2	$NHNH_2$
$R_2 = PO_3^{2-}$	**1a**	**2a**	**3a**	**4a**
$R_2 = H$	**1b**	**2b**	**3b**	**4b**

SCHEME 2 D-Arabinose derivatives synthesized.

TABLE I Inhibition properties of phosphate sugar derivatives on PGI from *T. brucei* and yeast with F6P as the substrate[a]

Cpd	*TbPGI*[b]		*YPGI*[c,d]		K_i^Y/K_i^{Tb}
	K_i (μM)	K_m/K_i	K_i (μM)	K_m/K_i	
1a	0.050 ± 0.009	2060	0.23 ± 0.02	300	4.6
2a	0.73 ± 0.06	141	2.1 ± 0.4	33	2.9
3a	3.3 ± 0.3	31	4.2 ± 0.9	16	1.3
4a	6.2 ± 0.3	17	8.0 ± 0.8	9	1.3
6PGA	140 ± 30[e]	0.7	123 ± 12	0.6	0.9

[a]50 mM Tris–HCl buffer, pH 8.0, 30°C (see Ref. [17] for details on the experimental conditions). [b]K_m (F6P) = 103 ± 9 μM. [c]K_m (F6P) = 69 ± 6 μM. [d]Values from Ref. [16,17]. [e]100 mM triethanolamine buffer, pH 7.6, 25°C (value from Ref. [10]).

of phosphorylated sugar derivatives, D-arabinonamide-5-phosphate **3a** and D-arabinonhydrazide-5-phosphate **4a** (Scheme 2) were subsequently synthesized and evaluated on YPGI.[17] In the present study, we wish to report the inhibitory properties of all four compounds on the purified TbPGI with F6P as the substrate (Table I). For comparison, the corresponding non-phosphorylated analogues **1b–4b** (Scheme 2), respectively D-arabinonhydroxamic acid, D-arabinonate,[18] D-arabinonamide[19] and D-arabinonhydrazide,[20] were also synthesized and their inhibitory properties evaluated on F6P isomerization catalyzed by TbPGI.

RESULTS AND DISCUSSION

Compounds **1a–4a** were designed as potential analogs of the postulated 1,2-cis-enediol(ate) HEI thought to be involved in the isomerization reaction catalyzed by PGI (Scheme 1).[15–17] As shown in Figure 1, **1a** proved to be a competitive inhibitor for TbPGI with a K_i value of 50 nM (Table I). To date, this inhibition constant makes **1a** the best known competitive inhibitor for TbPGI with F6P as the substrate. When compared to our previously reported K_i values for YPGI, RmPGI and BsPGI (respectively 228, 195 and 98 nM) determined under the same experimental conditions,[16] this inhibition constant of 50 nM is also the lowest value ever obtained for a PGI. The inhibition constants are approximately of the same order of magnitude, a result in agreement with the proposed relatively high-degree of conservation of the active site of these enzymes.[10]

As compared to the known TbPGI inhibitor 6PGA,[10] the K_i value for **1a** is about 3000-fold lower. The ratios K_m/K_i determined for **1a** and 6PGA (respectively 2060 and 0.7, Table I) clearly indicate that **1a** behaves as a

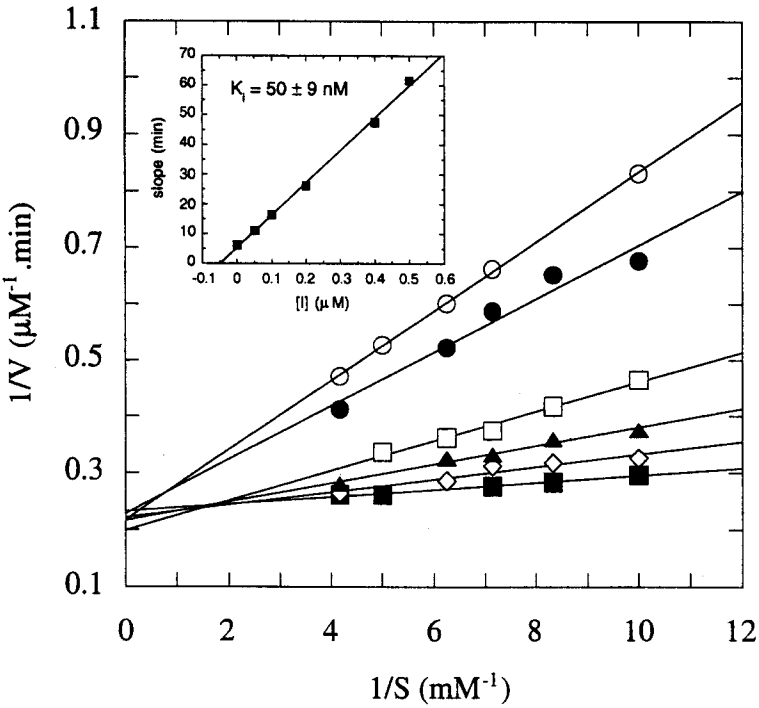


FIGURE 1 Inhibition of *T. brucei* PGI by D-arabinonhydroxamic acid-5-phosphate **1a**. Double reciprocal plot of initial reaction velocity versus F6P concentration obtained at various concentrations of inhibitors (50 mM Tris–HCl buffer, pH = 8.0, 30°C): ■, no inhibitor; ◇, $[I] = 0.05\,\mu M$; ▲, $[I] = 0.10\,\mu M$; □, $[I] = 0.20\,\mu M$; ●, $[I] = 0.40\,\mu M$; ○, $[I] = 0.50\,\mu M$. The inset represents a secondary plot of the slopes of the curves against inhibitor concentration.

strong HEI analog inhibitor of TbPGI, while 6PGA rather behaves as weak substrate analog competitive inhibitor. Interestingly the ratio of 4.6 for the inhibition constants of **1a** determined for YPGI and TbPGI (K_i^Y/K_i^{Tb}, Table I), suggests a weak specificity of the **1a** for TbPGI versus YPGI, while for 6PGA, the K_i^Y/K_i^{Tb} ratio of 0.9 indicates no specificity at all. Although the difference between the two ratios is not that large (it corresponds to a 0.9 kcal/mol increase in binding affinity), we do think that the observed specificity of TbPGI versus YPGI for the HEI analogue **1a** is significant.

The inhibition constant of D-arabinonate-5-phosphate for TbPGI **2a**[16] is about 15 times higher than that for **1a** (Table I). With a K_m/K_i ratio of 141, **2a** also behaves as a new and good HEI inhibitor of TbPGI, although it is much weaker than **1a**. The K_i^Y/K_i^{Tb} ratio of 2.9 also makes it more specific for TbPGI than for YPGI. The amide and hydrazide derivatives, **3a** and **4a**, competitively inhibit TbPGI with K_m/K_i ratios of 31 and 17, respectively.

While not as good inhibitors as **1a**, these two new HEI inhibitors are still much better inhibitors than 6PGA. Contrary to **1a** and **2a**, **3a** and **4a** have K_i^Y/K_i^{Tb} ratios approximately equal to unity, which make these inhibitors not specific for TbPGI versus YPGI. Owing to their respective properties **1a** and **2a** are most likely bound to the PGI active site in an anionic form, while **3a** and **4a** are considered to bind in a neutral form; this had led us to conclude that electrostatic stabilization of a 1,2-cis-enediolate (rather than 1,2-cis-enediol) HEI plays a significant role in the catalytic power of YPGI.[17] The same conclusion holds for TbPGI. In addition, the K_i^Y/K_i^{Tb} ratios determined for compounds **1a–4a** suggest that anionic HEI inhibitors are more specific than neutral ones in the case of TbPGI when compared to YPGI. This would indicate that anionic HEI is more stabilized by electrostatic interactions in the active site of TbPGI than in YPGI, i.e. that some positively charged residue could be present in the TbPGI active site which is absent in the YPGI active site.

To check for the contribution of the charged phosphoryl group, IC_{50} values for the TbPGI-catalyzed isomerization of F6P were measured for the phosphorylated D-arabinose derivatives **1a–4a** (100 mM triethanolamine buffer, pH 7.6, 25°C) as well as for the non-phosphorylated D-arabinose derivatives **1b–4b** (50 mM Tris–HCl buffer, pH 8.0, 30°C). D-Arabinonhydroxamic acid **1b** was synthesized from D-arabinono-1,4-lactone[18] using the procedure reported for the synthesis of aldonhydroxamic acids from aldonolactones.[21] D-Arabinonate **2b**[18] was synthesized from D-arabinose by the hypoiodite method.[22] D-Arabinonamide **3b**[19] and D-arabinonhydrazide **4b**[20] were obtained as described. IC_{50} values obtained for compounds **1a**, **2a**, **3a** and **4a** (respectively 0.4, 0.9, 8 and 9 μM) are smaller by a factor ranging from 15000 to 2100 to those for compounds **1b**, **2b**, **3b** and **4b** (respectively 6, 9.5, 22 and 19 mM). This means that the non-phosphorylated analogues **1b–4b** are quite weak inhibitors of the TbPGI-catalyzed isomerization of F6P. Interestingly, the respective order of the IC_{50} values obtained for the phosphorylated derivatives, IC_{50} (**1a**) < IC_{50} (**1b**) < IC_{50} (**1c**) ≈ IC_{50} (**1d**) is approximately the same as for the non-phosphorylated derivatives. This would indicate that compounds **1b–4b** bind at the enzyme active site in much the same way as do compounds **1a–4a**.

In conclusion, we report four new strong HEI analog competitive inhibitors of TbPGI, with D-arabinonhydroxamic acid-5-phosphate **1a** as the most potent inhibitor ever evaluated on a PGI. Our results show that anionic HEI analogues, and more particularly **1a**, display a weak but significant specificity for TbPGI versus YPGI, while neutral HEI analogues are not selective at all. This would indicate the presence of more positively charged

residues in the active site for TbPGI as compared to that of YPGI. Our observations are encouraging for structure-based drug design, where the identification of such differences could lead to the synthesis of more specific and effective TbPGI inhibitors, and eventually, to antitrypanosomal drugs. Biological activities of compounds **1a–4a** and **1b–4b** on live trypanosomes are presently being evaluated and structural studies on TbPGI will be undertaken.

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