The molecular dynamics of Trypanosoma brucei UDP-galactose 4'-epimerase a drug target for African sleeping sickness

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Figure S1. Thermal scanning fluorimetry of *HsGalE*. 5 μM *HsGalE* in 10 mM HEPES-NaOH, pH 8.8, 1% (v/v) DMSO, 5× Sypro orange showed a clear melting curve resulting in a $T_m$ of 51.5±0.3 °C.
Table S1. *Tb*GalE Agonists

| NSC ID | Structure | % inhib. @ 100 mM |
|--------|-----------|------------------|
| 91395  | ![Structure](image1) | -167             |
| 61610  | ![Structure](image2) | -169             |
| 7524   | ![Structure](image3) | -191             |
| 91396  | ![Structure](image4) | -194             |
Specific methods can be found in Durrant et al. (2010) *J Med Chem* 53, 5025-5032.
Table S2. Percentage activity of 20 nM HsGalE in the presence of different DTP compounds

| DTP Compound | % Activity |
|--------------|------------|
| No compound  | 100 ± 17   |
| 91395        | 102 ± 32   |
| 61610        | 89 ± 27    |
| 7524         | 112 ± 3    |
| 91396        | 124 ± 30   |
| 260594a      | 30 ± 190   |
| 146771a      | 104 ± 57   |
| 202386a      | 41 ± 59    |

The reactions contained 100 μM DTP compound, 100 μM UDP-Galactose, 10 mM NAD+, 1.2 μM HsUGDH, 10 mM HEPES-NaOH, pH 8.8, 1% (v/v) DMSO. Data are reported as the mean ± SD determined from three separate experiments. No compound resulted in a statistically significant (Student’s t-test) change in activity.

*Compounds 260594, 146771 and 202386 gave large errors due the formation of a coloured precipitate, which prevented accurate determination of activity.
Table S3. Melting temperatures of HsGalE in the presence of different DTP compounds

| DTP Compound | Tm (°C)  | ∆Tm (K)  |
|--------------|----------|----------|
| No compound  | 51.5 ± 0.3 | N/A |
| 91395        | 51.3 ± 0.3 | -0.2 ± 0.6 |
| 61610        | 51.4 ± 0.1 | -0.1 ± 0.4 |
| 7524         | 51.3 ± 0.4 | -0.2 ± 0.7 |
| 91396        | 51.3 ± 0.3 | -0.2 ± 0.6 |
| 260594a      | N/D      | N/D      |
| 146771a      | N/D      | N/D      |
| 202386a      | N/D      | N/D      |

The reactions contained 5 μM HsGalE, 100 μM DTP compound, 10 mM HEPES, pH 8.8, 1% (v/v) DMSO, 5× Sypro orange. The change of melting temperature, ∆Tm, due to ligand binding was calculated according to:

\[
\Delta T_m = (T_m \text{ of protein without compound}) - (T_m \text{ of protein with compound})
\]

Data are reported as mean ± SD determined from three experiments. If a compound bound to the enzyme, it would be expected to stabilize the protein’s structure resulting in an increase in Tm. However, none of the compounds tested here resulted in a statistically significant (Student’s t-test) change in Tm.

a Compounds 260594, 146771 and 202386 formed a colored precipitate, preventing determination of the melting temperature.