

## **Supporting Information.**

### **Rational Modification of a Candidate Cancer Drug for Use Against Chagas Disease**

**Kraus, JM, Verlinde, CLMJ, Karimi, M, Lepesheva, GI, Gelb, MH, Buckner, FS**

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***T. cruzi* and human 14DM assays.** Reconstitution of enzymatic activity *in vitro* and inhibitory assay of *T. cruzi* and human 14DM were performed as described previously<sup>1,2</sup>. We tested the ability of tipifarnib and its analogs to inhibit the *in vitro* turnover of *T. cruzi*<sup>1</sup> and human 14DM<sup>3</sup>. The enzymes were electrophoretically pure, had spectrophotometric indexes of OD<sub>417</sub>/OD<sub>278</sub> of 1.55 and 1.45 and specific heme content of 17 and 16.5 nmol/mg for *T. cruzi* and human 14DM, respectively. Cytochrome P450 concentration was determined from the difference spectra of the reduced carbon monoxide complexes using the extinction coefficient of 91,000 M<sup>-1</sup> cm<sup>-1</sup> (450-490 nm)<sup>4</sup>. The reaction conditions were optimized to give maximal turnover and reproducibility<sup>1,2</sup>. Low solubility of the sterols limits maximal substrate concentration while decrease in the enzyme concentration dramatically affects stability and protein-protein interactions with the electron donor, cytochrome P450 reductase. The final reconstituted reaction mixture contained 1 μM 14DM, 2 μM cytochrome P450 reductase and 50 μM [<sup>3</sup>H]-labeled substrate in 20 mM MOPS (pH 7.4), 50 mM KCl, 5 mM MgCl<sub>2</sub>, 10% glycerol, 0.4 mg/ml isocitrate dehydrogenase and 25 mM sodium isocitrate. For the inhibition assay, the reaction was performed in the presence of increasing concentrations of the compounds tested as 14DM inhibitors (concentration range 0.5-100 μM). After 5 min of preincubation with the inhibitors at 37°C, the reaction was initiated with the addition of NADPH (5 μM). Sterols were extracted with ethyl acetate and analyzed by reverse phase HPLC in the linear gradient of methanol:acetonitrile:H<sub>2</sub>O, 9:9:2 (solution A) and methanol (solution B) (0-100%) using a Waters C18 column and a β-RAM radioactivity detector. The inhibitory potency was estimated as molar ratio inhibitor/enzyme at which the activity decreased 2-fold (I/E<sub>2</sub>).

The data show that the tested compounds are species-specific: they strongly inhibit activity of 14DM from *T. cruzi* with essentially no effect on the orthologous human enzyme. In the case of *T. cruzi* 14DM, the value for  $I/E_2$  remains  $<1$  even after one hour reaction in the presence of the 50-fold molar excess of the substrate, indicating potent inhibition.

Compound	<i>T. cruzi</i> 14DM		Human 14DM	
	* $I/E_2$	Substrate conversion at 1/1 molar ratio inhibitor/P450, % (1 hour reaction)	$I/E_2$	Substrate conversion at 100/1 molar ratio inhibitor/P450, % (5 min reaction)
Tipifarnib	0.48±0.11	0.6±0.1	>100	100
Enantiomer of tipifarnib	0.74±0.06	40±3.5	>100	100
2c	0.65±0.09	28±1.7	>100	100
2f	0.85±0.07	37±4.0	>100	100
2g	0.62±0.06	24±1.0	48	93
Ketoconazole	0.75±0.07	33±1.4	2.3	0

\*molar ratio inhibitor/enzyme which causes 50% decrease in the turnover number;

the data are presented as mean ±SD turnovers: *T. cruzi* 14DM 2.8 nmoles/nmol P450/min; Human 14DM 33 nmoles/nmol P450/min;

We also tested the ability of **2g** to directly bind to recombinant *T. cruzi* 14DM<sup>5</sup> *in vitro* by monitoring the change in heme Soret absorbance band. Incremental amounts of **2g** were added to 1 μM recombinant *T. cruzi* 14DM as described previously<sup>5</sup>. A plot of the extent of Soret band spectral shift versus the total concentration of **2g** present showed a liner increase with a sharp break when 1 enzyme equivalent of inhibitor was present (data

not shown). This indicates that the equilibrium dissociation constant for the enzyme-**2g** complex is much less than the total enzyme concentration of 1  $\mu$ M, and it is likely less than 10 nM based on computer simulation of the binding curve.

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