Supplementary material

Activation of benznidazole by trypanosomal type I nitroreductases results in glyoxal formation

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Figure S1. Structure of the nitroimidazoles compounds.

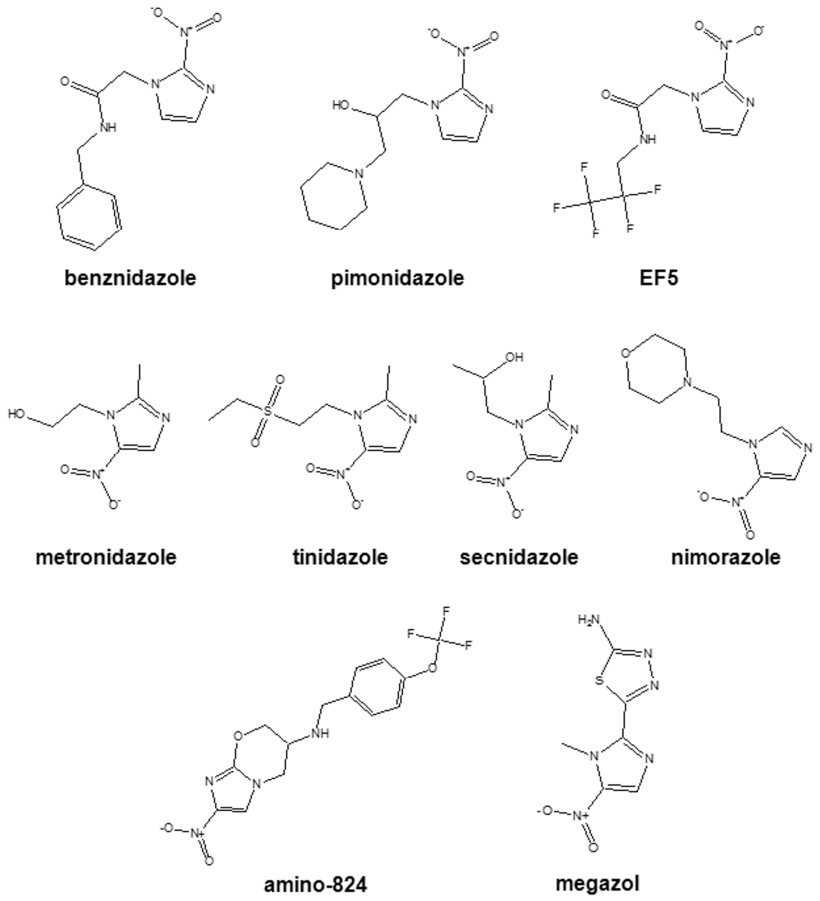


Figure S2. Elevated levels of the trypanosomal type I nitroreductase, but not other enzymes, enhances susceptibility of bloodstream form *T. brucei* to benznidazole. The susceptibility of bloodstream form *T. brucei* control cells (2T1) and derivatives induced to over express TbNTR, TbPGS (*Trypanosoma brucei* prostaglandin F2α synthase), TbCPR2 or TbCPR3 (*Trypanosoma brucei* cytochrome P450 reductase 2 and 3, respectively) to benznidazole was evaluated. The concentration of nitroimidazole that inhibits parasite growth by 50% (IC_{50} in μM) was determined using alamarBlue (see Experimental). The data are means from 4 experiments ± standard deviations. Susceptibility of parasites over expressing TbPGS, TbCPR2 or TbCPR3 was equivalent to the parental control line whereas elevated levels of TbNTR resulted in increased sensitivity. The difference in susceptibility between parasites with elevated levels of TbNTR and the other lines was shown to be statistically significant (P <0.01), as assessed by Student's *t* test.

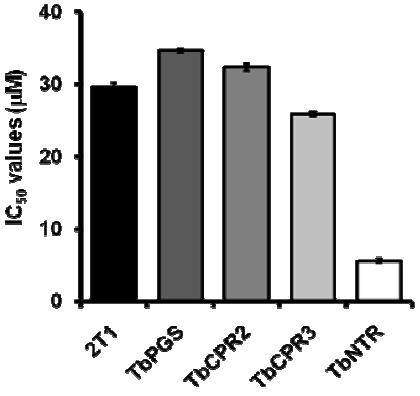


Figure S3. Susceptibility of bloodstream form *T. brucei* with elevated levels of NTR to nitroimidazoles. Over expression of TbNTR in bloodstream form *T. brucei* (cell line designated as $TbNTR^{RV}$) confers hypersensitivity to benznidazole and megazol but not to amino-824. Cells induced to express elevated levels of TbNTR are labeled as $TbNTR^{RV}$ +tet (tetracycline) while non-induced cultures are labeled as $TbNTR^{RV}$-tet. Data are expressed as the drug concentration that inhibits parasite growth by 50% (IC_{50}) and are the means from four experiments $\pm$ standard deviation. The difference in susceptibility for benznidazole and megazol were statistically significant ($p < 0.01$), as assessed by Student's *t*-test. The small difference observed using amino-824 was not significant under this analysis.

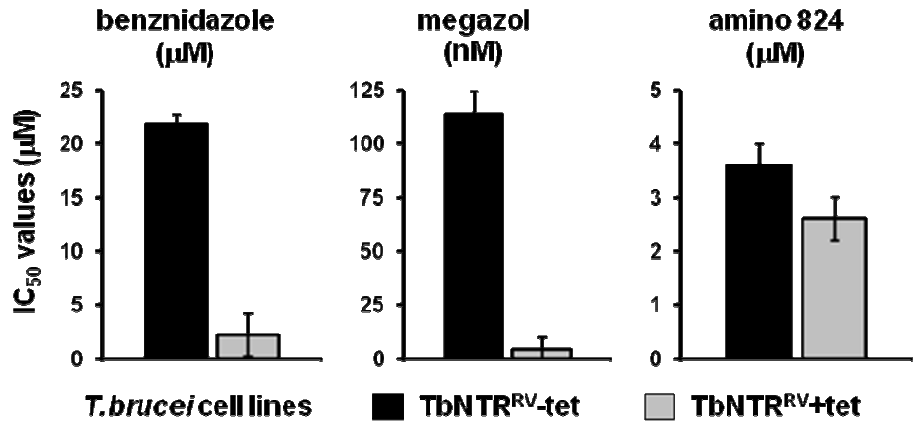
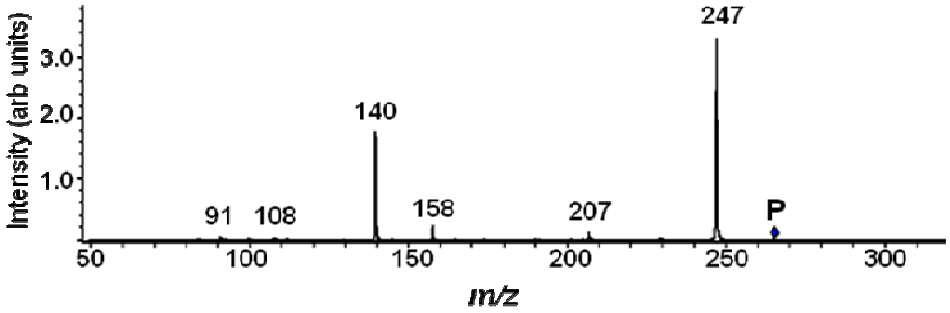
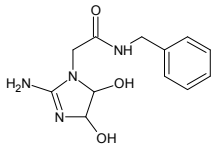
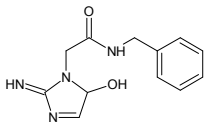
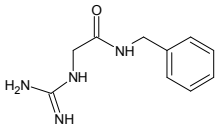
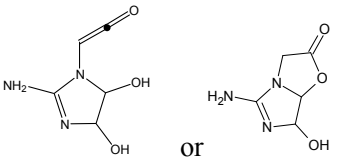
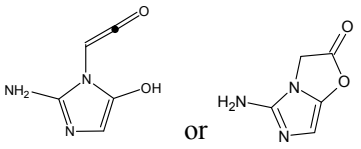
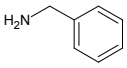


Figure S4. Analysis of the dihydro-dihydroxyimidazole. A. Positive tandem MS of the ion labeled as "peak d" in Fig. 3A (*m/z* value of 265). The series of ions generated is identical to that observed for peak c (Fig. 3C). B. Structure of the fragments obtained by tandem positive ESI-MS (see Fig. 3A and Fig. S3A). All adducts are $[M+H]^+$

A



B

m/z	Structure	*m/z*	Structure
265		247	
207		158	 or
140	 or	108	
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