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Discovery of 2-iminobenzimidazoles as a new class of trypanothione reductase inhibitor by high-throughput screening

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Abstract—A high-throughput screening campaign of a library of 100,000 lead-like compounds identified 2-iminobenzimidazoles as a novel class of trypanothione reductase inhibitors. These 2-iminobenzimidazoles display potent trypanocidal activity against *Trypanosoma brucei rhodesiense*, do not inhibit closely related human glutathione reductase and have low cytotoxicity against mammalian cells.

Parasitic protozoa of the family Trypanosomatidae are the causative agent of many significant tropical diseases including African trypanosomiasis, Chagas disease and Leishmaniasis. In the 2004 world health report[1] African trypanosomiasis was reported to cause 48 thousand deaths and a disease burden of 1525 thousand DALYs (disability adjusted life years) annually, Chagas disease 14 thousand deaths and a disease burden of 667 thousand DALYs and Leishmaniasis 51 thousand deaths and a disease burden of 2090 DALYs. There are currently nine key drugs in use for the treatment of these disease states (Fig. 1): suramin and pentamidine against early stage African trypanosomiasis, and eflornithine and melarsoprol against late stage disease; nifurtimox and benznidazole against early stage Chagas disease; meglumine antimoniate and sodium stibogluconate against Leishmaniasis, and amphotericin B against antimony-resistant strains. All of these drugs have severe limitations including administration difficulties, long treatment regimes, life-threatening side effects, varied parasitological cure rates for different strains, lack of efficacy against late stage diseases, and increasing incidence of drug resistance.[2–5]

The intracellular reducing environment of trypanosomatids is maintained by a unique thiol redox system where the glutathione/glutathione reductase (GR) couple found in mammalian cells is replaced by the (bis-glutathionyl)spermidine trypanothione/trypanothione reductase (TR) couple.[6] TR is a key enzyme of the parasite antioxidant defence,[7,8] does not occur in the mammalian host and has been found to be essential for all trypanosomatids currently studied.[9,10]

TR and human GR have similar catalytic mechanisms with 14 of the 19 amino acid residues close to the substrate binding site being conserved. However, they are specific to their respective disulfide substrates (Fig. 2).[11] GR has a hydrophilic, positively charged region in its active site that interacts with the glycine carboxylates of glutathione disulfide, while TR has a larger binding site with a negatively charged region with which the spermidine moiety of trypanothione disulfide binds.[12] The absence of TR from the mammalian host and the sensitivity of trypanosomatids to oxidative stress makes TR an attractive target for trypanosomiasis therapeutics.[13] The objective of this work, therefore,

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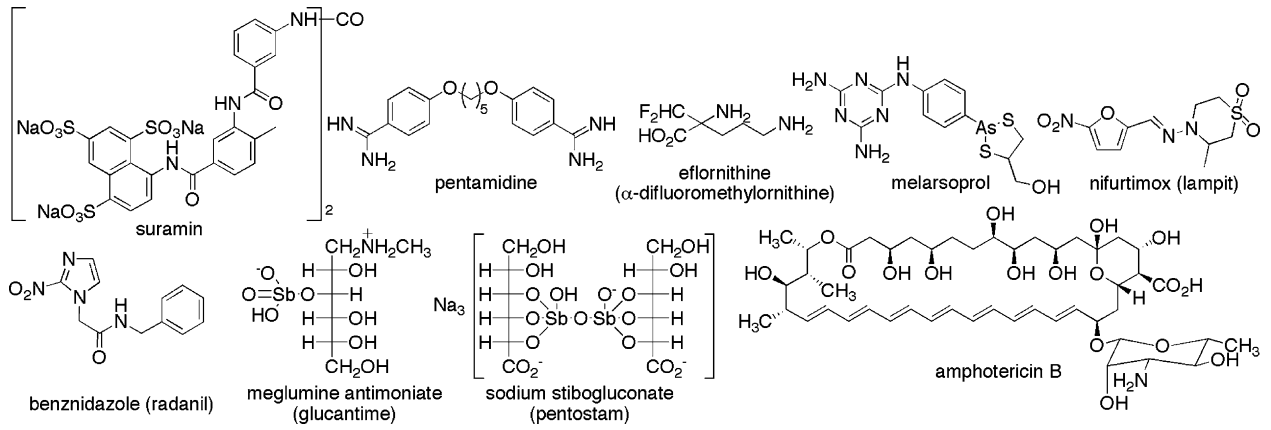

Figure 1. Current trypanosomiasis drugs on the market.

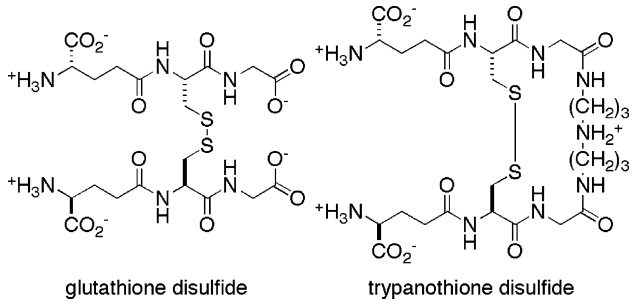

Figure 2. Trypanothione disulfide and glutathione disulfide.

was to identify novel classes of TR inhibitors by high-throughput screening (HTS) of a diverse chemical library.

Our chemical compound library is a collection of approximately 100,000 compounds purchased from commercial vendors. The compounds in this chemical library were selected to provide 'lead-like' chemical structures with a guiding philosophy that most successful drug development projects have started from leads that are smaller and more polar than the drug itself. 'Lead-like' chemical structures were defined as having simple molecular structures that are chemically unreactive, synthetically accessible and have 'drug-like' properties. The 100,000 compounds in our 'lead discovery library' represent a diverse set of molecules as judged by Tanimoto dissimilarity analysis ($T \leqslant 0.85$), and although simple filters based on the Lipinski criteria were not used in the selection process, 89% of the compounds in the library are Lipinski compliant[14] and 81% conform with Oprea's criteria for 'lead-likeness'.[15]

An automated screening protocol for TR was developed using the photometric assay described by Hamilton et al.[16,17] The assay was adapted for operation in 384-well microtitre plates and was found to be exceptionally robust and reproducible. The average Z' and Z values[18] achieved over the entire primary screen were 0.72 and 0.54, respectively. The primary screen of 100,000 compounds identified 120 compounds that inhibited TR activity by more than 50% at a concentration of 25 μM. The potencies of the hits were confirmed by assaying compounds as 11 point titrations. In summary, the hit set contained compounds from 13 distinct structural classes, and the IC_{50} values of the hits ranged from 1 to 67 μM. A focus set of 43 compounds was selected from the population of primary screen hits based on inhibitory potency, synthetic accessibility and compound novelty. These compounds were re-ordered from the original chemical vendors and the TR inhibitory potency, structural identity and purity of the re-supplied material confirmed. The focus set contained compounds from nine distinct structural classes and the 2-iminobenzimidazoles were prominent having four close analogues in the focus set. The development of this 2-iminobenzimidazole structural class will be discussed in this communication.

The 2-iminobenzimidazoles are a novel class TR inhibitors that are chemically suitable for optimization and scored well in a drug-likeness analysis. A search of the patent literature revealed few 2-iminobenzimidazoles, none of which were reported to have anti-trypanosomal activity. A number of other 2-iminobenzimidazoles were contained within the lead discovery library; eight were selected and their potency determined to investigate structure–activity relationships (SAR) (Table 1).

Defined SAR were observed with the highest inhibitory activity obtained when one side chain contained a basic moiety (R^2) and the other (R^1) an aromatic ring (**3**, **5**, **7**). Replacement of the basic group in the R^2 side chain with a hydrophobic group (**1**, **2**, **4**), or removal of the phenyl ring in the R^1 side chain (**6**, **8**) resulted in a significant loss of inhibitory activity. None of the 2-iminobenzimidazoles in the patent literature possessed the general structure **I** (Table 2). A search of chemical vendors revealed 26 analogues with the general structure **I** that were purchased and tested, revealing further SAR (selected compounds: Table 2). The most potent compounds ($IC_{50} \leqslant 10$ μM) possessed a piperidine (**9**, **11**, **14**, **16**, **20**) or diethylamine (**13**, **15**, **7**, **22**) basic amine moiety. The inhibitory activity was significantly reduced by extension of the R^2 side chain (**18**) and completely lost when the basic amine group was a morpholine moiety (**10**, **12**). A variety of alcohol substituents (**9**, **11**, **15**,