Biochemical investigations revealed the inhibitory mechanisms of novel inhibitors of Trypanosome Alternative Oxidase active against human and animal African trypanosomiasis

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Abstract

African trypanosomiasis caused by the protozoan parasite, *Trypanosoma brucei* is a neglected parasitic disease of public health importance and undermine food security in endemic areas, and parasites' resistance to the available drugs is widespread. Nevertheless, the pathogens possess certain unique metabolic features amenable to developing new efficient drugs. Particularly, they rely on an indispensable, mitochondrially-localized enzyme, Trypanosome Alternative Oxidase (TAO), which is involved in the respiration of the bloodstream form trypomastigotes of the parasite. Interestingly, TAO is absent in the mammalian hosts and hence an attractive target for designing safe trypanocides. We recently cloned, expressed, and purified the physiologically relevant form of TAO, which is devoid of the N-terminal 25 amino acid mitochondrial targeting sequence (ΔMTS-TAO). A newly designed and synthesized class of cationic and non-cationic 4-hydroxybenzoate and 4-alkoxybenzaldehyde inhibitors enabled the first structure-activity relationship (SAR) studies on ΔMTS-TAO. Remarkably, we obtained compounds with *in vitro T. brucei* inhibition of up to 1.4 nM and enzyme inhibition values (IC50) as low as 2 nM, which were also active against multidrug-resistant strains of *T. brucei* and *T. congolense*. The inhibitors designed with a mitochondrion-targeting lipophilic cation tail displayed trypanocidal potencies comparable to the reference drugs diminazene and pentamidine and exhibited no cross-resistance with the critical diamidine and melaminophenyl arsenical based trypanocides. The cationic inhibitors were also much more selective over human cells than the non-targeted neutral derivatives. A preliminary *in vivo* study showed that modest doses of the inhibitors were effective against parasitaemia of mice infected with *T. b. rhodesiense* (STIB900).

Conclusion

We have successfully developed a new class of potent and selective hits active against veterinary (*T. congolense*) and human (*T. brucei spp*.) African trypanosomes and confirmed their designed mode of action as inhibition of TAO using a combination of chemical and biochemical tools. This was achieved by efficiently targeting the compounds to the parasite's mitochondrion, thus increasing the potency of the original small molecule inhibitors against *T. brucei*. These compounds represent a promising new class of potent and selective hits against human and animal African trypanosomes.

Key words: SHAM, Triphenylphosphonium salt (TPP), Quinolinium salt, Lipophilic cation, Trypanosomiasis, Trypanocide, Mitochondrial targeting, Parasite respiration, Trypanosome alternative oxidase (TAO), Trypanosoma brucei, T. b. rhodesiense, T. congolense.