In Vitro Pharmacodynamics and Mechanism of Action Studies of Oxaborole 6-Carboxamides: A New Class of Compounds for the Treatment of African Trypanosomiasis

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Abstract

Human African Trypanosomiasis (HAT) is a fatal disease caused by Trypanosoma brucei. There is a need for new treatment for HAT because current treatments are costly, difficult to administer and frequently toxic. We have identified several oxaborole-6-carboxamides that demonstrate potent activity against T. brucei brucei in vitro and exhibit efficacy against both the acute and chronic CNS stages of HAT in mouse models.

The trypanosome life cycle is complex, involving a bloodstream stage and a CNS stage. Oxaborole-6-carboxamides are potent and selective inhibitors of parasite viability and DNA replication. The boron atom is essential for trypanocidal activity. AN3520-Induced Morphological Changes in T. brucei. Exposure to AN3520 leads to loss of the typical slender form to a more round morphology and appearance of detached flagella in T. brucei bloodstream parasites. The mixed population of trypanosomes and bloodstream parasites is evident within 0.5-1 hours after compound addition. There is evidence of detached flagella in some of the round-shaped parasites. The morphological changes are consistent with loss of parasite viability observed through determination of ATP content following exposure to AN3520.

Kinetics of Trypanosome Killing – Time Kill Studies

Trypanosome killing in vitro was determined by measurement of ATP content (luciferase assay) over time. The boron atom was necessary for trypanocidal activity. Oxaboroles exhibit potent inhibitory activity against T. brucei bloodstream parasites in vitro (IC50 = 0.04 – 0.26 µg/ml) and very low or no activity against a proliferating mammalian cell line, L929 fibroblast (3.77 – >50 µg/ml). A lactone derivative, in which the boron atom is replaced with carbon, does not exhibit trypanocidal activity when tested up to 50 µg/ml against T. brucei in vitro. The exact mechanism through which oxaboroles act to kill trypanosomes is unknown, but it is likely that the boron atom plays a role in the mechanism of action (MOA). Oxaboroles were immobilized on agarose matrices for use in affinity capture of parasite target proteins which will be identified by mass spectrometry and database searches. Collectively, these studies which use AN3520 as a model will provide a better understanding of how oxaboroles exert their trypanocidal activity and enable us to develop valuable PK/PD models to ensure appropriate drug delivery for treatment of HAT.

Assessment of Reversibility of Trypanocidal Activity

Intracellular Localization of Oxaboroles

Summary

Oxaborole carboxamides are potent and selective inhibitors of T. brucei. The boron atom is essential for trypanocidal activity.

Microscopic analysis shows morphologic changes of trypanosomes within several hours following exposure to oxaboroles in vitro.

Time kill studies demonstrate fast killing of T. brucei by oxaboroles in vitro and in vivo. T. b. brucei parasites do not recover from oxaborole effects following a transient exposure. Agomere immobilized ligands interact with several proteins in T. brucei. Both active and inactive analogues accumulate in the same region.