Innovative strategies in the development of nucleoside-based therapeutics for animal trypanosomiasis: expanding the focus from efficacy to environmental safety

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Animal trypanosomiasis (AT) is a widespread disease caused by *Trypanosoma spp.* and has a devastating effect on animal husbandry all over the world due to the scarcity of efficient drugs and development of drug resistance, hence emphasizing the need for novel treatment options. Following previous identification of 3'-deoxytubercidin as a highly potent trypanocide with curative activity in mouse models of both stage-1 and stage-2 Human African Trypanosomiasis (HAT), we now present

a comprehensive preclinical evaluation of new 6-amino substituted tubercidin analogues with promising activity against a broad range of AT species. Potent hits were identified *in vitro* across all important AT species, *i.e. Trypanosoma brucei brucei*, isometamidium (ISM)-resistant and -susceptible *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma evansi* (type A and B) and *Trypanosoma equiperdum*. Selected 'hits' were further tested for *in vitro* metabolic stability (using bovine, horse and piglet liver microsomes), *in vivo* mouse models for each AT species, genotoxicity assays and mode-of-action studies (*i.e.* genome-wide RNA interference library screening, metabolomics). Analogue **3** was highly active in *T. vivax*, *T. congolense*, *T. equiperdum*, *T. evansi* and *T. brucei* curative mouse models. Furthermore, there was no indication of *in vivo* toxicity or *in vitro* genotoxicity in Vitotox®, micronucleus and comet assays. Mode-of-action studies for **3** revealed that the P1 nucleoside transporter and adenosine kinase are involved in drug uptake and activation, respectively. Ecotoxicological assessments on *Daphnia* and green alga *Desmodesmus* revealed that the compound has an acceptable ecotoxicological footprint. Given the preferred target product profile for a broad-spectrum drug against AT, analogue **3** represents an advanced lead candidate for treatment of animal trypanosomiasis, regardless of the causative species.