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Safe, effective and oral antiparasitics against protozoal infections

Background

Current antiprotozoal treatments are often ineffective, require a suboptimal compound administration route, face increasing resistance and are endowed with concerns about toxicity (direct and indirect). Therefore, novel treatment options are needed, to treat a large variety of protozoan-caused infections (1).

Protozoa are unable to synthesize purines *de novo* and rely solely on the uptake and interconversion from the host, constituting purine nucleoside analogues as a potential source of antiprotozoal agents (1).

Target market for turkeys

The incidence and severity of histomoniasis outbreaks in turkeys is increasing in the EU and US (2,3). Histomoniasis has a high morbidity and mortality in turkeys, sometimes approaching 100 % of a flock (2). In 2015, 233 million turkeys were raised in the US, resulting in approximately 5.7 billion dollars in revenu. The average turkey flock size is ~20,000 birds (4).

Whilst disease in chickens is less severe, they are a significant contributor to the spread *H. meleagridis* to other poultry including wild bird reservoirs. This disease represents a severe and ongoing animal health and welfare problem to the poultry population and therefore to a significant sector of the poultry industry (2).

There are currently no authorised medicines for treatment or prevention in the EU, whilst nitarsone is the only product approved by the FDA for the prevention of histomoniasis (2,3). The lack of any legal treatment for histomoniasis is of concern, especially in the case of valuable turkey breeder candidate flocks. Losses to blackhead have been severe in several areas of Europe, and sporadic cases are occurring in North America (3).

Technology

We have prepared a purine nucleoside platform in the Laboratory for Medicinal Chemistry (Ghent University, BE), based on known and novel sugar- and purine ring scaffolds. We have demonstrated their ability to act as antiprotozoal agents (primarily against trypanosomes, but also other, see below).
Furthermore, nucleoside chemistry has already been extensively validated for its safety in antiviral indications in human medicine.

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Proof of concept

As an example, we found 3'-deoxytubercidin analogues to be highly potent trypanocides against *T. brucei* spp. One analogue served as proof-of-concept in relevant mouse model of *T. brucei* infection. This derivative, 3'-deoxytubercidin, displays curative activity in both acute and CNS-stage infection in mice, highlighting that it is able to **cross the blood-brain-barrier**, likely a class effect.
Nucleoside analogue **compound 9** shows excellent efficacy in murine human African trypanosomiasis (HAT) models (acute and central nervous system (CNS)).

Mouse model of acute HAT
Intraperitoneal administration at 10 mg kg−1 once a day for 5 days, or oral dosing at 25, 12.5 and 6.25 mg kg−1 twice a day for 5 days resulted in **negative blood parasitaemia** and survival of all treated mice at the pre-set endpoint of 21 days post infection (dpi).
No adverse reactions have been noted nor was weight loss recorded in any of the treatment schedules, indicating that compound 9 was well tolerated by the test animals.

CNS mouse model of HAT.
Oral dosing at 25 mg kg−1 twice a dahy for 5 days resulted in excellent activity, comparable to the reference drug for CNS clearance, melarsoprol.
All treated animals showed **negative blood parasitaemia** and survived the pre-set endpoint of 9 weeks post infection (wpi).
Bioluminescent imaging (BLI) confirmed a total clearance of all organs, including the CNS (Fig. 1). Quantification of the luminescent signal in the head region showed a rapid decline of the signal in both melarsoprol and compound 9-treated animals to levels similar to those detected in a non-infected control mouse. Dose titration at 25, 12.5 and 6.25 mg kg−1 once per day for 5 days also resulted in negative blood parasitaemia up to 9 wpi, with BLI confirming a total clearance in all organs including the CNS. Additional follow-up of surviving mice with BLI up to 14 wpi did not reveal any signs of relapse. All treated (melarsoprol and compound 9) animals showed complete absence of parasite burdens as assayed in several tissue samples (brain, spleen and fat) by a highly sensitive spliced-leader RNA (SL-RNA) quantitative PCR (qPCR) detection method.

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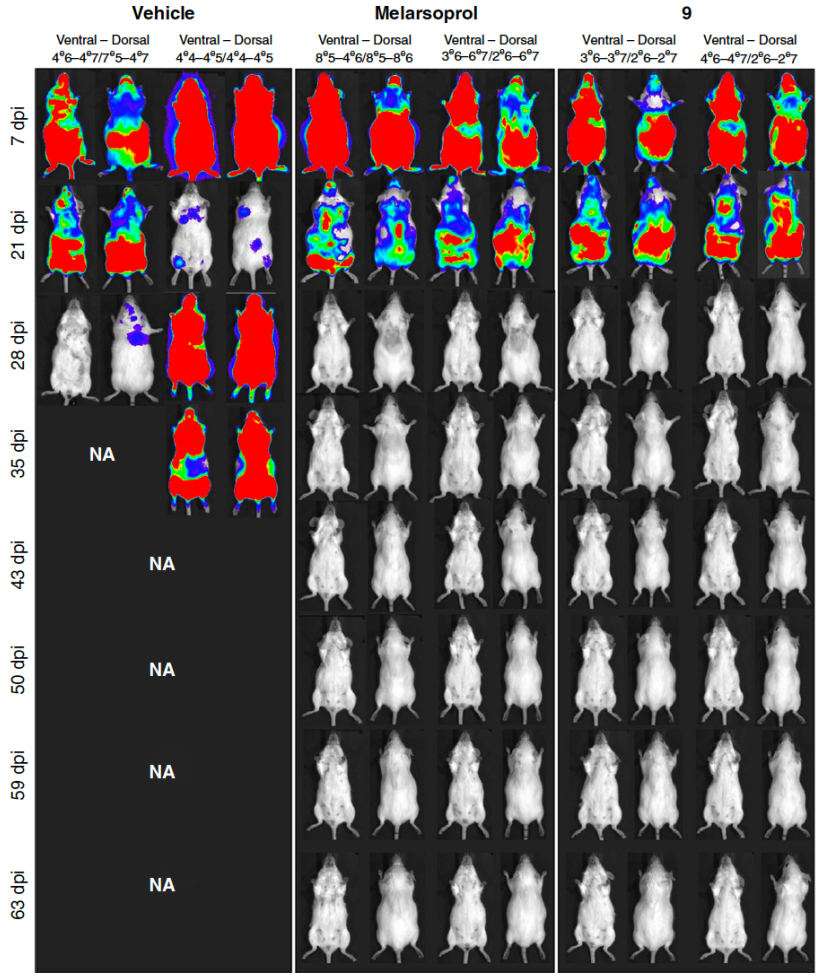

Fig. 1: *In vivo* evaluation of **9** in a stage 2 mouse model of HAT. BLI images of stage 2 T. b. brucei-infected mice treated at 21 dpi with vehicle (n =2), melarsoprol (n =2) or **9** (n = 2). Images were acquired from the dorsal and ventral sides of the animal. Image scales are indicated for each animal. NA = data not available due to animals in the control group succumbing to infection.

Additionally, *in vivo* proof-of-concept for several other protozoal infections (*T. cruzi*, *T. vaginalis*) has been obtained.

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Other indications

Development status of listed indications:

- **Leishmania** (*L. infantum* & *L. donovani*) / dogs: *in vitro* proof-of-concept, *in vivo* tests ongoing
- **Chagas** (*T. cruzi*) / mammals: *in vitro* proof-of-concept, *in vivo* proof-of-concept for acute stage disease.
- **Nagana disease**
  - *T. brucei* / cattle, goat, sheep, horse, dog: *in vitro* proof-of-concept, *in vivo* tests demonstrated full cure in a phase 2 mouse model
  - *T. congolense* / cattle, goat, sheep, horse, dog: *in vitro* proof-of-concept, *in vivo* tests demonstrated full cure in an acute mouse model.
  - *T. vivax* / cattle, goat, sheep, horse, dog: *in vitro* proof-of-concept, *in vivo* tests ongoing
- **Dourine** (*T. equiperdum*) / horse: *in vitro* proof-of-concept
- **Surra** (*T. evansi*) / camel, horse, cow, water buffalo: *in vitro* proof-of-concept
- **Blackhead disease** (*H. meleagridis*) / chicken, turkey, duck, geese, pheasant, quail: in vitro proof-of-concept (equal to dimetridazole)
- **Cryptosporidium** / neonatal calves: *in vitro* proof-of-concept
- ***Toxoplasma gondii*** / cat: *in vitro* proof-of-concept, ongoing
- ***Trichomonas foetus*** / cattle: *in vitro* proof-of-concept, *in vivo* mouse model: comparable to metronidazole

IP-position

Patent pending (national phase).

Partnering

We seek development and marketing partners interested in developing nucleoside analogues as anti-parasitic against protozoal infections.

We can offer a focused purine nucleoside library, containing >600 diverse nucleoside analogues in 96-well plate format for screening purposes.

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