AN11736, A Benzoxaborole Clinical Candidate for Treatment of *T. congolense* and *T. vivax* African Animal Trypanosomosis

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Abstract

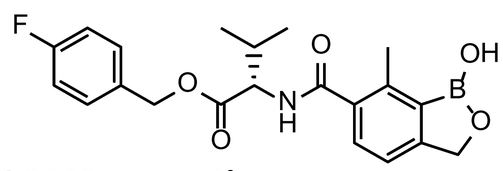
Animal African Trypanosomosis (AAT) is a potentially fatal parasitic wasting disease of livestock and wild animals in sub-Saharan Africa. It is caused primarily by the two parasites, *Trypanosoma congolense* and *Trypanasoma vivax,* which are spread by tsetse flies and other biting flies. AAT is responsible for 3 million cattle deaths annually in sub-Saharan Africa and costs African livestock farmers approximately US $1- 5 billion per year. There are few available drugs for effective treatment and prophylaxis and most have a narrow therapeutic index. Standard-of-care drugs such as diminazene, isometamidium and homidium are old, often ineffective and both drug resistance and safety are of concern.[1,2]

Screening of the Anacor Pharmaceuticals library of novel boron-containing compounds identified a series of amino acid ester amides of benzoxaborole 6-carboxylic acids as subnanomolar potency inhibitors of *T. congolense* and *T. vivax* in *in vitro* and *ex vivo* assays. A lead optimization effort resulted in AN11736 which demonstrated 100% cure (>60 days parasite-free) with a single IP dose of 10 mg/kg against both *T. congolense* (N=4/grp, 20 experiments) and *T. vivax* (N = 4, 12 experiments) in mouse models. 100% cure was also observed IM in the target animal species, cattle (>100 days parasite-free, N = 3 or 6/grp in 2 experiments). AN11736 showed no issues in Ames, *in vitro* micronucleus, hERG and receptor panel assays. A seven-day repeat dose oral toxicity study in dogs showed a NOAEL of 200 mg/kg for female (AUC =19.1 h*µg/mL) and 600 mg/kg for male (AUC=31.6 h*µg/mL) dogs.

AN11736 is a novel chemical entity which is being advanced to clinical development as the first novel drug for treatment of AAT in 50 years.

[1]http://www.hyperhistory.net/apwh/essays/comp/cw23diseaseafrica.htm; http://www.galvmed.org
[2]The Animal Trypanosomiases and Their Chemotherapy: A Review. F Giordani et al. Parasitology 1-28. 2016 Oct 10

In vitro IC_{50} and Physiochemical Properties of AN11736



In vitro and ADME properties	AN11736
T. congolense/T. vivax IC_{50} (nM)	0.15/1.3
Cytotoxicity in mouse fibroblasts, L929 (µM)	> 20
Molecular weight (Da)	399
cLog D	3.05
Solubility pH 7.4 PBS after 24h at 20°C (µg/mL)	4
Solubility 50:50 Propylene glycol: Glycerol formal with 1.5 mg/mL BHA (mg/mL) – formulation for *in vivo* efficacy	50
Metabolic stability + NADPH (mouse, bovine S9) Cl_{int} (µL/min/mg protein)	5.4/9.3
Mouse Plasma Stability (% at 4 h)	90.4
Bovine Plasma Stability (% at 4 h)	79.3
Permeability in MDCK-MDR1 ± p-gp inhibitor (x 10^{-6} cm/s)	26/33 (rec >15x10^{-6})
Plasma protein binding: f_u (mouse, bovine)	0.002/0.023

In vivo efficacy against *T. congolense* and *T. vivax* in Mice

NMRI female

Infection
1 x 10^5 Tc
1 x 10^4 Tv

Start treatment IP
Tc – 7 dpi
Tv – 4 dpi

Twice weekly parasitemia checks to 60 days

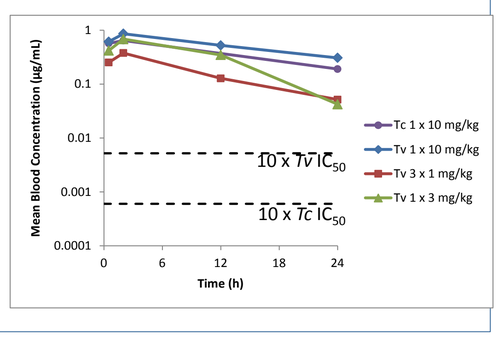
Day 60

Infection

Parasite	Dose Tested (days x mg/kg)	Absence of parasites at 60 days (# of experiments)	Average Survival Days
T. congolense	1 x 10	4/4 (20)	60
T vivax	1 x 10	4/4 (12)	60
	3 x 1	4/4	60
	1 x 3	4/4	60

Parameter	1 x 10 mg/kg *Tc*	1 x 10 mg/kg *Tv*
Cmax (µg/mL)	0.725	0.857
AUC_{0-24} (µg*h/mL)	12.0	13.1

Blood Exposures in Infected Mice Following IP Dosing of AN11736



In vivo efficacy against *T. congolense* and *T. vivax* in Cattle

Friesen-Holstein

Infection
1 x 10^5 Tc or Tv

IM Treatment when PCV drop by proportionately >0.2 of pre-infection values, with clinical signs and parasitemia

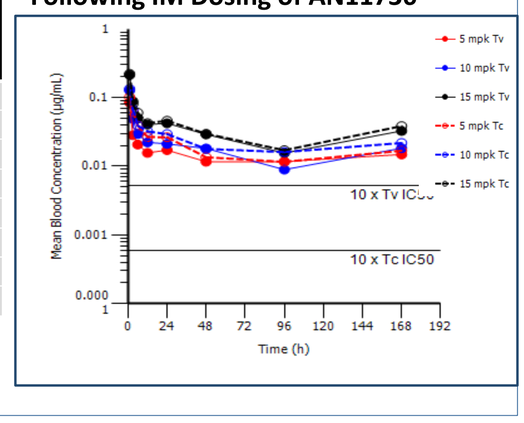
Twice weekly parasitemia check to 100 days

Day 100

Parasite	Treatment (mg/kg)	Route of Treatment	Cure at 100 Days Post Treatment/Total in Group
T. congolense	1 x 15	IM	6/6
T. congolense	1 x 10	IM	6/6
T. congolense	1 x 5	IM	6/6
T. congolense	1 x 10	SC	5/5*
T. vivax	1 x 15	IM	6/6
T. vivax	1 x 10	IM	6/6
T. vivax	1 x 5	IM	3/6**
T. vivax	1 x 10	SC	5/5

Vehicle: 50:50 PG:GF +1.5 mg/mL BHA
* Rescue treatment of 2 donor and 3 saline controls,
** relapsed Day 29- 30 post-treatment

Blood Exposures in Infected Cattle Following IM Dosing of AN11736



AN11736 Exhibits No Genotoxicity and a Favorable Profile in Preliminary Safety Pharmacology Studies

Safety Pharmacology Testing	AN11736
Ames Assay	Non-mutagenic
In vitro micronucleus	negative
ExpesSProfile 48 Receptor/enzyme panel screening (@ 10 µM)	All <50% except GABA-gated Cl^- IC_{50} = 6 µM
Cardiotoxicity, ion channels (% Inhibition at 10 µM)	All <50% inhibition
CYP450 inhibition at 10 µM (CYP isoforms 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4/5)	negative

Exploratory Studies to predict Human Safety following consumption of AN11736-treated Cattle

Target: Withdrawal period in milk < 1 day and meat < 14 days
Dose Range Finding (DRF) followed by repeat dose for 5-7 days in Rat and Dog

Rat 7-day oral acute tox: NOAEL = 120 mg/kg/day
Dog 5-day oral acute tox: NOAEL = 200 (F) and 600 (M) mg/kg/day
(saw reduced feed intake, decreased reticulocytes at higher doses)

Design of Milk Residue study:
1. Single 10 mg/kg IM dose to 3 lactating dairy cows (50 mg/mL, PG:GF)
2. Milked twice daily. Sampled milk up to 14 days after single administration

Provisional Results:
1. Parent AN11736 only found in 1h milk sample (<0.07 ppm). AN14667, the acid metabolite present until D14 but shown to be non toxic in dog tox study
2. Milk withholding period estimated 0 to 1 day

Meat residue study in progress.

Preliminary Target Animal Safety Studies Suggest Sufficient Safety Margin in Cattle

Target : Demonstrate safety at 3x therapeutic dose (3x 10mg/kg) repeated 3 times

Design:
1. Conducted at Clinvet, South Africa to facilitate local breed selection
2. N=4 Nguni cattle (*Bos taurus* X *Bos indicus* genetics) + 4 control (untreated)
3. 30 mg/kg IM given 3 times at 14-day intervals (50 mg/mL in PG:GF formulation)

Results:
An adequate margin of safety was indicated based on clinical signs, gross pathology, hematology, clinical chemistry and histopathology

AN11736 meets TPP requirements and could be the First Novel Drug for AAT in 50 Years

Target Product Profile	AN11736
Novel Chemical Entity with no pre-existing Resistance	✓ yes
Single dose cure of *T. congolense* and *T. vivax*	✓ yes
No genotox or safety issues based on studies to date	✓ yes
Target animal safety studies predict no issues	✓ yes
Milk and meat residue studies suggest short withdrawal times	✓ yes

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