Efficacy and Pharmacokinetics of SCYX-7158 (AN5568): a Novel and Potent Oxaborole-6-Carboxamide Selected as a Pre-Clinical Candidate for Once-Daily Oral Treatment for Stage 2 Human African Trypanosomiasis

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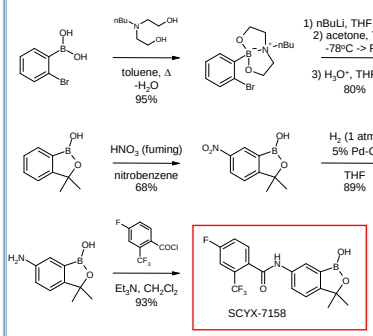
Abstract

SCYX-7158, a 3,3-dimethyloxaborole-6-carboxamide, is distinguished from earlier trypanocidal oxaboroles by enhanced pharmacokinetic and CNS disposition properties allowing for a once per day (QD) oral dosing regimen at a markedly lower efficacious dose in a Stage 2 murine Human African Trypanosomiasis (HAT) model. The discovery of SCYX-7158 was achieved through application of integrated lead optimization strategies across medicinal chemistry, parasitology and pharmacokinetic disciplines. SCYX-7158 is active *in vitro* against relevant strains of *Trypanosoma brucei*, including *T. b. rhodesiense* and *T. b. gambiense* and is efficacious in both Stage 1 and Stage 2 murine HAT models. Physicochemical and *in vitro* ADME properties of SCYX-7158 are consistent with the compound being orally available, metabolically stable, readily CNS permeable and with low risk for drug-drug interactions. In an ongoing murine Stage 2 study, SCYX-7158 is effective orally at doses as low as 12.5 mg/kg (QD x 7 days). *In vivo* pharmacokinetic characterization of SCYX-7158 demonstrates that the compound is highly bioavailable in rodents, has low intravenous plasma clearance, a 24 hr elimination half-life and a volume of distribution that indicates good tissue distribution. Most importantly, SCYX-7158 readily distributes into brain and CSF and crosses the blood-testicular barrier to achieve therapeutically-relevant concentrations in potential trypanosomal sanctuary sites. Based on these properties, which promise lower rates of recrudescence than with current standard of care, SCYX-7158 has been selected as a pre-clinical candidate for treatment of Stage 2 HAT.

Synthesis of SCYX-7158



Synthesis of SCYX-7158 is achieved in five steps from 2-bromo phenylboronic acid. Protection of the boronic acid as the n-butyl borocan facilitates generation of an aryllithium reagent, which is trapped by acetone. Acid-catalyzed hydrolysis and ring formation efficiently delivers the oxaborole ring. Nitration at C(6), followed by reduction of the nitro function and acylation of the resultant amine provides the final drug candidate in 42% overall yield. The current synthetic route requires no chromatographic purification and is anticipated to be operable on multi-kilogram scale.

In vitro activity against *Trypanosoma brucei* strains

Strain	IC_{50} (μM)	Comments
T. b. brucei SBRI 427	1.10 ± 0.22 (n= 5)	Routine screening strain.
T. b. rhodesiense STIB 900	0.80	Isolated from a patient in Tanzania in 1982, adapted to cell culture at Swiss Tropical Institute.
T. b. gambiense 40R	0.99	Isolated from a patient in DRC in 2005, relapse 6 mo. after melarsoprol treatment
T. b. gambiense 108R	0.45	Isolated from a patient in DRC in 2005, relapse 8 mo. after melarsoprol treatment
T. b. gambiense DAL 1402	0.18	Isolated from a patient in Cote d'Ivoire in 1990
T. b. gambiense ITMAP 141267	0.25	Isolated from a patient in DRC in 1960.
T. b. gambiense Drani	0.35	Isolated from a patient in Uganda in 1995

SCYX-7158 exhibited sub-micromolar activity opposite a range of *Trypanosoma brucei* strains, including *T. b. gambiense* strains isolated from patients who had relapsed after treatment with melarsoprol. Profiling of SCYX-7158 against additional drug-resistant strains of both *T. b. gambiense* and *T. b. rhodesiense* is ongoing at Pace University and Swiss Tropical Institute.

In vitro ADMET profile of SCYX-7158

Absorption/Permeability (MDR1-MDCK monolayer)

P_{app} (A-B), nm/sec	P_{app} (+GF 120918) (A-B), nm/sec	AQ
415	427	0.03

Protein Binding

Matrix	[SCYX-7158], μM	Fraction bound (%)	Mass balance (%)
Human plasma	2.5	98.7	112
Mouse plasma	0.5	99.7	98
Mouse brain	2.0	94.6	114

Metabolism

Matrix (Parameter)	Mouse	Rat	Dog	Monkey	Human
Liver microsomes ($t_{1/2}$, min)	>350	NT	NT	NT	>350
Liver S9 fraction ($t_{1/2}$, min)	>350	>350	>350	>350	>350
Brain homogenate (% remaining @ 5h)	77-80	NT	NT	NT	NT

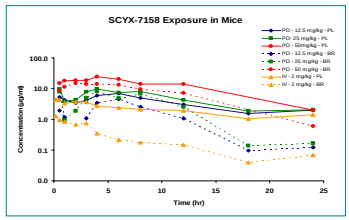
Inhibition of Cytochrome P450 Enzymes

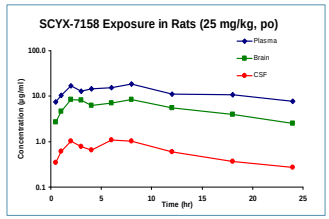
Isoform	1A2	2C19	2C9	2D6	3A4
IC_{50} (μM)	>100	23.1	23.5	21.1	47.4

SCYX-7158 exhibits high permeability and metabolic stability, modest protein binding and low levels of inhibition of CYPs. These properties suggest that SCYX-7158 can achieve sufficient exposure levels in the CNS to be effective in Stage 2 HAT.

SCYX-7158 was non-cytotoxic to a mouse fibroblast L929 cell line up to 136 μM.

In vivo PK of SCYX-7158 in rodents





SCYX-7158 achieves drug concentrations in excess of the in vitro IC_{90} for >12 hours in rodents at a dose of 25 mg/kg in both plasma and brain. In rats, SCYX-7158 also achieves therapeutically relevant drug concentrations in the CSF for 8-10 hours. These data are consistent with observed levels of efficacy in the murine Stage 2 CNS HAT model and form the basis for development of PK-PD relationships for allometric scaling to select doses for human trials.

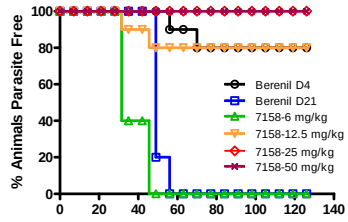
In vivo efficacy in murine HAT models

Stage 1 non-CNS Model (Acute)

Group	Dose (mg/kg)	Route	Treatment Duration (days)	MSD[1]	Survival @ Day 30
1	25	i.p.	4	>30	3/3
2	10	i.p.	4	9.3	0/3
3	5	i.p.	4	6	0/3
4	10	p.o.	4	>30	3/3
5	25	p.o.	1	8.6	4/6
6	10	p.o.	1	8.3	0/3
Pentamidine – historical positive control					
7	4	i.p.	4	>30	27/30
Vehicle – historical negative control					
8	N/A			4.2	0/30

Mice were each infected intraperitoneally (i.p.) with $2.5x10^5$ *T. b. brucei* (EATRO 110) parasites isolated from infected rats. SCYX-7158 was dosed once daily starting 24 hours after parasite infection for the duration shown. Mice were checked for parasitemia twice weekly by microscopic examination of smears prepared from tail vein blood. Mice found moribund were sacrificed. [1]Mean survival time in days.

Stage 2 CNS Model (Chronic)



- Infection on Day 0 with 10,000 *T. b. brucei* (TREU 667)
- 10 mice per dose group
- SCYX-7158 dosed orally once-daily starting on Day 21 for 7 days
- Berenil dosed as a single 10 mg/kg i.p. dose on either Day 4 (positive control) or Day 21 (negative control).
- Parasitemia measured in tail vein blood weekly starting on day 35.

SCYX-7158 has exhibited 100% efficacy at 50 and 25 mg/kg, with ~80% efficacy at 12.5 mg/kg through Day 125.

Summary

- SCYX-7158 exhibits good *in vitro* activity against *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense*.
- *In vitro* physicochemical and ADME properties of SCYX-7158 are consistent with expectations for oral availability and CNS exposure.
- Pharmacokinetics of SCYX-7158 in rodents suggest sufficient exposure in plasma, brain and CSF.
- An ongoing Stage 2 (CNS) murine HAT model has demonstrated efficacy of SCYX-7158 at a dose of 25 mg/kg, p.o., q.d. for 7 days.
- SCYX-7158 has been selected for further development as a pre-clinical candidate for Stage 2 HAT.