Lead Optimization Investigation of Oxaboroles for the Treatment of Human African Trypanosomiasis

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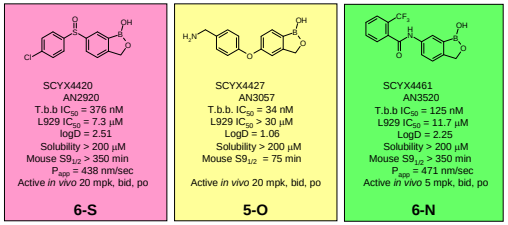
Abstract

Human African Trypanosomiasis (HAT), also known as sleeping sickness, is a fatal disease caused by the kinetoplastid parasite *Trypanosoma brucei* that threatens millions of people in sub-Saharan Africa. Current treatments for HAT are either toxic and/or complicated to administer, particularly in patients who have progressed to stage II disease, in which the parasite has migrated to the brain. Major efforts are urgently needed to facilitate the discovery and development of safe, effective and innovative drugs to treat HAT.

Collaborative research between SCYNEXIS, Inc., Anacor Pharmaceuticals, Inc., Pace University and DND*i* has identified small molecule oxaboroles as a chemotype of interest for potential treatment of HAT. Oxaborole 6-benzamides have been identified that inhibit *in vitro* growth of *Trypanosoma brucei brucei* with IC_{50}'s in the range ~ 100 - 200 nM, are not cytotoxic to mammalian cells, exhibit good physicochemical and pharmacokinetic (PK) properties, and are orally active in both acute (stage I) and chronic (stage II) murine models of the disease. SCYX-7158 represents an optimized balance of potency and PK properties that eliminates potential efflux and allows for sustained brain exposure.

Oxaborole Development: Early Hit-to-Lead

Objective – To develop chemistry and explore structure activity relationships for three lead oxaborole scaffolds (6-S, 5-O and 6-N series)



SCYX4420
AN2920
T.b.b IC_{50} = 376 nM
L929 IC_{50} = 7.3 µM
logD = 2.51
Solubility > 200 µM
Mouse $S9_{1/2}$ > 350 min
P_{app} = 438 nm/sec
Active *in vivo* 20 mpk, bid, po
6-S

SCYX4427
AN3057
T.b.b. IC_{50} = 34 nM
L929 IC_{50} > 30 µM
logD = 1.06
Solubility > 200 µM
Mouse $S9_{1/2}$ = 75 min
Active *in vivo* 20 mpk, bid, po
5-O

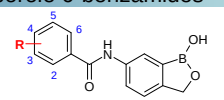
SCYX4461
AN3520
T.b.b IC_{50} = 125 nM
L929 IC_{50} = 11.7 µM
logD = 2.25
Solubility > 200 µM
Mouse $S9_{1/2}$ > 350 min
P_{app} = 471 nm/sec
Active *in vivo* 5 mpk, bid, po
6-N

- *In vitro* ADME Methods:
  - Solubility: pH 7.4 PBS; estimated by nephelometry
  - LogD: Chromatographic Hydrophobicity Index (CHI) method
  - Mouse S9 $t_{1/2}$: Incubation for 60 min @ 1 µM
  - MDR1-MDCK: Monodirectional (A-B) +/- GF120918 (Pgp inhibitor)
- *In vivo* mouse model (Acute) – *T.b.brucei* (250K innoculum)
  Drug candidate was dosed orally 24 hrs post-infection, b.i.d., for 4 days

Selection of Benzamides (6-N) for Lead Optimization

- **6-N (Benzamides):**
  - High *In vitro* activity vs. *T.b.brucei* and *T.b.rhodesiense*
  - Excellent physicochemical properties
  - Good chemical and metabolic stability
  - Oral bioavailability and good pharmacokinetics
  - Low cytotoxicity, no observed acute *in vivo* toxicity, AMES and hERG negative
  - Efficacious *in vivo* against both acute (Stage I) and chronic (Stage II) infection
- **5-O:** Activity appears to be dependent on chemical reactivity
- **6-S:** Modest activity and poor pharmacokinetic profiles

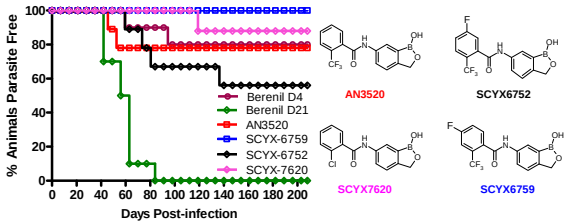
Activity of Oxaborole 6-benzamides



SCYX id	R	T. b. brucei IC_{50} (nM)	L929 Cytotox IC_{50} (µM)	Animal cured* (10mg/kg, p.o.,b.i.d.)
4424	H	160	> 39.5	NT
7582	2-F	130	> 36.9	1/3
5896	2,6-F2	159	> 34.5	1/3
4461	2-CF3	125	11.8	3/3
7620	2-Cl	70	> 34.8	3/3
6835	2-Cl-4-F	147	> 32.7	3/3
6752	2-CF3,5-F	130	15.5	3/3**
6759	2-CF3,4-F	180	> 29.5	3/3**

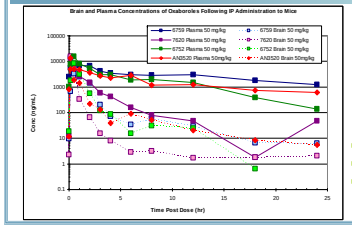
- *Number of alive/total animals after 30 days in the acute mouse model of HAT
- ** also active at 5 mg/kg, p.o., b.i.d. (3/3 cured)

6N-Benzamides Cure CNS HAT Infection



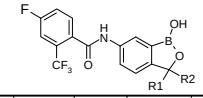
- Infection on Day 0 with 10,000 TREU 667 parasites; 10 mice per dose group
- Compounds dosed i.p. starting on Day 21 (50 mg/kg, b.i.d. for 14 days).
- Parasitemia measured via tail vein weekly starting on day 35.
- **SCYX 6759 has exhibited 100% cure of CNS infected mice**
- **SCYX 7620 has exhibited 90% cure of CNS infected mice**

Oxaborole Benzamides Are Brain Penetrant



- Robust plasma exposure
- Modest brain exposure
- **More rapid decay of brain concentrations relative to plasma suggest active "efflux" from brain**
- Compounds dosed to mice i.p.
- One mouse per time point
- Plasma and brain samples analyzed by LC/MS/MS

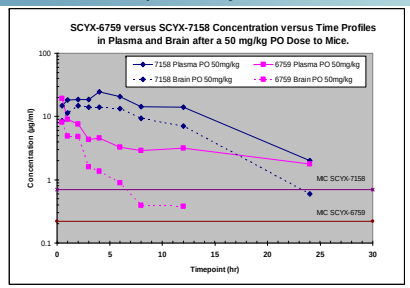
Addressing Brain Efflux: C(3) Optimization

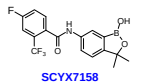


SCYX id	R1	R2	T.b.b IC_{50} (nM)	Cytotox (µM)	$t_{1/2}$, mouse S9 (min)	logD	Solubility (µM)	MDR1-MDCK Permeability	
								P_{app} (nm/sec)	AQ
6759	H	H	180	> 29.5	>350	2.57	>200	380	0.02
9308	H	CH_3	258	3.4	>350	3.20	50	683	0.03
8258	H	$-CH_2CH(CH_3)_2$	6099	>25.3	NT	NT	NT	NT	NT
8240	H	Cyclopentyl	7810	>24.6	NT	NT	NT	NT	NT
7158	CH_3	CH_3	556	>27.2	>350	3.51	25	415	0.03
7265	Spirocyclobutyl		892	>26.4	NT	NT	NT	NT	NT
1124	Spirocyclopentyl		13940	>25.4	97	4.34	25	377	-0.02

- Steric limitation for C(3) substituents: larger R = reduced activity
- Cytotoxicity problematic for C(3) mono-methyl analogs
- **Increased LogD (ranges 3.0-3.6)**
- **Decreased aqueous solubility**
- SCYX9308 & 7158 *maintain In vivo* efficacious at 10 mg/kg (4 days, p.o., b.i.d.)

SCYX-7158: Therapeutically Relevant Concentration in Brain



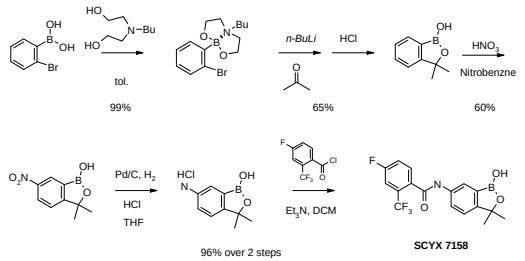


SCYX7158
T.b.brucei IC_{50} = 556 nM

SCYX6759
T.b.brucei IC_{50} = 180 nM

- Mouse data
  - **SCYX-7158 sustains >MIC in brain for ~24hr**
  - SCYX-6759 sustains >MIC in brain for only ~12hr
- SCYX-7158 : Excellent PK and good CNS disposition at some cost to potency

Synthesis of SCYX7158



- Only one chromatographic purification throughout the synthesis
- Further optimization of the lithium-bromide exchange reaction planned

Summary

- Oxaboroles have been discovered which exhibit high *in vitro* potency vs. *Trypanosoma brucei*, good PK and physicochemical properties
- Lead optimization of the 6-N benzamide region has afforded compounds that are orally active in both an acute (stage I) and chronic (stage II) mouse model of HAT
- C(3) modification of oxaborole ring has significantly optimized overall PK properties, particularly in addressing retention in the CNS
- Current lead compound (SCYX-7158) is being evaluated at various dosing levels (p.o., q.d., 7 days) in the chronic (stage II) mouse model
- Further biological, toxicological and DMPK profiling of SCYX-7158 is in progress