



Novel Oxaborole 6-Carboxamides Demonstrate Potential for Treatment of CNS-Stage Human African Trypanosomiasis

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Abstract

Human African Trypanosomiasis (HAT) afflicts hundreds of thousands of individuals in sub-Saharan Africa. An urgent need exists for the discovery and development of new, safe and effective drugs to treat HAT, as existing treatments suffer from poor safety profiles, difficult treatment regimens and high cost. From a collaborative effort between SCYNEXIS, Anacor, Pace University and DNDi, we report here our efforts to discover small molecule oxaborole 6-carboxamides for treatment of HAT. We have discovered that oxaborole 6-carboxamides, exemplified by AN3520 and SCYX-6759, inhibit *in vitro* growth of *T. b. brucei* with IC₅₀'s ~ 100 nM, are not cytotoxic to mammalian cells, and exhibit good physicochemical properties. In initial evaluations of this class of compounds in a CNS-stage disease model, where mice were infected with the TREU 667 strain of *T. b. brucei*, treatment with 50 mg/kg of drug candidate, BID for 7-14 days, starting at Day 21 post-infection, resulted in absence of blood parasites for >100 days. Concurrent *in vivo* pharmacokinetic evaluation of selected analogs has demonstrated that this class of compounds has good oral bioavailability and modest CNS penetration. On-going evaluation of this chemotype in the CNS-stage disease model suggests improved pharmacokinetics will provide greater efficacy.

Oxaborole 6-carboxamides: Amide SAR

Benzamides	Heterocyclic amides	Aliphatic amides
 AN 2951 SCYX 4424 T.b.b. IC ₅₀ = 160 nM T.b.r. IC ₅₀ = 160 nM Cytotox IC ₅₀ > 30 μM	 AN3121 SCYX 4437 T.b.b. IC ₅₀ = 200 nM T.b.r. IC ₅₀ = 2000 nM Cytotox IC ₅₀ > 30 μM	 AN3509 SCYX 5546 T.b.b. IC ₅₀ = 3300 nM T.b.r. IC ₅₀ = 23000 nM Cytotox IC ₅₀ > 30 μM
 AN 3518 SCYX 4459 T.b.b. IC ₅₀ = 70 nM T.b.r. IC ₅₀ = 250 nM Cytotox IC ₅₀ > 30 μM	 AN3511 SCYX 4454 T.b.b. IC ₅₀ = 550 nM T.b.r. IC ₅₀ = NT Cytotox IC ₅₀ > 30 μM	 AN4933 SCYX 7395 T.b.b. IC ₅₀ = 950 nM T.b.r. IC ₅₀ = NT Cytotox IC ₅₀ > 30 μM

Selection of Benzamides for Further Exploration

Based on SAR which emerged from initial evaluation of the oxaborole 6-carboxamides in both the *in vitro* *T. b. brucei* and *T. b. rhodesiense* viability assays, benzamides such as AN2951 were attractive for further exploration. Heterocyclic amides such as the 3-pyridyl derivative AN3121 were of similar potency against *T. b. brucei*, but this activity did not translate as well to *T. b. rhodesiense*. Aliphatic amides were generally less potent against both parasites and were not explored further at this time.

None of the initial derivatives in the oxaborole 6-carboxamide series exhibited significant cytotoxic activity in a mouse L929 cell line, with IC₅₀ values in excess of 30 μM, the highest concentration tested, affording Selectivity Indices of at least 100 (SI = Cyto IC₅₀/T.b.b. IC₅₀).

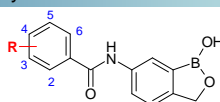
Summary and Acknowledgements

A series of oxaborole 6-carboxamides have been discovered which exhibit high *in vitro* potency vs. *Trypanosoma brucei*, good PK and physicochemical properties. Optimization of the series through systematic variation of the benzamide region has afforded compounds that are orally active in a Stage 1 HAT *in vivo* model.

The best compound examined to date, SCYX-6759, has demonstrated activity in a Stage 2 CNS HAT *in vivo* model at a dose of 50 mg/kg, bid.

The contributions of Prof. Reto Brun and Dr. Marcel Kaiser (STI), and Prof. Louis Maes (Univ. Antwerp) for provision of *T. b. rhodesiense* data are acknowledged.

In vitro Activity of Oxaborole 6-benzamides



SCYX id	AN id	R	T. b. brucei IC ₅₀ (nM)	T. b. rhodes. IC ₅₀ (nM)	L929 Cytotox IC ₅₀ (μM)
4424	AN 2951	H	160	160	>30
4455	AN 3512	4-CH ₃	110	220	>30
4460	AN 3519	4-F	275	420	>30
4461	AN 3520	2-CF ₃	125	120	11.8
5547	AN 3521	4-Cl	520	383	>30
7582	AN 4063	2-F	130	NT	>30
7620	AN 4074	2-Cl	70	NT	>30
7627	AN 4080	3,4-Cl ₂	220	810	>30
3361	AN 4108	4-CN	560	NT	>30
6752	AN 4164	2-CF ₃ -5-F	130	NT	15.5
6759	AN 4169	2-CF ₃ -4-F	180	225	>30
0581	AN 4214	4-OPr	3375	NT	>30
5894	AN 4931	2,4-F ₂	155	NT	>30

Oxaborole 6-Benzamide SAR

Further development of the series through systematic modification of the benzamide ring suggested that a diverse array of substituents on the benzamide ring were tolerated, though more sterically demanding (e.g. isopropoxy), or electron withdrawing (e.g. cyano) groups at the 4-position resulted in loss of activity. Modest cytotoxicity was observed for several 2-CF₃ derivatives, but Selectivity Indices remained near 100.

Key compounds were further evaluated in an array of *in vitro* ADME and physicochemical property assays to prioritize candidates for *in vivo* evaluation.

In vitro ADME and Physicochemical Properties

SCYX id	Solubility (μM)	logD	Mouse S9 t _{1/2} (min)	Protein binding	MDR1-MDCK Permeability P _{app} (nm/sec)	AQ
4461	>200	2.25	217	95.3	424	0.02
7620	100	2.00	67	97.9	569	-0.06
6752	>200	2.23	>350	97.3	424	-0.01
6759	>200	2.57	>350	97.7	379	0.02

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

Protein binding: Human serum albumin

MDR1-MDCK: Monodirectional (A-B) +/- GF120918 (Pgp inhibitor)

Activity in an in vivo Mouse Model of Stage 1 HAT

SCYX id	AN id	R	Dose (mg/kg)	Days to Relapse* (avg)	Animals Cured
4461	AN 3520	2-CF ₃	20	>30	3/3
			10	11	2/3
7620	AN 4074	2-Cl	10	>30	3/3
			5	12	0/3
6752	AN 4164	2-CF ₃ -5-F	10	>30	3/3
			5	>30	3/3
			10	>30	3/3
			5	>30	3/3
6759	AN 4169	2-CF ₃ -4-F	5	>30	3/3
			2	11	2/3

*excluding cured animals

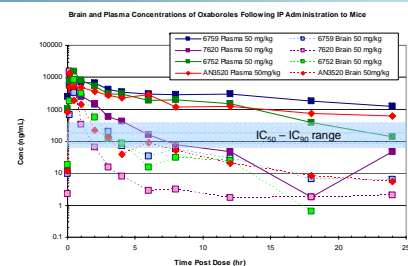
Mice were infected with 250,000 *T. brucei* EATRO 110 parasites on Day 0.

Drug candidates were dosed orally, b.i.d., for 4 days starting on Day 1.

Mice were examined each day for 30 days, animals found moribund were sacrificed.

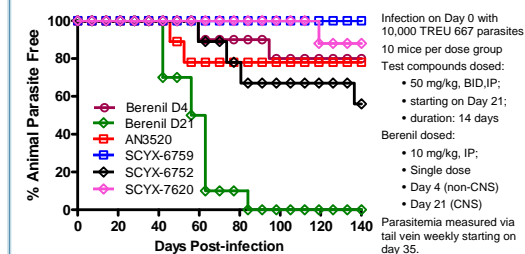
In this model, pentamidine is curative at 2 mg/kg, i.p. (4 days, b.i.d.)

Oxaborole 6-Carboxamides Are Brain Penetrant



Compounds dosed to mice i.p. One mouse per time point, plasma and brain samples analyzed by LC/MS/MS.

Oxaborole 6-Carboxamides Cure CNS HAT Infection



SCYX 6759 has exhibited 100% cure of CNS infected mice through Day 140
 SCYX 7620 has exhibited 90% cure of CNS infected mice through Day 140
 This study is ongoing.