

## A New Class of Benzoxaborole-based Potent Antitrypanosomal Agents: Probing the Effect of Different Linkage Groups on *Trypanosoma brucei* Growth Inhibition

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### Abstract

Initial screening of a benzoxaborole library in a growth inhibition assay against *Trypanosoma brucei* identified AN2901 [6-(phenylthio)-benzoxaborole] as a hit compound. The effect on *in vitro* potency of different central linkage groups was investigated by comparing an array of functional groups linking the two aromatic rings. The thioether, sulfoxide, ketone, CHOH and sulfone linking groups showed comparable potency, although they have different bond angles and hydrogen bond forming capability. The introduction of a carboxamide or sulfonamide linking group significantly increased the potency of the compound. The most potent compound showed an *in vitro* *T. brucei* growth inhibition IC<sub>50</sub> as low as 0.02 µg/mL. We present here the synthesis and *in vitro* SAR data for this new chemical class of potential antitrypanosomal agents.

### Introduction

Human African trypanosomiasis (HAT), or African sleeping sickness, is caused by protozoal parasite *T. brucei*. HAT affects approximately 150,000 individuals around the world and represents a significant cause of mortality in the developing world. There is urgent need to discover new antitrypanosomal agents due to the poor safety profile or high cost of the existing drugs. We report the discovery of a new class of potent antitrypanosomal agents based on novel benzoxaboroles.

### Methods

#### *In vitro* *T. b. brucei* growth inhibition assay

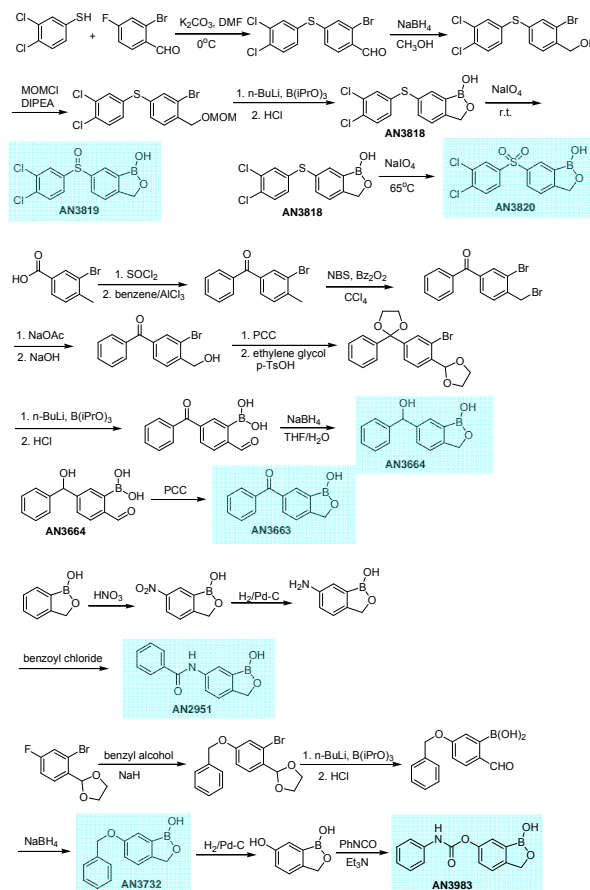
Compounds were tested for their ability to inhibit growth of free-living *T. b. brucei*. From a starting concentration of 5 µg/ml, the compounds were serially 2-fold diluted in DMSO and added to 96-well plates in triplicate. Parasites were added, incubated for 72 hours, then resazurin was added and fluorescence was read 3-5 hours later. Data from the plates was averaged/analyzed to generate an IC<sub>50</sub> value for each test compound.

#### *In vivo* murine infection assay

Mice were infected by intraperitoneal injection of 600 *T. brucei* or 10<sup>4</sup> *T. b. rhodesiense* parasites. Starting 24 hours after infection, test compound was administered at 100 mg/kg, bid, i.p. for 5 days. Parasitemia was evaluated by weekly sampling of blood. Mice were deemed "cured" of the infection if free of parasitemia at 40 days post-infection.

### Results and Discussion

The synthesis of representative antitrypanosomal benzoxaboroles are shown in **Figure 1**.



**Figure 1.** Synthesis of antitrypanosomal agents based on benzoxaboroles.

The growth inhibition activity against *T. b. brucei* is described in **Table 1** and **Table 2**.

**Table 1.** *T. brucei* growth inhibition (µg/mL)  
Effect of linkage group L.

	L	IC <sub>50</sub> (µg/mL)		L	IC <sub>50</sub> (µg/mL)
AN2901		0.51	AN3732		1.62
AN3810		0.17	AN2990		1.21
AN3811		0.24	AN2951		0.04
AN2896		1.11	AN3983		0.35
AN3663		0.15	AN2965		0.02
AN3664		0.16	AN3451		0.59

**Table 2.** *T. brucei* growth inhibition (µg/mL)  
Effect of Cl substitution.

	L	X	IC <sub>50</sub> (µg/mL)		L	X	IC <sub>50</sub> (µg/mL)
AN3769		ortho-Cl	0.39	AN3819		m,p-diCl	0.30
AN3369		meta-Cl	0.12	AN3817		ortho-Cl	0.10
AN2919		para-Cl	0.15	AN3729		meta-Cl	0.22
AN3818		m,p-diCl	0.23	AN2922		para-Cl	0.16
AN3770		ortho-Cl	0.32	AN2920		m,p-diCl	0.30
AN3735		meta-Cl	0.15				

### *In vivo* Activity

When evaluated in a model of acute murine *T. b. brucei* infection, treatment with AN2920 at a dose of 100 mg/kg, bid, i.p. resulted in 100% survival and no parasitemia 40 days post infection. At 100 mg/kg, bid i.p., AN2920 also cured mice infected with the human pathogen *T. brucei rhodesiense* in this model.

### Conclusion

- A novel series of 6-substituted benzoxaboroles has been discovered as potent anti-trypanosomal compounds
- Structure-activity studies measuring growth inhibition of *T. brucei* probed the linking group between the two aromatic rings and revealed that a range of structural variants show good inhibitory activity
- Trends in potency for different linking groups suggested that hydrogen bonding capability and/or geometric effects of the linking group effected growth inhibition potency
- The most potent linking groups were the sulfonamide and carboxamide groups
- AN2920 showed *in vivo* efficacy in an acute murine *T. brucei* infection model