

A Novel Oxaborole, AN3520, Shows Efficacy Against Human African Trypanosomiasis (HAT) *In Vitro* and *In Vivo*, Including Promise in a Murine CNS Model of *T. brucei* Infection

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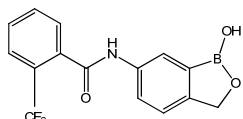
Abstract

Trypanosoma brucei is the cause of Human African Trypanosomiasis (HAT), a protozoan disease endemic to sub-Saharan Africa. Poor safety profiles, emergence of resistance and high cost of existing therapies makes developing new treatments for HAT imperative. Anacor Pharmaceuticals Inc., Scynexis Inc., Pace University and DNDi are developing small molecule, boron-containing compounds with anti-parasitic activities. One of these novel oxaboroles, AN3520, inhibits *in vitro* growth of *T. b. brucei* with an IC₅₀ of 125 nM and shows a 94-fold selectivity index when cytotoxicity is measured on murine L929 fibroblasts. AN3520 is not extensively metabolized *in vitro* and does not inhibit cytochrome P450 enzymes. MDCK-MDR1 cell permeability results suggest potential for CNS penetration and low likelihood of P-glycoprotein efflux in the blood-brain barrier. When AN3520 was dosed by oral, intraperitoneal or intravenous routes, compound was detectable in mouse plasma at 24 h with a t_{1/2} of 3.7 h. AN3520 showed good efficacy in the Eatro 110 murine model of acute *T. b. brucei* infection, following oral dosing at 5 mg/kg BID for 4 days, starting 24 h post-infection. In a CNS-stage disease model where mice were infected with the TREU 667 strain of *T. b. brucei*, treatment with 50 mg/kg AN3520 BID for 7 days, starting at Day 21 post-infection, resulted in absence of blood parasites through Day 99 in 70% of the mice. In contrast, all animals treated on Day 21 with the non-CNS penetrant drug Diminazene relapsed to exhibit blood parasitemia by Day 53. These results indicate that AN3520 has potential for development to treat late-stage HAT, since it appears to be able to control parasites in the CNS.

Methods

In vitro efficacy was measured by HTS against the SBRI 427 strain of *T. b. brucei*. Parasites were incubated for 72 h with 5 serial 2-fold dilutions of compounds, starting at 10 µg/mL. Parasite viability was measured using resazurin. Fluorescence was read 3-5 h after resazurin addition. Cytotoxicity was measured in a 3-day L929 growth inhibition assay, using the resazurin assay. Data from triplicates was averaged to generate IC₅₀ values for each compound. Cell permeability was measured in Madin Darby canine kidney cells transfected with the MDR1 gene coding for the P glyco-protein (P-gp) transporter. GF-120918 was used to block effects of P-gp.

Figure 1. AN3520. Structure and Physicochemical Properties.



MW = 321.1
Log D = 2.25
Solubility in PBS, pH 7.4 >200 µM.

Results

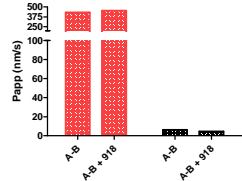
Table 1. AN3520 Shows Good *In vitro* Efficacy and Selectivity against *T. b. brucei*.

<i>In Vitro</i> Efficacy	AN3520
<i>T.b.brucei</i> IC ₅₀ (µM)	0.125
Cytotoxicity on murine L929 IC ₅₀ (µM)	11.7
Selectivity Index (SI)	94

Table 2. AN3520 does not Inhibit Cytochrome P450 Enzymes and is not Rapidly Metabolized by Mouse/Human Liver Microsomes or S9 Fractions.

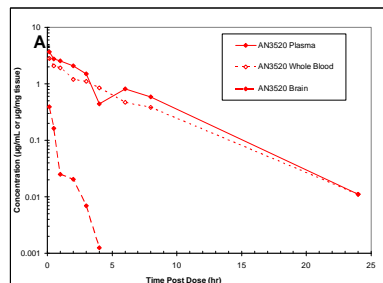
<i>In vitro</i> Metabolism	AN3520
t _{1/2} (Mouse/human liver microsomes, min)	342/>350
t _{1/2} (mouse S9, min)	217
Inhibition of Cytochrome P450s IC ₅₀ (µM): CP1A2, 2C9, 2C19, 2D6, 3A4	>10

Figure 2. MDCK-MDR-1 Cell Permeability Assay



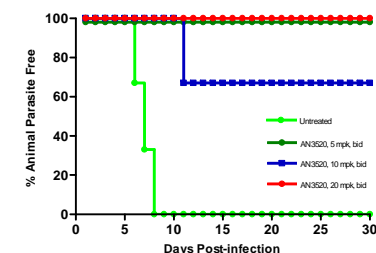
AN3520 is not a substrate for the P-gp transporter. Data suggests potential for blood brain barrier penetration.

Figure 3. (A) Disposition of AN3520 Following IV Dose of 2 mg/kg to CD-1 mice. (B) PK Data Summary – PO Dosing.



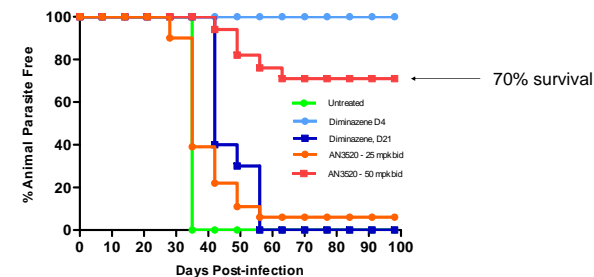
Poster will be available at www.anacor.com

Figure 4. Oral Administration of AN3520 at 5 mg/kg BID Allows 100% Survival in a 30-day Acute *T. b. brucei* Infection Model.



Mice were infected with 250,000 parasites of the EATRO 110 strain of *T. b. brucei*. 24 h post-infection, mice were treated for 4 days, BID, with various concentrations of AN3520. Survival was monitored for 30 days.

Figure 5. Administration of AN3520 Allows 70% survival after 99 days against a *T. b. brucei* CNS Infection in Mice.



Mice were infected IP with 10,000 parasites of the TREU667 strain of *T. brucei*. BID treatment for 7 days was started on Day 21. Dosing was IP. Study is ongoing.

Conclusion

- AN3520, a novel 6-substituted benzoxaborole, has demonstrated sub micromolar activity in an *in vitro* model of *T. b. brucei* infection and a 94X selectivity index.
 - AN3520 is not actively metabolized by mouse or human liver microsomes or mouse S9 fractions, and does not inhibit cytochrome P450 enzymes.
 - AN3520 shows sustained presence in plasma with a t_{1/2} of 3.7 h and good bioavailability.
 - Oral administration of AN3520 given at 5 mg/kg BID for 4 days results in 100% survival after 30 days
 - AN3520 allows 70% survival in a CNS model of *T. b. brucei* infection after 99 days
- This compound shows promise for development as treatment for early and late stage HAT.