

## Novel Boron-Containing Small Molecules Demonstrate Potent Activity Against Malaria Parasites with Excellent Drug-like Properties.

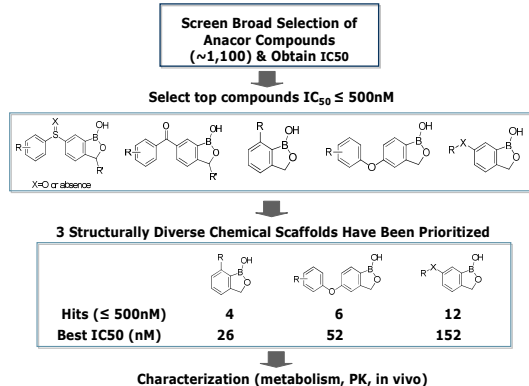
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### Abstract

There is an urgent need to discover new medications to treat falciparum malaria. New drugs must counter resistance to older drugs, be active orally, be effective in short-course therapy, be relatively inexpensive to produce, and be safe for use in developing world populations. We have developed boron-containing compounds with potential as anti-parasitic drugs, with potent activity, specificity and excellent drug-like properties. A panel of 1,100 oxaborole compounds was screened *in vitro* against *P. falciparum*. This resulted in 42 potent hits, with IC<sub>50</sub>s below 1 μM. Three unique chemical scaffolds were prioritized. IC<sub>50</sub>s for the best compound in each of these series were 26 nM, 52 nM and 156 nM, respectively. For the best compound, AN3661, with an IC<sub>50</sub> of 26 nM, further experiments were conducted to assess drug-like properties, including solubility, cytotoxicity and pharmacokinetic profile in mice. AN3661 was soluble at 750 μg/ml in 10 mM PBS at pH of 7.0. A safety index of >100 fold was observed in human KB carcinoma cells, murine J774 macrophages, and murine L929 fibroblasts. Pharmacokinetic studies of AN3661 in mice showed an oral bioavailability of 53%, an AUC<sub>0-last</sub> of 3.55 h\*μg/ml, a C<sub>max</sub> of 2.66 μg/ml, and a t<sub>1/2</sub> of 1.42 hours; all leading to an optimal oral exposure, when dosed at 30 mg/kg, that was well above the IC<sub>50</sub> for greater than 8 hours. Blood-to-plasma partitioning of AN3661 in non-infected mice showed equal distribution between blood and plasma, a 93% blood:plasma ratio. Two lead compounds from different scaffolds showed efficacy in a mouse model of *P. berghei* infection. Our results suggest that new oxaboroles have excellent potential as novel antimalarial agents.

### In vitro Screening and Scaffold Prioritization



**HTS in vitro screening:** W2 (chloroquine-resistant) *P. falciparum* parasites were cultured in human erythrocytes in RPMI media + 2% human serum/ 0.5% albumax, 100 μM hypoxanthine in 3% O<sub>2</sub>, 5-6% CO<sub>2</sub>. Compounds were initially screened at 10 μM. Hits were screened at 1 μM and IC<sub>50</sub>s determined on compounds showing >50% inhibition. 42 compounds had IC<sub>50</sub>s < 1 μM and 21 had IC<sub>50</sub>s < 0.5 μM. Parasite viability was assessed after 1 life cycle (48 h) with flow cytometry and YOYO-1 staining. Three scaffolds were prioritized based on potency and drug-like parameters.

### Results

**Table 1. Each of 3 series chosen for optimization showed good in vivo efficacy, selectivity and drug-like characteristics**

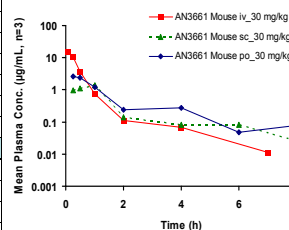
	Series 1 AN3661	Series 2 AN3232	Series 3 AN3330
<i>Efficacy: P. falciparum</i> (W2) IC <sub>50</sub> (μM)	0.026	0.052	0.163
Cytotoxicity: L929 fibroblast IC <sub>50</sub> (μM)	49	NT	>100
Selectivity Index (SI)	>1000	NT	>600
Molecular Weight	206	286	270
Solubility PBS pH 7 (μg/mL)	750	880	NT
cLogD at pH 7	-1.63	-0.39	-3.47
Oral bioavailability (%)	53	51	84

**Table 2. AN3661 showed good t<sub>1/2</sub>, AUC and C<sub>max</sub> after IV and PO dosing of Swiss Webster mice**

AN3661 IV <sup>1</sup>	IV-30 mg/kg (n=3)
Plasma C <sub>max</sub> (μg/mL) @ 5 min	15.1 ± 0.9
CL (mL/h/kg)	4513
V <sub>c</sub> (mL/kg)	1332
V <sub>ss</sub> (mL/kg)	2051
MRT (h)	0.454
AUC <sub>0-inf</sub> (h*μg/mL)	6.65
a-t <sub>1/2</sub> (h) [%AUC]	0.188 [91]
b-t <sub>1/2</sub> (h) [%AUC]	1.55 [9]
AN3661 PO <sup>2</sup>	PO-30 mg/kg (n=3)
Plasma C <sub>max</sub> (μg/mL)	2.66
T <sub>max</sub> (h)	0.25
AUC <sub>0-inf</sub> (h*μg/mL)	3.55
AUC <sub>0-last</sub> (h*μg/mL)	3.71
Terminal t <sub>1/2</sub> (h)	1.42
Bioavailability <sup>3</sup> (%)	53

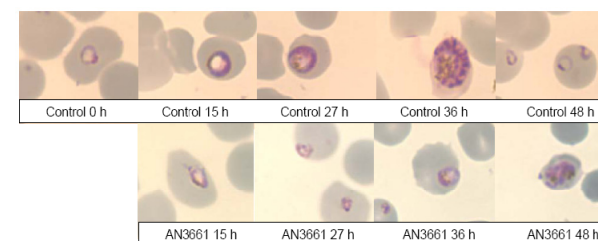
<sup>1</sup> WinNonlin two-compartment analysis with iterative weighting  
<sup>2</sup> WinNonlin non-compartment analysis with uniform weighting  
<sup>3</sup> Calculated by AUC<sub>last</sub> (PO or SC)/AUC<sub>0-inf</sub> (IV) with dose normalization

AN3661 partitioned equally between blood and plasma after oral dosing



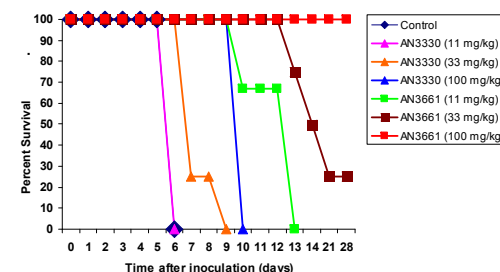
PK parameter	AN3661 in plasma	AN3661 in blood
C <sub>max</sub> (μg/mL)	2.66	2.73
T <sub>max</sub> (h)	0.25	0.50
AUC <sub>last</sub> (h*μg/mL)	3.55	3.55

**Figure 2. Abnormal morphology of *P. falciparum* parasites at various time points after treatment with AN3661.**



Parasites treated with 500 nM AN3661 and control untreated parasites were stained with Giemsa and photographed at the indicated time points. Controls showed progression from rings (0 h), to early (15 h) and late (27 h) trophozoites, to multinucleated schizonts (36 h), to new rings (48 h). Treated parasites were grossly abnormal at each time point, and did not progress to new ring forms.

**Figure 3. In vivo efficacy of orally administered AN3330 and AN3661 against *P. berghei* infection in Swiss Webster Mice (Ongoing to Day 42).**



Swiss Webster mice (~20 g) were infected with 10<sup>6</sup> *P. berghei*-infected murine erythrocytes from a previously infected mouse and oral treatment of infected animals was begun 1 h after infection. Treatment was BID for 4 days. All animals treated with 100 mg/kg AN3661 were parasite-free at 28 days post-infection.

### Summary

- Three separate series of oxaborole compounds showed excellent *in vitro* efficacy against Chloroquine-resistant *P. falciparum*, with IC<sub>50</sub>s less than 200 nM.
- All 3 series showed excellent drug-like qualities, optimal PK parameters and oral bioavailability.
- AN3661 treatment resulted in significant changes in parasite morphology which can be seen starting by 15 h post-infection.
- AN3661 provided potent antimalarial activity in a murine model, with 100% survival after oral treatment with 100 mg/kg BID for 4 days.
- The AN3661 series shows promise for lead optimization. Other series will be pursued as backups.