

Novel Boron-Containing Small Molecules Demonstrate Potent Activity Against *Trypanosoma cruzi*

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Abstract

Chagas disease (American Trypanosomiasis) is a parasitic infection mostly occurring in Central and South America and currently affecting more than 8 million people. There are estimated 50,000 or more new clinical cases and 20,000 deaths attributable to Chagas disease each year. The causative parasite *Trypanosoma cruzi* is transmitted to humans by blood-sucking triatomine insects, by congenital infection, by blood transfusions, or by organ transplant. While there are two drugs to treat the acute phase of Chagas disease, these drugs have side effects, incomplete efficacy, and resistance issues. No therapeutic agents are available for the chronic phase of the disease. Anacor Pharmaceuticals has been working with DNDi, Murdoch University, and EpiChem to identify a new class of oxaborole compounds for treatment of Chagas. We recently screened our boron-containing compound collection *in vitro* against *T. cruzi* and found a number of compounds showing IC₅₀ values of less than 1 μM, which were more potent than benznidazole (IC₅₀ 2.5 μM). These compounds also have good drug-like properties. Active compounds have a hydrophobic or electron rich pendant aromatic group connected to the benzoxaborole scaffold with an amide linkage. Compounds with electron deficient aromatic group tended to be less active. The *in vitro* structure-activity relationship, cytotoxicity, and drug-like property predictors for these new compounds are described.

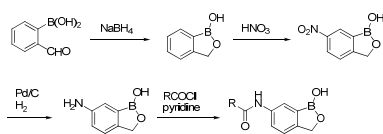


Figure 1. Synthetic route for acylaminobenzoxaborole derivatives

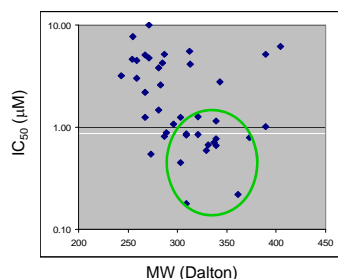


Figure 2. Relationship between molecular weight and IC₅₀

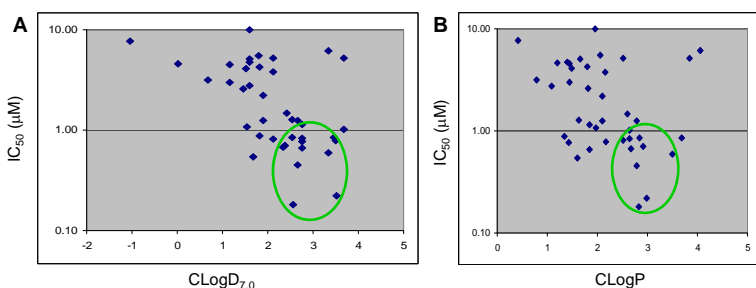


Figure 3. Relationships between physical property and activity; (A) CLogD_{7.0} and IC₅₀, (B) CLogP and IC₅₀

Trypanosoma cruzi in vitro assay

On Day 1, all wells of a 96-well plate were seeded with 100 μL medium (RPMI1640 + 10% fetal calf serum + 15 mM HEPES + 20 mM L-glutamine without phenol red) containing 1.5 × 10⁵ L6 rat myoblast cells (European Collection of Animal Cell Cultures, ECACC, UK) per well and incubated at 37 °C and 5% CO₂. On Day 2, 5 × 10³ *T. cruzi* trypomastigotes (MHOM/CL/00/Tulahuen) transfected with β-galactosidase (Lac Z) gene were added to the appropriate wells. On Day 4, media was removed from all wells and replaced with fresh medium. Compounds were serially diluted on the plate to the desired final concentrations. On Day 8, 50 μL of 2.5 × CPRG/Nonidet (5 × stock = 500 mL Nonidet P40 + 30.38 mg CPRG in 100 mL 1 × PBS) was added to all wells. The color reaction was read at 2-6 h using an absorbance reader at 530 nm, IC₅₀ values were calculated using a four parameter logistic equation.

Table 1: Structure-activity relationships of acylaminobenzoxaboroles

Compound	R	<i>T. cruzi</i> IC ₅₀ (μM)	L929 IC ₅₀ (μM)	CLogP	CLogD _{7.0}	MW
1		0.18	27	2.8	2.6	309
2		0.22	< 1.7	3.0	3.5	362
3		0.45	> 33	2.8	2.7	303
4		0.54	> 37	1.6	1.7	273
5		0.59	< 1.9	3.5	3.3	329
6		0.66	16	1.8	2.8	339
7		0.71	> 30	2.9	2.4	337
8 (AN4169)		0.78 (0.43-1.2)	28	1.8	2.8	339
9		1.0	14	2.6	3.7	389
10		1.3	> 33	2.8	2.7	303
11		2.6	> 35	1.8	1.5	283
12		3.2	> 41	0.78	0.67	243
13		4.1	NT ^a	1.5	1.5	313
14		5.2	> 26	3.8	3.7	389
15		7.7	>100	0.42	-1.0	255
16		> 10	>100	0.15	-0.89	257
17		> 10	>39	0.85	0.020	254
18		> 10	>100	0.12	-0.05	191
	Benznidazole	2.5	NT ^a	0.90	0.95	260

^a Not tested

Conclusions

- Pendant aromatic group was essential for the activity
- Fused aryl and biaryl analogs showed most potent activity
- Benzamide derivatives with halogen, alkyl, or alkoxy groups showed moderate activity
- Compounds with electron deficient heteroaromatic ring (pyridine, pyrazine, etc.) were less active or inactive
- Potent compounds had CLogD_{7.0} and CLogP values between 2.5 and 3.5 and molecular weight between 300 and 370, which would be the guide for further optimization

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