ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

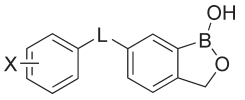
Discovery of Novel Benzoxaborole-Based Potent Antitrypanosomal Agents

Dazhong Ding,[†] Yaxue Zhao,[†] Qingqing Meng,[†] Dongsheng Xie,[†] Bakela Nare,[‡] Daitao Chen,[‡] Cyrus J. Bacchi,[§] Nigel Yarlett,[§] Yong-Kang Zhang,[∥] Vincent Hernandez,[∥] Yi Xia,[∥] Yvonne Freund,[∥] Maha Abdulla,[⊥] Kean-Hooi Ang,[⊥] Joseline Ratnam,[⊥] James H. McKerrow,[⊥] Robert T. Jacobs,[‡] Huchen Zhou,*[,†] and Jacob J. Plattner[∥]

[†]School of Pharmacy and E-Institute of Chemical Biology, Shanghai Jiao Tong University, Shanghai, 200240, China, [‡]SCYNEXIS, Inc., P.O. Box 12878, Research Triangle Park, North Carolina 27709, [§]Haskins Laboratory, Pace University, New York, New York 10038, [∥]Anacor Pharmaceuticals Inc., 1020 East Meadow Circle, Palo Alto, California 94303, and [⊥]Small Molecule Discovery Center and Sandler Center for Basic Research in Parasitic Diseases, University of California, San Francisco, California 94158

ABSTRACT We report the discovery of benzoxaborole antitrypanosomal agents and their structure−activity relationships on central linkage groups and different substitution patterns in the sulfur-linked series. The compounds showed in vitro growth inhibition IC_{50} values as low as 0.02 μg/mL and in vivo efficacy in acute murine infection models against *Tryapnosoma brucei*.

KEYWORDS *Tryapnosoma brucei*, African trypanosomiasis, benzoxaborole



African sleeping sickness (African trypanosomiasis), a fatal disease, is caused by the protozoan parasite *Trypanosoma brucei* and is transmitted by the bite of the tsetse fly.[1] Although it affects a large population in Africa, drug discovery has been largely neglected during the past half century.[2] The currently available treatments for early stage infection, pentamidine and suramin, and melarsoprol and eflornithine for late stage infection, have the problems of high toxicity, high cost, or low efficacy.[3] There is an urgent need to develop new therapies with low toxicity, improved efficacy, and affordable cost.[4,5]

We report here the discovery and structure−activity relationship (SAR) of novel benzoxaborole antitrypanosomal agents. During an initial screening of a focused library of antiinfective benzoxaboroles, compound **12** was found to inhibit in vitro *T. brucei* growth (IC_{50} = 0.12 μg/mL). There was no previous report on benzoxaboroles as effective antiprotozoals, although they had been studied as antifungal[6] and antiinflammatory[7] agents. In this study, we explored the effect of a variety of linkage groups at C(6) and different substitution patterns in the 6-sulfur linked series on *T. brucei* growth inhibition.

The synthesis of benzoxaboroles with thioether, sulfoxide, and sulfone linkage groups at C(6) is outlined in Scheme 1. Nucleophilic substitution of 2-bromo-4-fluorobenzaldehyde by phenylthiol gave thioether **1**, where an ice bath was necessary in some cases to minimize side reactions due to the substitution of bromide. After the aldehyde was converted to MOM-protected hydroxyl, the oxaborole ring was installed by halogen−metal exchange with *n*-butyllithium followed by in situ trapping with triisopropylborate and deprotection with HCl to give benzoxaboroles **4**−**8**. An alternative route utilized a palladium-mediated boronylation of **1** to provide aldehyde **19**, followed by reduction with $NaBH_4$ and acid-catalyzed cyclization to the benzoxaboroles **20**−**24**. Thioethers were oxidized to their sulfoxide analogues either by heating with $NaIO_4$ at 60 °C for an hour or by treatment with an equivalent of *m*-CPBA at −20 °C. Sulfones were obtained by treatment of thioethers with $NaIO_4$ at 60 °C for 12 h or by treatment with 2 equiv of *m*-CPBA at −60 °C. Analogously, ether **28** was obtained from 2-bromo-4-fluorobenzaldehyde and phenol as depicted in Scheme 2.

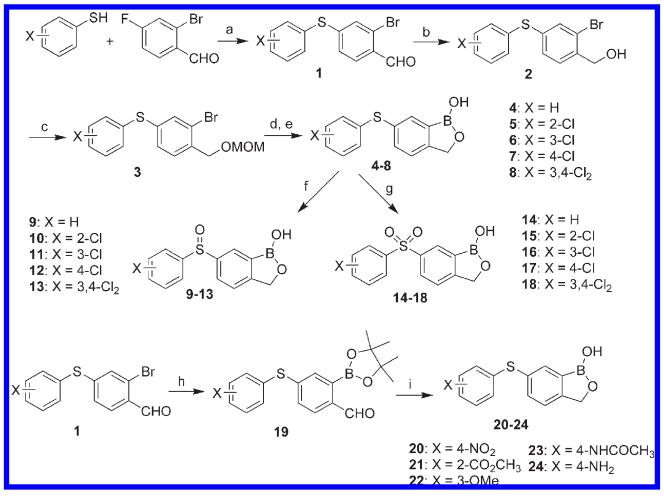
Benzoxaboroles with carbonyl and carbinol linkage groups were synthesized as shown in Scheme 3. Diphenyl ketone **29** was prepared by Friedel−Crafts reaction and was subsequently brominated with NBS in the presence of Bz_2O_2 and converted to hydroxymethyl group by treatment with sodium acetate followed by basic hydrolysis to give compound **30**. After oxidation with PCC, both carbonyl groups were protected as acetals (**32**). Introduction of the boronic acid functionality via lithiation and trapping with triisopropyl borate afforded boronic acid **33** after hydrolysis of the acetal groups. Reduction of compound **33** led to the formation of carbinol **34**. Oxidation of compound **34** with PCC resulted in ketone **35**.

Benzoxaboroles with linkage groups derived from 6-OH and 6-NH_2 were also synthesized (Scheme 4). First, compound **37** was prepared from acetal **36** by treatment with benzyl alcohol and NaH. Benzoxaborole **39** was obtained after boronylation and reduction as described above. Hydrogenation of compound **39** in the presence of Pd/C resulted in the 6-OH benzoxaborole **40**, which was coupled with phenyl

Received Date: January 20, 2010
Accepted Date: March 19, 2010
Published on Web Date: April 06, 2010

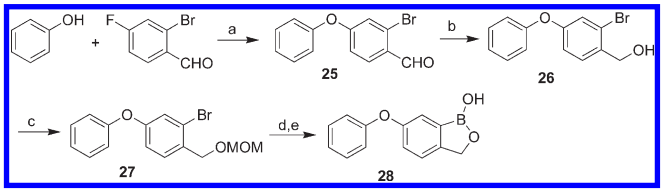
DOI: 10.1021/ml100013s | *ACS Med. Chem. Lett.* **2010**, *1*, 165–169

Scheme 1. Synthesis of Benzoxaboroles with Thioether, Sulfoxide, and Sulfone Linkage Groups[a]



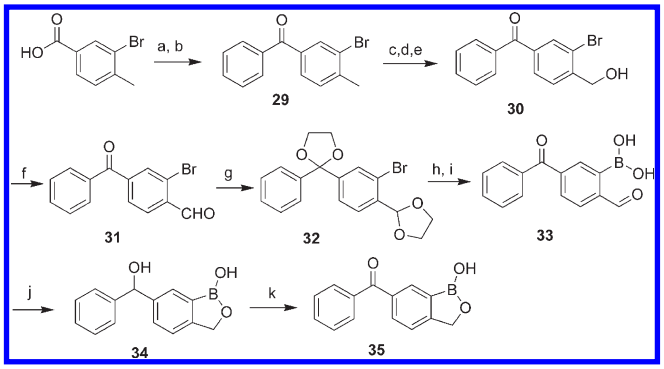
[a] Reagents and conditions: (a) K_2CO_3, DMF, 0 or 100 °C. (b) $NaBH_4$, CH_3OH. (c) MOMCl, DIPEA. (d) *n*-BuLi, B(*i*-PrO)$_3$, −78 °C to room temperature. (e) HCl. (f) $NaIO_4$, MeOH−H_2O, 60 °C, 1 h or 1 equiv of *m*-CPBA, DCM−THF, −20 °C. (g) $NaIO_4$, 60 °C, 12 h or 2 equiv of *m*-CPBA, −60 °C to room temperature. (h) Bis(pinacol-diboron), $PdCl_2(dppf)_2$, KOAc, dioxane. (i) $NaBH_4$, EtOH.

Scheme 2. Synthesis of Benzoxaboroles with Ether Linkage Group[a]



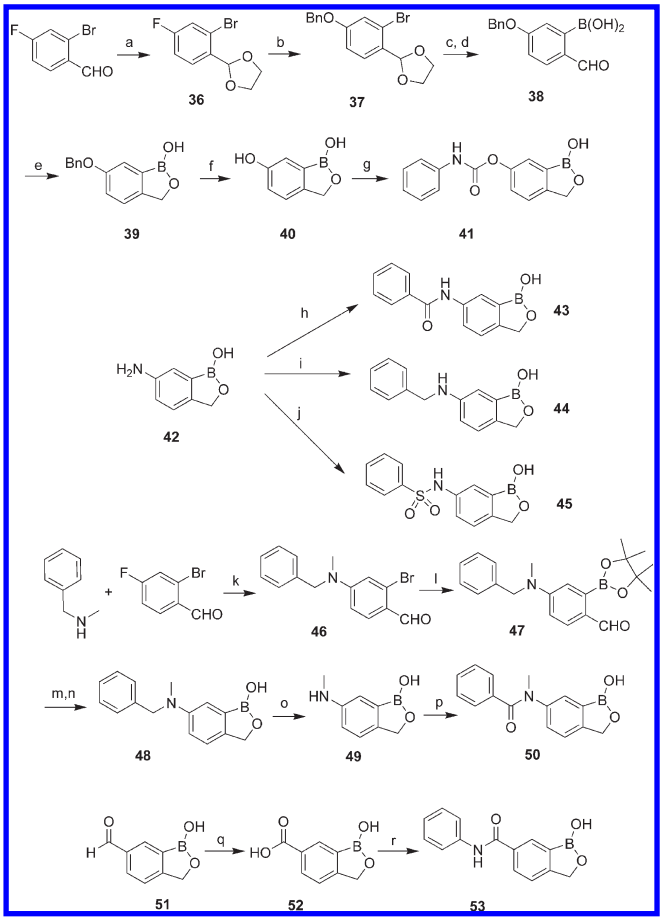
[a] Reagents and conditions: (a) K_2CO_3, DMF, 100 °C. (b) $NaBH_4$, MeOH. (c) MOMCl, DIPEA, DCM. (d) (*i*-PrO)$_3$B, *n*-BuLi, THF, −78 °C to room temperature. (e) HCl.

Scheme 3. Synthesis of Benzoxaboroles with Carbonyl and Carbinol Linkage Groups[a]



[a] Reagents and conditions: (a) $SOCl_2$. (b) Benzene, $AlCl_3$, 50 °C. (c) NBS, Bz_2O_2, CCl_4, reflux. (d) NaOAc, DMF, 60 °C. (e) NaOH, MeOH−H_2O, reflux. (f) PCC, DCM. (g) Eethylene glycol, *p*-TsOH, toluene, reflux, 96 h. (h) B(*i*-PrO)$_3$, *n*-BuLi, −78 °C to room temperature. (i) HCl. (j) $NaBH_4$, THF−H_2O. (k) PCC, DCM.

Scheme 4. Synthesis of Benzoxaboroles with 6-OH and 6-NH_2 Derived and Reversed Amide Linkage Groups[a]



[a] Reagents and conditions: (a) Ethylene glycol, TsOH, toluene, reflux. (b) BnOH, NaH, DMF, 0−65 °C. (c) *n*-BuLi, B(iPrO)$_3$, −78 °C to room temperature. (d) HCl. (e) $NaBH_4$, THF, 0 °C. (f) Pd/C, H_2, MeOH. (g) PhNCO, Et_3N, DMF, 0 °C to room temperature. (h) PhCOCl, $NaHCO_3$, CH_3CN. (i) BnBr, $NaHCO_3$, DMF, 100 °C. (j) $PhSO_2Cl$, K_2CO_3, CH_3CN. (k) K_2CO_3, DMF. (l) Bis(pinacol-diboron), $PdCl_2(dppf)_2$, KOAc, dioxane. (m) $NaBH_4$, MeOH. (n) Aqueous HCl. (o) Pd/C, HCO_2NH_4. (p) PhCOCl, Et_3N, CH_2Cl_2. (q) $AgNO_3$, NaOH, H_2O, 0 °C. (r) Analine, EDCI, DCM, room temperature, 60 h.

isocyanate to give carbamate **41**. Coupling of amine **42**[8] with benzoic acid, benzyl bromide, or phenylsulfonyl chloride led to the formation of benzoxaboroles with amide (**43**), aminomethylene (**44**), and sulfonamide (**45**) linkage groups, respectively. The *N*-methylbenzyl amine **48** was prepared in three steps from 2-bromo-4-fluorobenzaldehyde. Hydrogenolysis of compound **48** afforded the *N*-methyl benzoxaborole **49**, which was treated with benzoyl chloride to provide the *N*-methylbenzamide **50**. The reversed amide **53** was synthesized by coupling of aniline with carboxylic acid **52** that was prepared from 6-formyl benzoxaborole **51**.[9]

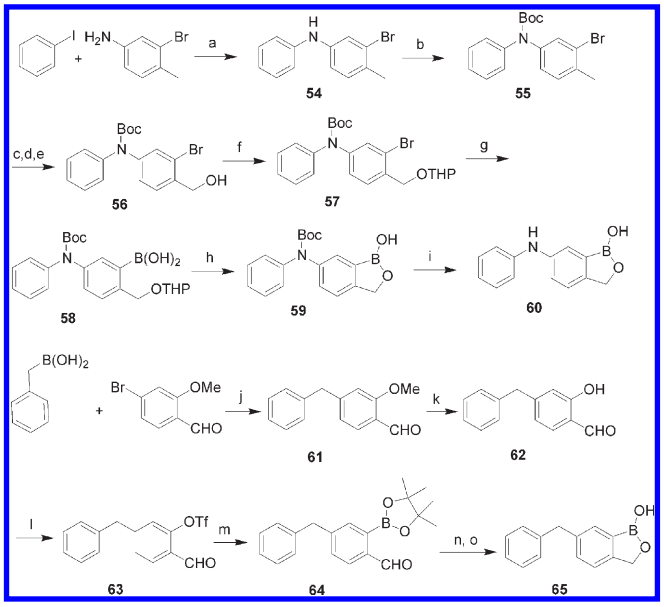
As shown in Scheme 5, benzoxaboroles with NH and methylene linkage groups were also synthesized. Diphenylamine **54** was obtained by the coupling of iodobenzene and 3-bromo-4-methylanaline in the presence of CuI and L-proline.[10] After Boc protection, compound **55** was converted to

DOI: 10.1021/ml100013s | ACS Med. Chem. Lett. 2010, 1, 165–169

ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

Scheme 5. Synthesis of Benzoxaboroles with NH and CH_2 Linkage Groups[a]

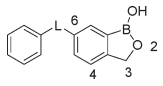


[a] Reagents and conditions: (a) CuI, L-proline, *t*-BuONa, DMSO, 50 °C. (b) LiHMDS, Boc_2O, THF, −80 °C to room temperature. (c) NBS, Bz_2O_2, CCl_4, reflux. (d) NaOAc, DMF, 70 °C. (e) NaOH, MeOH−H_2O, reflux. (f) DHP, pyridine, *p*-TsOH, CH_2Cl_2. (g) *n*-BuLi, $B(iPrO)_3$, −78 °C to room temperature. (h) *p*-TsOH, pyridine, EtOH, 50 °C. (i) TFA, CH_2Cl_2, 0 °C to room temperature. (j) $Pd(dppf)Cl_2$, CsF, K_2CO_3, dioxane, 80 °C. (k) $CeCl_3$, NaI, CH_3CN, reflux. (l) Tf_2O, Et_3N, CH_2Cl_2, −78 °C. (m) Bis-(pinacol-diboron), $Pd(dppf)Cl_2$, KOAc, dioxane, 80 °C. (n) $NaBH_4$, MeOH−THF, 0 °C. (o) HCl.

alcohol **56** as described above. Subsequent THP protection and boronylation gave boronic acid **58**. Attempts to remove the Boc and THP protecting groups simultaneously with HCl resulted in a complex mixture. Thus, THP was first removed with pyridinium *p*-toluenesulfonate to give compound **59**, which was treated with trifluoroacetic acid at 0 °C to give amine **60**. Compound **65** with a methylene linkage group was synthesized from benzyl boronic acid and 2-methoxy-4-bromobenzaldehyde. Suzuki coupling gave biarylmethane **61**, which was converted to triflate **63** by demethylation using $CeCl_3$/NaI followed by treatment with Tf_2O. Boronylation followed by reduction and acidic treatment gave benzoxaborole **65**.

To probe the effect of different C(6) linker groups on antitrypanosomal activity, the above benzoxaboroles were tested for their ability to inhibit growth of *T. brucei*. They showed good inhibitory effect with IC_{50} values ranging from 1.62 to 0.02 μg/mL. They also showed a satisfactory (> 10 μg/mL) cytotoxicity profile against mouse lung fibroblast cells (L929) in a 72 h in vitro assay. First, the oxaborole functionality was essential for the observed antitrypanosomal activity as demonstrated by the loss of activity (IC_{50} > 10 μg/mL) upon removal of the oxaborole ring from compounds **4** and **35**. Second, the length and hydrogen-bonding properties of the linkage group "L" at C(6) had a significant effect on the antitrypanosomal activity (Table 1). The mechanism of action of these benzoxaboroles is unclear,

Table 1. Effect of Linkage Group "L" on *T. brucei* Growth Inhibition and Cytotoxicity[a]



	L	IC_{50}	L929		L	IC_{50}	L929
4		0.51	3.1	**48**		0.83	>10
9		0.17	>10	**43**		0.04	>10
14		0.33	>10	**50**		0.32	>10
28		1.11	>10	**53**		0.44	>10
35		0.15	>10	**41**		0.35	>10
34		0.16	>10	**45**		0.02	3.48
39		1.62	>10	**60**		0.70	>10
44		1.21	>10	**65**		0.59	>10

[a] IC_{50}: growth inhibition of *T. b. brucei* 427 strain (μg/mL). L929: IC_{50} against L929 cells (μg/mL). References: suramin and pentamidine.

so the possibility of interaction with multiple biomolecular targets remains, and their membrane permeability and serum-binding properties may also have contributed to cellular activity.

Compounds with thioether (**4**), ether (**28**), methylene (**65**), and amino (**60**) linkage groups showed IC_{50} values in the range of 0.51−1.11 μg/mL. The methylene linker is the most flexible with a wide range of allowed conformations, while amino is the most rigid and prefers a near planar conformation.[11]

The benzoxaboroles bridged with sulfoxide (**9**), sulfone (**14**), carbonyl (**35**), and carbinol (**34**) represent the category of linkage groups with a hydrogen bond acceptor that is two covalent bonds away from C(6) and showed improved potency (0.15−0.33 μg/mL). It is known that benzophenones such as **35** are a rather rigid chemical motif with sp^2 geometry and closely clustered conformations,[11,12] while sulfoxide **9**, sulfone **14**, and carbinol **34** have sp^3 geometry and more conformational flexibility. Carbinol **34** showed comparable activity, suggesting that the hydroxyl group may serve as a hydrogen bond acceptor as well.

Benzoxaboroles with amide (**43**) and sulfonamide (**45**) linkers showed further improvement of antitrypanosomal activity and represent the most potent compounds among the series (IC_{50}: 0.04 and 0.02 μg/mL). The *N*-methylbenzamide **50** is 8-fold less potent than amide **43**. It is interesting that benzylamine **44** and *N*-methylbenzyl amine **48** are significantly less active (IC_{50}: 1.21 and 0.83 μg/mL), indicating that carbonyl is essential for high potency and may contribute as a strong hydrogen bond acceptor. Benzyl ether **39** lacking a carbonyl also showed low potency. The reversed

DOI: 10.1021/ml100013s | *ACS Med. Chem. Lett.* **2010**, *1*, 165–169

Table 2. SAR of S-Linked Series[a]

	X	IC_{50}	L929		X	IC_{50}	L929
				thioether			
5	2-Cl	0.58	>10	**21**	2-CO_2Me	0.14	>10
6	3-Cl	0.12	1.11	**22**	3-OMe	0.12	2.64
7	4-Cl	0.15	2.02	**23**	4-NHCOMe	0.04	>10
8	3,4-Cl_2	0.49	0.56	**24**	4-NH_2	0.03	>10
20	4-NO_2	0.32	>10				
				sulfoxide			
10	2-Cl	0.18	>10	**12**	4-Cl	0.12	8.87
11	3-Cl	0.15	>10	**13**	3,4-Cl_2	0.30	2.67
				sulfone			
15	2-Cl	0.10	>10	**17**	4-Cl	0.16	>10
16	3-Cl	0.22	>10	**18**	3,4-Cl_2	0.52	>10

[a] IC_{50}: growth inhibition of *T. b. brucei* 427 strain (μg/mL). L929: IC_{50} against L929 cells (μg/mL). References: suramin and pentamidine.

amide **53** has a 10-fold lower activity than amide **43**, suggesting that the distance between the hydrogen bond acceptor O and the benzoxaborole C(6) has a significant effect. Compounds **9**, **14**, **34**, and **35** have an O−C(6) distance in the range of 2.38−2.70 Å and IC_{50} values of 0.15−0.24 μg/mL. Amide **43** and sulfonamide **45** have an O−C(6) distance of 2.96 and 3.52 Å and improved IC_{50} of 0.04 and 0.02 μg/mL. With the exception of thioether **4** and sulfonamide **45**, the benzoxaboroles described in Table 1 exhibited very little cytotoxicity in a L929 cell line.

We next explored the SAR of the 6-sulfur-linked benzoxaboroles (Table 2). In the thioether oxidation state, the 3-chloro (**6**) and 4-chloro (**7**) analogues improved the antiparasite potency approximately 3-fold, but cytotoxicity was also increased relative to the unsubstituted thioether (**4**). Cytotoxicity was further increased in the 3,4-dichloro (**8**) analogue, but the antiparasite potency was diminished. By contrast, the 2-chloro analogue (**5**) was approximately equipotent with the unsubstituted phenyl but did not exhibit cytotoxicity. In the sulfoxide oxidation state, the antiparasitic potency was not significantly increased by chloro substitution (**10**−**13**), but cytotoxicity was observed for the 4-chloro (**12**) and 3,4-dichloro (**13**) analogues. In the sulfone oxidation state, the antiparasite potency was similar for the three analogues (**15**−**17**) and slightly diminished for the 3,4-dichloro analogue (**18**). No cytotoxicity was observed at the sulfone oxidation state. Furthermore, a few more thioethers 4-nitro (**20**), 2-carbomethoxy (**21**), 4-acetamido (**23**), and 4-amino (**24**) analogues exhibited good antiparasite activity and low cytotoxicity, while the 3-methoxy (**22**) analogue was cytotoxic.

The 4-chlorophenylsulfoxide **12** was evaluated in a murine model of blood stage *T. brucei* infection. Treatment of mice infected with 600 *T. b. brucei* (EATRO 221 strain) at 50 mg/kg, b.i.d., i.p. × 5 days resulted in 100% survival and no parasitemia 40 days after infection. Treatment of *T. b. rhodesiense* (strain IL1852, 10^4 parasites)-infected mice at the same dose also cleared parasites 60 days after infection (Figure 1).

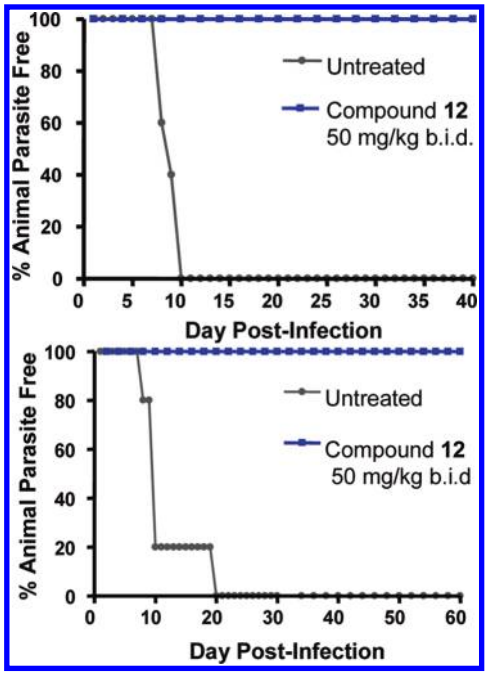


Figure 1. (a) Female BALB/c mice were inoculated IP with 600 *T. b. brucei* (EATRO 221) parasites. Treatment with compound **12** (50 mg/kg, b.i.d.) cured 100% of mice. (b) Treatment of *T. b. rhodesiense* (IL1852, 10^4/mouse)-infected mice with compound **12** (50 mg/kg, b.i.d.) also gave parasite-free survival of the mice. Reference compound: suramin.

We further explored the efficacy of several 6-S-linked benzoxaboroles in the murine model of blood stage *T. b. brucei* infection, using the EATRO 110 strain. The sulfoxide **12** was effective at 20 mg/kg, i.p., but failed to show complete cure of infection via an oral route. The sulfone **17** was more efficacious, with complete cure observed at 20 mg/kg, p.o., but with only limited efficacy at 10 mg/kg, p.o. None of the thioether analogues (**7**, **23**, or **24**) demonstrated meaningful reduction of parasitemia in this model.

In summary, we report novel benzoxaborole-based antitrypanosomal agents. Their in vitro antitrypanosomal IC_{50} values ranged from 0.02 to 1.62 μg/mL, and they also showed satisfactory cytotoxicity above 10 μg/mL against L929 cells. The SAR of the linkage groups suggested that while most linkers can provide compounds with acceptable antiparasite potency, those containing a hydrogen bond acceptor offer superior potency. The effects of substitution and sulfur oxidation state were investigated, and it was found that they had a significant effect on cytotoxicity. Finally, compounds **12** and **17** showed efficacy in the mice infected with *T. brucei*. Further optimization of the benzoxaboroles, in particular 6-carboxamides, exemplified by compound **43**, is underway and will be the subject of future communications. The discovery of benzoxaboroles as a novel class of antiparasitic agents offers a new opportunity in the war against pathogenic parasites.

SUPPORTING INFORMATION AVAILABLE Synthetic experimental details, analytical data of compounds, and biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

DOI: 10.1021/ml100013s | *ACS Med. Chem. Lett.* **2010**, *1*, 165–169

AUTHOR INFORMATION

Corresponding Author: *To whom correspondence should be addressed. E-mail: hczhou@sjtu.edu.cn.

Funding Sources: We thank The Sandler Foundation, Drugs for Neglected Diseases initiative, National Science Foundation of China (20702031), and Ministry of Science and Technology of China (2009CB918404) for financial support of this work.

REFERENCES

(1) Legros, D.; Ollivier, G.; Gastellu-Etchegorry, M.; Paquet, C.; Burri, C.; Jannin, J.; Buscher, P. Treatment of human African trypanosomiasis-present situation and needs for research and development. *Lancet Infect. Dis.* **2002,** *2*, 437–440.

(2) Hotez, P. J.; Molyneux, D. H.; Fenwick, A.; Kumaresan, J.; Sachs, S. E.; Sachs, J. D.; Savioli, L. Control of neglected tropical diseases. *N. Engl. J. Med.* **2007,** *357*, 1018–1027.

(3) Croft, S. L.; Barrett, M. P.; Urbina, J. A. Chemotherapy of trypanosomiases and leishmaniasis. *Trends Parasitol.* **2005,** *21*, 508–512.

(4) Renslo, A. R.; McKerrow, J. H. Drug discovery and development for neglected parasitic diseases. *Nat. Chem. Biol.* **2006,** *2*, 701–710.

(5) Pink, R.; Hudson, A.; Mouries, M. A.; Bendig, M. Challenges in antiparasitic drug discovery. *Nat. Rev. Drug Discovery* **2005,** *4*, 727–740.

(6) Rock, F. L.; Mao, W.; Yaremchuk, A.; Tukalo, M.; Crepin, T.; Zhou, H.; Zhang, Y. K.; Hernandez, V.; Akama, T.; Baker, S. J.; Plattner, J. J.; Shapiro, L.; Martinis, S. A.; Benkovic, S. J.; Cusack, S.; Alley, M. R. K. An antifungal agent inhibits an aminoacyl-tRNA synthetase by trapping tRNA in the editing site. *Science* **2007,** *316*, 1759–1761.

(7) Akama, T.; Baker, S. J.; Zhang, Y. K.; Hernandez, V.; Zhou, H.; Sanders, V.; Freund, Y.; Kimura, R.; Maples, K. R.; Plattner, J. J. Discovery and structure-activity study of a novel benzoxaborole anti-inflammatory agent (AN2728) for the potential topical treatment of psoriasis and atopic dermatitis. *Bioorg. Med. Chem. Lett.* **2009,** *19*, 2129–2132.

(8) Lennarz, W. J.; Snyder, H. R. Arylboronic acids. IV. Reactions of boronophthalide. *J. Am. Chem. Soc.* **1960,** *82*, 2172–2175.

(9) Ye, L.; Ding, D.; Feng, Y.; Xie, D.; Wu, P.; Guo, H.; Meng, Q.; Zhou, H. Convenient and versatile synthesis of formyl-substituted benzoxaboroles. *Tetrahedron* **2009,** *65*, 8738–8744.

(10) Ma, D.; Cai, Q. Copper/amino acid catalyzed cross-couplings of aryl and vinyl halides with nucleophiles. *Acc. Chem. Res.* **2008,** *41*, 1450–1460.

(11) Hao, M. H.; Haq, O.; Muegge, I. Torsion angle preference and energetics of small-molecule ligands bound to proteins. *J. Chem. Inf. Model.* **2007,** *47*, 2242–2252.

(12) Gough, K. M.; Wildman, T. A. Hindered internal rotation in benzophenone. *J. Am. Chem. Soc.* **1990,** *112*, 9141–9144.

DOI: 10.1021/ml100013s | *ACS Med. Chem. Lett.* **2010,** *1*, 165–169