Journal of Medicinal Chemistry

ARTICLE

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Design, Synthesis, and Structure−Activity Relationship of *Trypanosoma brucei* Leucyl-tRNA Synthetase Inhibitors as Antitrypanosomal Agents

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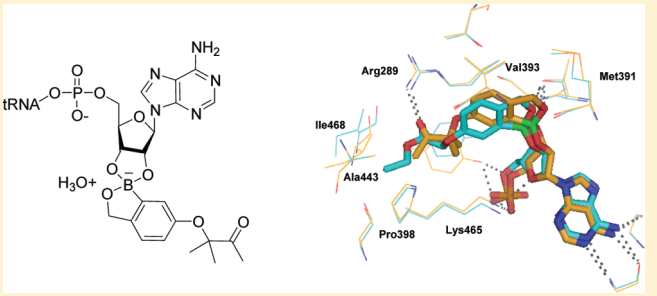
ABSTRACT: African trypanosomiasis, caused by the protozoal pathogen *Trypanosoma brucei* (*T. brucei*), is one of the most neglected tropical diseases that are in great need of new drugs. We report the design and synthesis of *T. brucei* leucyl-tRNA synthetase (TbLeuRS) inhibitors and their structure−activity relationship. Benzoxaborole was used as the core structure and C(6) was modified to achieve improved affinity based on docking results that showed further binding space at this position. Indeed, compounds with C(7) substitutions showed diminished activity due to clash with the eukaryote specific I4ae helix while substitutions at C(6) gave enhanced affinity. TbLeuRS inhibitors with IC_{50} as low as 1.6 μM were discovered, and the structure−activity relationship was discussed. The most potent enzyme inhibitors also showed excellent *T. brucei* parasite growth inhibition activity. This is the first time that TbLeuRS inhibitors are reported, and this study suggests that leucyl-tRNA synthetase (LeuRS) could be a potential target for antiparasitic drug development.



INTRODUCTION

African trypanosomiasis, also called sleeping sickness, is one of the most neglected tropical diseases that are in great need of new drug discovery and development.[1] The causative pathogen, *Trypanosoma brucei*, belongs to the *kinetoplastid* family and is transmitted via the bite of the tsetse fly. The disease is a daily threat to over 60 million people in 36 countries of sub-Saharan Africa. The four currently used drugs against trypanosomiasis are suramin, pentamidine, melarsoprol, and eflornithine.[2] Because of the lack of efficacy in late stage infection, high toxicity, and potential development of resistance, discovery of drugs in new chemical class with new mechanism of action is highly desired.[3,4]

Aminoacyl-tRNA synthetase (aaRS) enzymes have been a focus of antimicrobial research. These enzymes play critical roles in protein synthesis by catalyzing the attachment of specific amino acids to their cognate tRNAs. Thus, inhibition of aaRS will halt protein synthesis and attenuate microorganism growth.[5−7] Mupirocin, an isoleucyl-tRNA synthetase (IleRS) inhibitor, has been used as an antibacterial drug in clinical application. 2-(3-{[4-Bromo-5-(1-fluorovinyl)-3 -methylthiophen-2-ylmethyl]amino} propylamino)-1*H*-quinolin-4-one (REP8839), which is a bacterial methionyl-tRNA synthetase (MetRS) inhibitor, is in clinical trials.[8] Recently, **1** (AN2690) (Figure 1), which is under clinical investigation, was reported as an antifungal agent by inactivating fungal LeuRS.[9] We propose aaRS as a class of new targets in antiprotozoal drug discovery, and their inhibitor design should contribute to the discovery of antiprotozoal agents with mechanism of action that is likely to be different from that of current clinically used drugs.

LeuRS is responsible for charging leucine to its cognate tRNA correctly. This enzyme has a catalytic site where leucine is covalently attached to the 2′-hydroxy group of the 3′-terminal adenosine and an editing site where proofreading happens to preferentially hydrolyze an incorrect amino acid or release the correctly charged tRNA to fulfill its role in ribosomal protein synthesis.[10,11] The search for inhibitors directed toward the synthetic active sites of aaRSs has been undergoing for more than a decade.[5−7] Recently, the discovery of **1** and its mechanism of action demonstrated for the first time the feasibility of targeting the editing site.[9] Compound **1** has a benzoxaborole core structure with a boronic acid embedded in a five-membered ring. The sp^2 hybridized boron atom possesses an empty p-orbital that accepts electrons from the hydroxyl groups of the terminal adenosine and forms an adduct with the tRNA. The

Received: September 27, 2010
Published: February 15, 2011

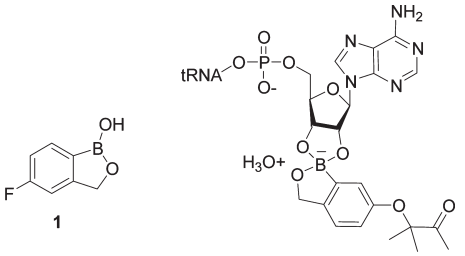


Figure 1. Chemical structures of **1** and the covalent adduct between compound **42** and the terminal AMP of tRNA.

benzoxaborole-tRNA adduct is illustrated in Figure 1 by compound **42**. Thus, tRNA is trapped in the editing site and the enzyme function is abolished. In this study, we hope to investigate if benzoxaboroles can effectively inhibit *T. brucei* LeuRS and affect parasite growth. There has not been any successful report of parasitic aaRS inhibitors in the past. Moderately active inhibitors of *Brugia malayi* asparaginyl-tRNA synthetase (AsnRS), resulting from a virtual screening, have been reported,[12] but the structure−activity relationship or antiparasitic effect was not described.

In this report, a homology model of *T. brucei* LeuRS was built as the basis of structure-guided inhibitor design and the enzyme was cloned and expressed for the first time. Substitutions at C(6) of the benzoxaborole core gave favorable activity, and their structure−activity relationship was further studied. Enzyme inhibitors with IC_{50} as low as 1.6 μM were discovered, and these enzyme inhibitors were demonstrated to inhibit *T. brucei* parasite growth. This work suggests that LeuRS may serve as a potential target in the exploration of new antitrypanosomal agents with new mechanism of action.

CHEMISTRY

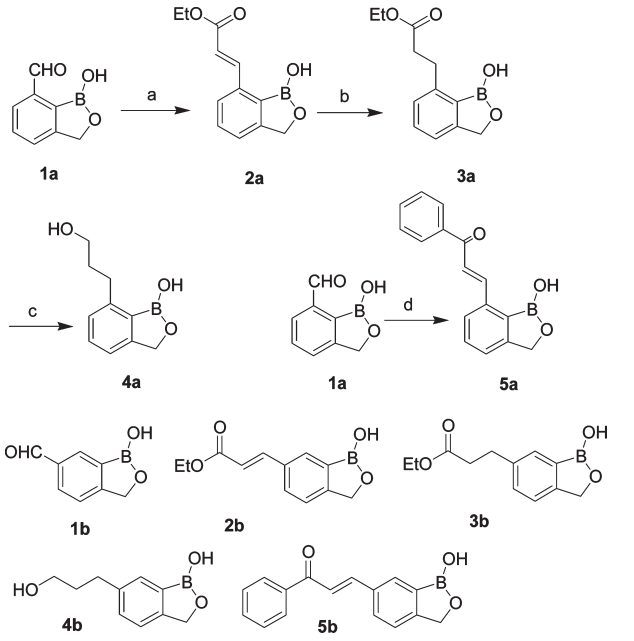
Compounds discussed in this study belong to two categories, those derived from formyl benzoxaboroles and those derived from hydroxyl benzoxaboroles.

The 7-formylbenzoxaborole and 6-formylbenzoxaborole **1a** and **1b** were prepared from the corresponding dimethylbromobenzenes according to the procedure we reported previously.[13] Wittig reaction on 7-formylbenzoxaborole **1a** gave α,β-unsaturated ester **2a**, which was subsequently reduced under catalytic hydrogenation to give ester **3a**. Compound **4a** was prepared from **3a** after reduction with DIBAL. Chalcone compound **5a** was prepared from aldehyde **1a** by aldol condensation. Compounds with 6-substitutions, **2b**, **3b**, **4b**, and **5b**, were synthesized from 6-formylbenzoxaborole **1b** following similar procedures described above (Scheme 1).

The 6-hydroxybenzoxaborole intermediate **11** was prepared from 2-bromo-4-fluorobenzaldehyde **6**.[14] The formyl group was protected with ethylene glycol, which was necessary for achieving good yield in the following step of neucleophilic replacement of fluorine by benzyl alcohol to result in compound **8**. Metal−halogen exchange using butyllithium followed by in situ trapping with triisopropylborate resulted in the boronylation of compound **8**; subsequent acidification gave boronic acid **9**. Compound **9** was reduced with $NaBH_4$ to give benzoxaborole **10** which underwent hydrogenation in the presence of Pd/C to give compound **11** (Scheme 2).

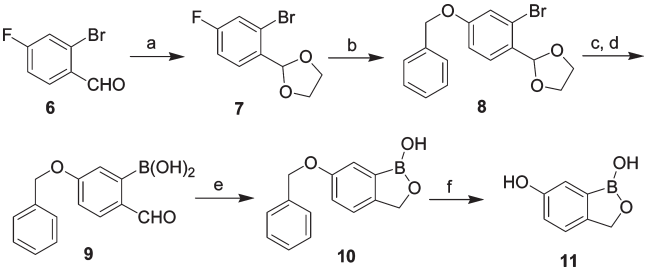
Compound **11** was treated with NaH at 0 °C and then coupled with the corresponding benzyl bromides, alkyl bromides, or α-bromoacetates to give derivatives as exemplified by benzyl ether **12**, alkyl ether **21**, and α-alkoxyacetate **26**. Diaryl ethers such as compound **13** were prepared from phenol **11** using NaH as the base under elevated temperature (70 °C). Treatment of phenol **11** with α-bromoacetamides under the condition of K_2CO_3 with NaI as catalyst gave α-bromoacetamides as shown by compound **35** as an example. Carbamates such as compound **27** were prepared from phenol **11** by coupling with corresponding isocyanates in the presence of Et_3N (Scheme 3).

Scheme 1. Synthesis of 7- and 6-Substituted Benzoxaboroles[a]



[a] Reagents and conditions: (a) $Ph_3PCH_2COOCH_2CH_3Br$, NaH, THF, 0 °C to room temp; (b) $PtO_2 \cdot 3H_2O$, H_2, room temp; (c) DIBAL, THF, 0 °C to room temp; (d) acetophenone, NaOH, $EtOH/H_2O$, room temp.

Scheme 2. Synthesis of 6-OH Benzoxaborole[a]

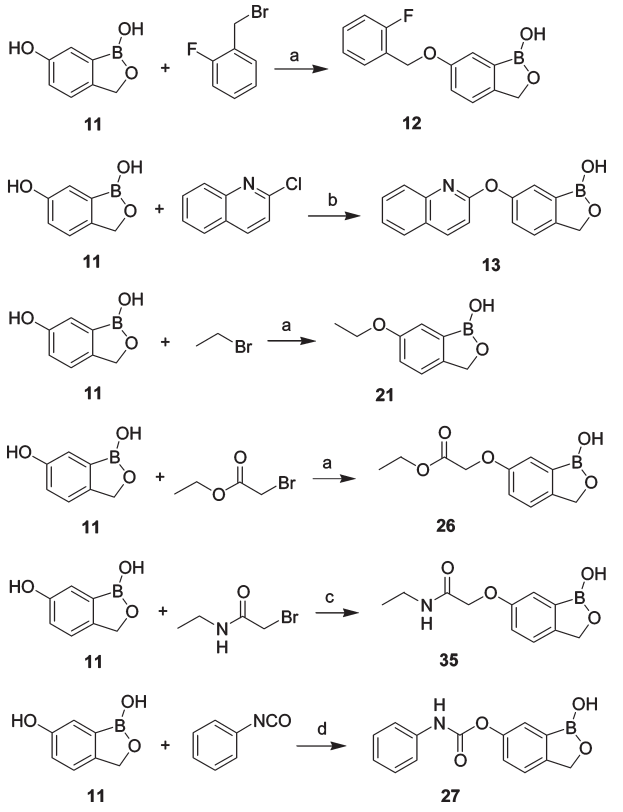


[a] Reagents and conditions: (a) ethylene glycol, *p*-TsOH, toluene, reflux; (b) BnOH, NaH, DMF, 0 to 65 °C; (c) *n*-BuLi, $B(iPrO)_3$, −78 °C to room temp; (d) HCl; (e) $NaBH_4$, THF, 0 °C; (f) H_2, Pd/C, CH_3OH, room temp.

RESULTS AND DISCUSSION

We first cloned and expressed the full length *T. brucei* LeuRS using pET21a vector in *E. coli* strain BL21(DE3)-RIPL. Attempts to obtain an X-ray crystal structure of *T. brucei* LeuRS have been

Scheme 3. Synthesis of Representative Derivatives from 6-OH Benzoxaborole[a]



[a] Reagents and conditions: (a) NaH, DMF, 0 °C; (b) NaH, DMF, 0 to 70 °C, 36 h; (c) K_2CO_3, DMSO, NaI (cat.), 90 °C, 36 h; (d) Et_3N, DMF, 0 °C to room temp, 48 h.

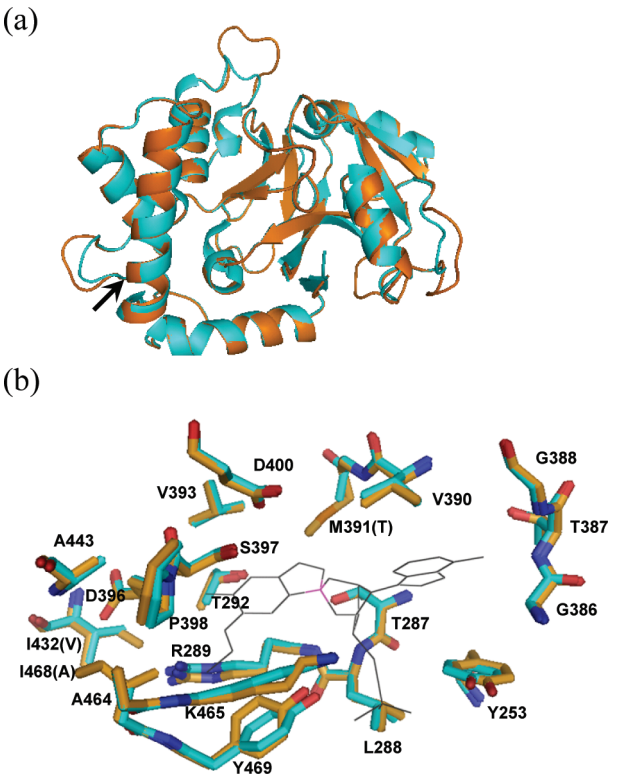


Figure 2. (a) Homology model of *T. brucei* LeuRS CP1 domain (orange) based on *C. albicans* LeuRS structure (cyan) (PDB code 2WFG). The arrow points to the inserted eukaryote specific helix. (b) Amino acid residues that line the editing pocket. TbLeuRS is in orange, and residues are all labeled. CaLeuRS is in cyan. Identical residues are not labeled, and different residues are indicated in parentheses. Ligand from 2WFG is represented by a gray line.

unsuccessful, so a homology model of *T. brucei* LeuRS editing domain (CP1) was constructed using *Candida albicans* LeuRS (CaLeuRS) CP1 domain (PDB code 2WFG) as the template (Figure 2a).[15] They shared a sequence identity of 36% (249 aa, rmsd of 0.24 Å on C(α)), and both possess very similar core structures consisting of seven β-sheets and three α-helices. Similar to CaLeuRS, TbLeuRS has an eukaryote specific I4ae helix[15] that partially blocks the entrance of the editing pocket by the two residues: Lys465 and Tyr469 (Figure 2b), which results in a half-closed binding pocket compared to the open pocket in prokaryote LeuRS such as *E. coli* LeuRS. The conserved active site features found in TbLeuRS include the "threonine-rich region" (T287 and T292), the "GTG region" (G386, T387, G388), and a strictly conserved catalytic aspartic acid (D400) (Figure 2b). Overall, the TbLeuRS editing site is very similar to CaLeuRS, but a limited number of residue variations also exist (Figure 2b). Among the 29 amino acid residues that line the editing pocket, variations were seen at eight residues.

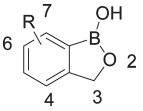
The benzoxaborole-AMP adduct, which does not include the whole tRNA, was docked into the editing pocket of *T. brucei* LeuRS by taking a similar binding pose as the benzoxaborole-AMP complexed in CaLeuRS.[15] Inspection of this pose suggested that substituents at C(7) might clash with the eukaryote helix, whereas substituents at C(6) vectoring toward a well-defined pocket could provide improvements in affinity. These predictions were explored via the compounds depicted in Table 1. The 7-substituted benzoxaboroles including ester, alcohol, and chalcone structures showed minimal inhibitory activity, while 6-substituted analogues showed improved activity. Compound **3b** with an ethyl propionate substitution from C(6) was the most active with an IC_{50} of 16.7 μM, which was slightly lower than that of the parent compound **1c** (IC_{50} = 22.1 μM). Similar to C(7), the model showed minimal space near C(5) and C(3), so we did not explore these two positions either. Thus, we next focused on the exploration of the effect of a variety of 6-substitutions on enzyme inhibition activity.

Encouraged by the inhibitory activity of compound **3b**, we synthesized its close analogue, ester **26**, which showed a 4-fold improvement of activity with IC_{50} of 3.5 μM (Table 2). The docking model of compound **26** (Figure 3) showed the formation of a hydrogen bond between its carbonyl and Arg289, with a predicted O−N distance of 2.738 Å. The pocket is rather small and hydrophobic, lined by nonpolar amino acid residues including Pro398, Ala443, Ile468, and Ala464 which showed favorable interactions with the terminal ethyl group of compound **26** (Figure 3). The carboxylic acid **18** showed completely diminished activity, suggesting the necessity of a nonpolar terminal group. The aldehyde **19** and its acetal **17** also showed diminished activity.

In order to probe the binding properties of the above-mentioned binding pocket, we designed and synthesized a variety of 6-O substituted compounds as shown in Table 2.

The parent phenol **11** showed IC_{50} of 30.9 μM. Benzyloxy compounds **10** and **12** showed comparable activity with IC_{50}

Table 1. Exploration of the Effect of 6- versus 7-Substitutions on *T. brucei* LeuRS Inhibition

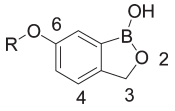


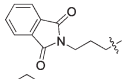
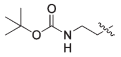
Compd	R	6- or 7- Substitution	LeuRS IC_{50} (μM)
2a		7-	>1000
3a		7-	>1000
4a		7-	541
5a		7-	412
2b		6-	578
3b		6-	16.7
4b		6-	688
5b		6-	209
1c	H		22.1

values of 30.6 and 25.6 μM. Quinolinyl compound **13** gave lower activity (IC_{50} = 63.0 μM). Pyridyl compound **14**, phthalimide **15**, and cyclohexyl compound **16** completely lost activity. Compound **20** with a relatively long chain and a bulky terminal *tert*-butyl group completely lost activity as well. On the other hand, the shorter alkyl substitutions in compounds **21**−**24** gave IC_{50} values ranging from 13.0 to 54.3 μM, with the butyl (**24**) having the weakest activity. Carbamates such as **27** and **28** were also explored, and their activity is similar to the activity of benzyl compounds **10** and **12**. These observations suggest that the binding pocket may have a limited space, so bulky or rigid structures result in steric clash and diminished affinity while relatively adaptable chain structures are preferred, which is not surprising considering the rather small and half closed editing pocket of *T. brucei* LeuRS. We previously reported[14] a series of C(6)-L-phenyl substituted benzoxaboroles including benzyl ether **10** and carbamate **27**. These compounds showed poor inhibitory activity against TbLeuRS, which corroborates with the above observation of the binding pocket. Their antitrypanosomal activity is due to unknown cellular targets, which is currently under investigation.

Led by the promising activity of ester **26**, we further explored the effect of different ester groups (Table 3). Methyl ester **29** has comparable activity as ethyl ester **26**, but *tert*-butyl ester **30** has a 2-fold reduced activity. Further analysis of the docking model of compound **26** (Figure 3) revealed the existence of space near the α-carbon of the ester. So α-substituted esters **31**−**34** were synthesized with the aim to improve activity. It was found that methyl-substituted compound **31** showed improved activity (IC_{50} = 2.8 μM) and ethyl compound **32** showed further

Table 2. Exploration of the Effect of Different 6-O Substitutions on *T. brucei* LeuRS Inhibition



Compd	R	LeuRS IC_{50} (μM)
11	H	30.9
10		30.6
12		25.6
13		63.0
14		>100
15		>100
16		>100
17		71.3
18		>100
19		>100
20		>100
21		13.0
22		30.9
23		15.8
24		54.3
25		>100
26		3.5
27		43.1
28		32.3

improved activity (IC_{50} = 1.6 μM). Dimethyl substitution also gave improved activity (**33**, IC_{50} = 2.1 μM), while a larger phenyl substituent gave much reduced activity (**34**, IC_{50} = 41.7 μM), indicating the space around α-C is limited as shown by the docking model (Figure 3).

We further explored the effect of replacing the ester bond with the more stable amide bond. The amide analogue of ester **26**, compound **35**, showed 22-fold reduced activity. At the same time, *tert*-butylamide **36** has completely reduced activity

dx.doi.org/10.1021/jm101225g |*J. Med. Chem.* 2011, 54, 1276–1287

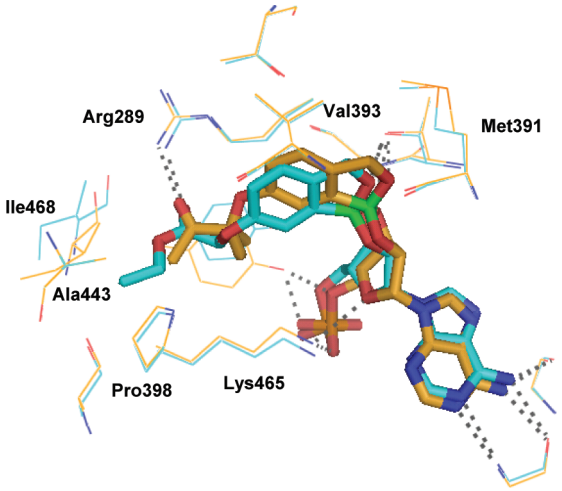


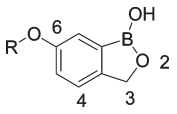
Figure 3. Binding of ester **26** (ligand in cyan sticks and binding site residues in cyan lines) and α-substituted ketone **42** (ligand in orange sticks and binding site residues in orange lines) in the editing pocket of TbLeuRS. Compounds **26** and **42** are shown as covalent adducts with the terminal AMP of tRNA. The boron atom is shown in green for clarity.

compared to its ester analogue **30**. It is not clear why amide bond gave much reduced activity, and investigation of the docking model did not explain the phenomenon.

As an effort to improve the stability of the lead ester compounds while retaining their activity, corresponding ketones were designed and synthesized (Table 3). First, ketones **37** and **38**, which are analogues of ethyl ester **26** and methyl ester **29**, were found to have 9-fold and 8-fold reduced activity. Next, α-substituents were installed in compounds **39**−**45** to capture additional hydrophobic interactions. Addition of methyl or ethyl substituents resulted in significant enhancement of activity, as demonstrated by compounds **39**−**41** (IC_{50} = 2.5, 2.9, 3.8 μM). Thus, the α-substituted ketones successfully achieved activity similar to that of the most potent ester compounds. To further explore the effect of different alkyl groups at α-C, acetone compounds **41**−**45** which vary in the size of α-substituents were synthesized. It was found that methyl (**41**, IC_{50} = 3.8 μM) and ethyl (**43**, IC_{50} = 3.5 μM) gave the best activity while dimethyl (**42**, IC_{50} = 5.0 μM) and isopropyl (**45**, IC_{50} = 4.5 μM) gave slightly reduced activity. When the length of the alkyl chain further increases, compound **44** with *n*-propyl group showed an almost 10-fold decrease of activity (IC_{50} = 31.4 μM). This observation corroborates with the dimension of space around α-C in the docking model (Figure 3). Enlargement of the terminal alkyl chain (**46**, IC_{50} = 67.6 μM) also resulted in further diminished activity compared to compound **37**. Finally, it is worth mentioning that conversion of the ketone (**39**) carbonyl to hydroxyl (**47**) led to complete loss of activity. At the same time, removal of the carbonyl resulted in complete loss of activity as shown by comparison of methyl ether **25** (IC_{50} > 100 μM) to methyl ester **29** (IC_{50} = 3.6 μM). These observations suggested the importance and steric/geometry constraint of a hydrogen bond between Arg289 and the carbonyl, as depicted in the docking model of compounds **26** and **42** in the enzyme pocket (Figure 3).

Next, we tested if these TbLeuRS inhibitors could effectively inhibit the parasite growth in vitro and if they are toxic against mammalian cells. These inhibitors generally showed good potency against bloodstream form of *T. brucei* parasites. For

Table 3. Effect of Different Esters, Amides, and Ketones on *T. brucei* LeuRS Inhibition



Compd	R	LeuRS IC_{50} (μM)
29		3.6
30		7.4
31		2.8
32		1.6
33		2.1
34		41.7
35		77.7
36		>100
37		30.8
38		27.2
39		2.5
40		2.9
41		3.8
42		5.0
43		3.5
44		31.4
45		4.5
46		67.6
47		>100

compounds with enzyme inhibitory IC_{50} below 10 μM, parasite growth inhibition IC_{50} values in the range 0.37−12.93 μM were observed (Table 4). The α,α-dimethyl ketone **42** showed the highest potency (IC_{50} = 0.37 μM), while its methyl (**41**, IC_{50} = 2.09 μM), ethyl (**43**, IC_{50} = 3.76 μM), and isopropyl (**45**, IC_{50} = 2.74 μM) analogues showed near 10-fold decrease in potency, although these α-substituted ketones have similar enzyme inhibitory activity. On the other hand, α-substituted ethyl esters **31**, **32**, and **33**, which have improved enzyme inhibitory activity compared to the unsubstituted ester **26**, were found to show near

dx.doi.org/10.1021/jm101225g |*J. Med. Chem.* 2011, 54, 1276–1287

Table 4. *T. brucei* Parasite Growth Inhibition by the *T. brucei* LeuRS Inhibitors[a]

compd	*T. brucei* IC_{50} (μM)	L929 (μg/mL)
26	0.72	>10
29	1.22	>10
30	2.61	>10
31	3.00	>10
32	11.32	>10
33	5.49	>10
39	8.25	>10
40	12.93	>10
41	2.09	>10
42	0.37	>10
43	3.76	>10
45	2.74	>10
suram[b]	0.005	
pent[c]	0.026	

[a] L929: IC_{50} against L929 mouse lung fibroblast cells. μg/mL is used as the measurement unit, and >10 μg/mL approximately converts to >40 μM. [b] Suramin. [c] Pentamidine.

10-fold decreased antiparasitic potency (IC_{50} = 3.00, 11.32, 5.49 μM) compared with **26** (IC_{50} = 0.72 μM). The indiscrepancy between enzymatic and cellular assays is often observed in medicinal chemistry research, which is generally caused by membrane permeability, serum binding properties, and off-target interactions of the compounds. Further detailed study of these properties would help provide better understanding in this regard. It is also worth pointing out that a number of compounds showed better cellular IC_{50} values than enzymatic values. This could be caused by the nature of the inhibition mechanism. Benzoxaborole LeuRS inhibitors are irreversible (half-life of 424 min for **1**) slow-tight-binding inhibitors due to the covalent locking of tRNA.[9,15] So enzyme inhibition is highly dependent on the enzyme–inhibitor preincubation time[9,15] (17-fold decrease of K_i when preincubation time increased from 2 to 20 min for **1**),[9] which renders the inhibitory IC_{50} a relative measurement that can only be used to rank compounds under a designated assay condition. Caution should be taken when it is being related to cellular IC_{50} values, where lengthened incubation time (72 h) is involved. This represents a different scenario from that of the synthetic site-targeting inhibitors. Lastly, the compounds showed excellent toxicity profile against mammalian cells with IC_{50} against L929 cells (mouse lung fibroblast cells) above 10 μg/mL (approximately above 40 μM). Taken together, these observations suggest that TbLeuRS may serve as a promising target for the development of chemotherapies against trypanosomiasis.

In summary, the potential of using TbLeuRS as a target for the development of antitrypanosomal agents was explored. LeuRS enzyme from *T. brucei* was cloned and expressed, and its homology model was built based on *C. albicans* LeuRS structure. Subsequent structure-guided design and synthesis of 6-OH benzoxaborole-derived inhibitors with different size or polarity of substituent groups were carried out. Alkyl esters, identified as the first leads, were further modified to amide and ketone analogues with the aim to improve the in vivo stability. While amides showed reduced activity, ketones proved to be equipotent compounds. The most potent TbLeuRS inhibitor we discovered showed IC_{50} = 1.6 μM. They also inhibited *T. brucei* parasite growth in vitro with IC_{50} as low as 0.37 μM. It is the first time that effective protozoal aaRS inhibitors and their structure–activity relationship were reported.

EXPERIMENTAL SECTION

NMR spectra were recorded on MercuryPlus 400 (Varian), Mercury 300 (Varian), or AVANCE 400 (Bruker) spectrometers. Chemical shifts are expressed in parts per million (δ) relative to residual solvent as an internal reference. High resolution mass spectra were obtained on a Micromass GCT (electron ionization) or an Agilent 6530 Accurate Mass Q-TOF LC–MS (electrospray ionization). High performance liquid chromatography analysis was performed on a Agilent 1200 with a flow rate of 1 mL/min and a gradient of 90% H_2O/10% MeCN to 100% MeCN in 15, 20, or 25 min using a DAD detector. An Agilent Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm) was used. Purity was based on the integrated UV chromatogram (220 and 254 nm). All compounds have purity of >95%. Column chromatography was performed using Huanghai silica gel (300–400 mesh). Melting points were measured on a SGW X-4 melting point apparatus. Radioactivity was measured using a scintillation counter (Beckman LS 6500). Brewer's yeast tRNA was purchased from Roche.

(*E*)-[3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-7-yl)]acrylic Acid Ethyl Ester (2a). To a solution of aldehyde **1a** (400 mg, 2.47 mmol) and (ethyloxycarbonylmethyl)triphenylphosphonium bromide (1.06 g, 2.47 mmol) in THF (25 mL) was added NaH (60% in mineral oil, 99 mg, 2.47 mmol) at −5 °C. The reaction mixture was stirred at room temperature for 12 h before another portion of NaH (50 mg, 1.24 mmol) was added at 0 °C and stirred at room temperature for 8 h. The reaction was quenched with water, acidified to pH 2–3, extracted with EtOAc, and dried over Na_2SO_4. The residue after rotary evaporation was purified by column chromatography and recrystallization (hexane/EtOAc) to obtain 200 mg of compound **2a** (34.9%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.33 (s, 1H), 8.10 (d, 1H, J = 16.2 Hz), 7.82 (d, 1H, J = 10 Hz), 7.52 (t, 1H, J = 7.5 Hz), 7.44 (m, 1H), 6.81 (d, 1H, J = 16.2 Hz), 5.02 (s, 2H), 4.19 (q, 2H, J = 7.1 Hz), and 1.26 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 168.8, 155.9, 144.9, 138.9, 132.3, 125.9, 123.7, 120.1, 71.9, 61.5, 14.6. HRMS-EI: $[M]^+$ $C_{12}H_{13}BO_4$ calcd 232.0907, found 232.0909. Mp: 151–152 °C. HPLC: purity 98.9% (220 nm, 10.7 min/15 min).

[3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-7-yl)]propionic Acid Ethyl Ester (3a). To a solution of compound **2a** (1.20 g, 5.17 mmol) in methanol (10 mL) was added $PtO_2 \cdot 3H_2O$ (73 mg, 0.26 mmol), and the reaction mixture was vacuumed and filled with hydrogen. After stirring overnight at room temperature, the reaction mixture was filtered and evaporated in vacuo to obtain 1.16 g of compound **3a** (96.0%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.34 (t,1H, J = 7.6 Hz), 7.18 (d, 1H, J = 7.6 Hz), 7.11 (d, 1H, J = 7.2 Hz), 5.02 (s, 2H), 4.06 (q, 2H, J = 7.2 Hz), 3.05 (t, 2H, J = 7.6 Hz), 2.61 (t, 2H, J = 7.6 Hz), and 1.19 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 174.9, 155.7, 146.1, 132.2, 128.2, 120.1, 72.1, 61.4, 37.1, 30.9, 14.5. HRMS-EI: $[M]^+$ $C_{12}H_{15}BO_4$ calcd 234.1063, found 234.1060. Mp: 93–95 °C. HPLC: purity 98.7% (220 nm, 10.2 min/15 min).

7-(3-Hydroxypropyl)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (4a). To a solution of compound **3a** (500 mg, 2.15 mmol) in THF (20 mL) was added dropwise DIBAL (1.0 M in hexane, 12.9 mL, 12.9 mmol) at 0 °C. The reaction mixture was stirred overnight at room temperature and quenched with 1 M HCl at 0 °C. The mixture was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. After rotary evaporation, the residue was purified by column chromatography to obtain 270 mg of compound **4a** (65.5%). ^{1}H NMR (400 MHz, CD_3OD, Na added): δ 7.03 (m, 1H), 6.91 (m, 1H), 6.83 (m, 1H), 4.81 (s, 2H), 3.48 (m, 2H), 2.77 (m, 2H), and 1.84 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 155.5, 147.9, 132.1, 128.1, 119.6, 72.1, 62.5, 35.9, 31.8. HRMS-ESI: $[M-H]^-$ $C_{10}H_{12}BO_3$ calcd 191.0879, found 191.0882. Mp: 91–93 °C. HPLC: purity 99.1% (220 nm, 10.5 min/20 min).

(*E*)-[3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-7-yl)-1-phenyl]propenone (5a). To a mixture of acetophenone (0.22 mL, 1.85 mmol), ethanol (5 mL), and water (8 mL) was added NaOH (296 mg, 7.41 mmol). After the mixture was stirred for for 5 min, aldehyde **1a** (300 mg, 1.85 mmol) was added. The reaction mixture was stirred at room temperature overnight before the pH was adjusted to ~2 with 1 M HCl. The mixture was evaporated, extracted with EtOAc, and dried over anhydrous Na_2SO_4. The residue after rotary evaporation was purified by column chromatography and recrystallization (hexane/EtOAc) to obtain 240 mg of compound **5a** (49.1%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.39 (s, 1H), 8.14 (m, 5H), 7.68 (t, 1H, J = 7.4 Hz), 7.58 (t, 3H, J = 8 Hz), 7.48 (m, 1H) and 5.05 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 192.8, 156.2, 145.3, 139.6, 139.4, 134.2, 132.4, 129.7, 127.4, 124.8, 123.9, 71.9. HRMS-EI: $[M]^+$ $C_{16}H_{13}BO_3$ calcd 264.0958, found 264.0964. Mp: 136−137 °C. HPLC: purity 97.1% (220 nm, 10.7 min/15 min).

(*E*)-[3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yl)]acrylic Acid Ethyl Ester (2b). Compound **2b** (153 mg, 62.5%) was prepared following a similar procedure to compound **2a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.26 (s, 1H), 7.96 (s, 1H), 7.85 (dd, 1H, J = 8.1 and 1.5 Hz), 7.70 (d, 1H, J = 16.2 Hz), 7.46 (d, 1H, J = 7.8 Hz), 6.59 (d, 1H, J = 16.2 Hz), 5.02 (s, 2H), 4.19 (q, 2H, J = 7.2 Hz), and 1.26 (t, 3H, J = 7.2 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 155.6, 144.6, 133.4, 130.4, 130.1, 121.5, 117.9, 71.2, 60.5, 14.2. HRMS-ESI: $[M + Na]^+$ $C_{12}H_{13}BO_4Na$ calcd 255.0805, found 255.0848. Mp: 129−131 °C. HPLC: purity 99.6% (254 nm, 15.4 min/20 min).

[3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yl)]propionic Acid Ethyl Ester (3b). Compound **3b** (89 mg, 90.0%) was prepared following a similar procedure to compound **3a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.10 (s, 1H), 7.55 (s, 1H), 7.32 (m, 2H), 4.94 (s, 2H), 4.03 (q, 2H, J = 7.2 Hz), 2.89 (t, 2H, J = 7.5 Hz), 2.61 (t, 2H, J = 7.5 Hz), and 1.14 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 151.7, 139.5, 131.4, 130.1, 121.1, 71.0, 60.5, 36.1, 30.8, 14.2. HRMS-ESI: $[M + Na]^+$ $C_{12}H_{15}BO_4Na$ calcd 257.0961, found 257.0973. Mp: 76−78 °C. HPLC: purity 95.0% (220 nm, 9.3 min/15 min).

6-(3-Hydroxypropyl)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (4b). Compound **4b** (53.6 mg, 65.0%) was prepared following a similar procedure to compound **4a**. ^{1}H NMR (400 MHz, CD_3OD): δ 7.49 (s, 1H), 7.31 (m, 2H), 5.04 (s, 2H), 3.56 (t, 2H, J = 8.8 Hz), 2.72 (t, 2H, J = 9.6 Hz), and 1.83 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 153.0, 142.2, 132.5, 131.0, 122.1, 72.2, 62.2, 35.8, 32.9. HRMS-ESI: $[M + Na]^+$ $C_{10}H_{13}BO_3Na$ calcd 193.1036, found 193.1058. Mp: 214−218 °C. HPLC: purity 97.2% (220 nm, 6.9 min/20 min).

(*E*)-[3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yl)-1-phenyl]propenone (5b). Compound **5b** (105 mg, 32.3%) was prepared following a similar procedure to compound **5a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (m, 3H), 7.92 (d, 1H, J = 15.9 Hz), 7.77 (d, 1H, J = 7.8 Hz), 7.56 (m, 4H), 7.41 (d, 1H, J = 8.4 Hz), 5.42 (s, 1H), and 5.15 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.9, 155.9, 144.9, 137.8, 133.7, 132.8, 131.1, 130.1, 128.5, 128.3, 121.6, 121.5, 71.2. HRMS-ESI: $[M + Na]^+$ $C_{16}H_{13}BO_3Na$ calcd 287.0856, found 287.0886. Mp: 146−148 °C. HPLC: purity 95.4% (220 nm, 10.6 min/15 min).

6-Benzyloxy-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (10). To a solution of compound **6** (50 g, 246 mmol) in toluene (500 mL) were added ethylene glycol (74.8 g, 1207 mmol) and *p*-toluenesulfonic acid monohydrate (2.5 g, 13 mmol). After heating to reflux and being stirred for 4 h, the mixture was washed with saturated $NaHCO_3$, water, and brine and dried over Na_2SO_4. The residue after rotary evaporation was purified by flash column over silica gel to give compound **7** (53.6 g, 87.8%) as a viscous oil.

Compound **7** (50.5 g, 204 mmol) was dissolved in DMF (400 mL) and cooled to 0 °C. To this solution under nitrogen were added in sequence benzyl alcohol (24.2 g, 224 mmol) and NaH (60% in mineral oil, 16.2 g, 408 mmol) in portions. The reaction mixture was stirred for 4 h at 65 °C and then treated with cold water (500 mL). After extraction with EtOAc, the organic layer was washed with water and brine and dried over Na_2SO_4. The residue after rotary evaporation was purified by flash column over silica gel to give compound **8** (43.3 g, 64.0%).

Compound **8** (20 g, 59.7 mmol) and B(*i*-PrO)$_3$ (15.7 mL, 68.6 mmol) were dissolved in anhydrous THF (400 mL) and cooled to −80 °C under nitrogen. To this solution was added dropwise 1.6 M *n*-BuLi (42.87 mL, 68.6 mmol) over 1 h. The mixture was allowed to warm to room temperature gradually and stirred overnight. After 6 M HCl (100 mL) was added and the mixture was stirred for 2 h, the mixture was evaporated and extracted with EtOAc and dried over Na_2SO_4. The residue after rotary evaporation was purified by crystallization to give compound **9** (13.4 g, 88.1%).

Compound **9** (23 g, 89.8 mmol) was dissolved in THF (300 mL) and cooled to 0 °C. To this solution was added $NaBH_4$ (4.3 g, 114 mmol) in portions. The reaction mixture was stirred for 1 h and then treated with 1 M HCl. After evaporation, the residue was extracted with EtOAc and the organic layer was washed with water and brine and dried over Na_2SO_4. The residue after rotary evaporation was purified by flash column over silica gel to give compound **10** (21 g, 97.0%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.15 (s, 1H), 7.48−7.28 (m, 7H), 7.13 (dd, 1H, J = 8.4 and 2.4 Hz), 5.12 (s, 2H), and 4.91 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.5, 146.4, 137.2, 128.8, 128.1, 127.7, 122.2, 119.8, 114.6, 71.3, 70.5. HRMS-EI: $[M]^+$ $C_{14}H_{13}BO_3$ calcd 240.0958, found 240.0963. Mp: 118−119 °C. HPLC: purity 97.9% (254 nm, 10.1 min/15 min).

6-Hydroxyl-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (11). Compound **10** (21 g, 87.5 mmol) was dissolved in MeOH (250 mL). To this solution under nitrogen was added 10% Pd/C (1.5 g). The reaction mixture was vacuumed and backfilled with hydrogen 3 times, then stirred overnight at room temperature. After filtration and rotary evaporation, the residue was purified by recrystallization (hexane/EtOAc) to give compound **11** (12.7 g, 97.0%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.30 (s, 1H), 9.04 (s, 1H), 7.18 (d, 1H, J = 8.0 Hz), 7.09 (d, 1H, J = 2.0 Hz), 6.87 (dd, 1H, J = 8.1 and 2.4 Hz), and 4.86 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 208.0, 158.3, 147.1, 123.9, 120.5, 117.5, 71.9. HRMS-EI: $[M]^+$ $C_7H_7BO_3$ calcd 150.0488, found 150.0483. Mp: 133−135 °C. HPLC: purity 95.7% (254 nm, 9.4 min/20 min).

6-(2-Fluorobenzyloxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (12). Compound **11** (150 mg, 1.0 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C. To this solution under nitrogen were added in sequence NaH (60% in mineral oil, 160 mg, 4.0 mmol) and 1-(chloromethyl)-2-fluorobenzene (0.485 mL, 4.0 mmol). The reaction mixture was stirred for 2 h and then treated with 1 M HCl (10 mL). After extraction with EtOAc, the organic layer was washed with water and brine and dried over Na_2SO_4. The residue after rotary evaporation was purified by column chromatography over silica gel to give compound **12** (143.6 mg, 55.7%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.13 (s, 1H), 7.58−7.12 (m, 7H), 5.16 (s, 2H) and 4.92 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ157.9, 146.3, 129.6, 129.5, 124.1, 124.0, 122.0, 119.4, 115.3, 115.1, 114.2, 70.9, 63.9. HRMS-EI: $[M]^+$ $C_{14}H_{12}BFO_3$ calcd 258.0864, found 258.0865. Mp: 129−131 °C. HPLC: purity 98.7% (254 nm, 16.3 min/20 min).

6-Quinolin-2-yloxy-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (13). Compound **11** (100 mg, 0.667 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C. To this solution under nitrogen were added in sequence NaH (60% in mineral oil, 80 mg, 2.00 mmol) and 2-chloroquinoline (119 mg, 0.734 mmol). The reaction mixture was stirred for 36 h at 70 °C and then treated with 1 M HCl (10 mL). After extraction with EtOAc the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The residue after rotary evaporation was purified by column chromatography over silica gel to give

compound **13** (24.8 mg, 13.5%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (s, 1H), 8.42 (d, 1H, J = 9 Hz), 7.95 (d, 1H, J = 8.4 Hz), 7.65−7.27 (m, 7H), and 5.04 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.7, 163.1, 149.7, 146.0, 140.0, 129.8, 127.2, 125.5, 124.8, 124.3, 122.3, 122.2, 112.3, 70.9. HRMS-EI: $[M]^+$ $C_{16}H_{12}BNO_3$ calcd 277.0910, found 277.0907. Mp: 173−175 °C. HPLC: purity 95.0% (254 nm, 9.9 min/15 min).

6-(Pyridin-2-ylmethoxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (14). Compound **14** was prepared following a similar procedure to compound **12**. Yield: 50.0%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.14 (s, 1H), 8.59−8.56 (m, 2H), 7.46−7.42 (m, 2H), 7.33 (d, 1H, J = 8.4 Hz), 7.29 (d, 1H, J = 2.4 Hz), 7.15 (dd, 1H, J = 8.4 and 2.4 Hz), 5.21 (s, 2H), and 4.92 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 150.0, 149.8, 148.0, 123.4, 123.3, 123.2, 120.2, 115.6, 115.5, 72.0, 69.2. HRMS: $[M]^+$ $C_{13}H_{12}BNO_3$ calcd 241.0910, found 241.0911. Mp: 167−170 °C. HPLC: purity 95.9% (220 nm, 6.1 min/15 min).

2-[3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxyl)-propyl]isoindole-1,3-dione (15). Compound **15** was prepared following a similar procedure to compound **12**. Yield: 53.6%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.01 (s, 1H), 7.84 (d, 4H, J = 2.7 Hz), 7.24 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 2.4 Hz, 1H), 6.87 (dd, 1H, J = 8.4 and 2.4 Hz), 4.89 (s, 2H), 4.01 (t, 2H, J = 6.2 Hz), 3.77 (t, 2H, J = 6.5 Hz), and 2.12−2.02 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 158.0, 145.9, 133.9, 131.9, 123.2, 121.8, 119.2, 113.6, 70.9, 65.7, 35.4, 28.1. HRMS-EI: $[M]^+$ $C_{18}H_{16}BNO_5$ calcd 337.1121, found 337.1122. Mp: 159−162 °C. HPLC: purity 96.4% (220 nm, 17.4 min/20 min).

6-Cyclohexylmethoxy-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (16). Compound **16** was prepared following a similar procedure to compound **12**. Yield: 66.0%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 7.29 (d, 1H, J = 8.4 Hz), 7.23 (d, 1H, J = 2.4 Hz), 7.03 (dd, 1H, J = 8.4 and 2.7 Hz), 4.90 (m, 2H), 3.78 (d, 2H, J = 6.3 Hz), and 1.83−1.01 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.7, 145.6, 121.9, 119.4, 113.8, 73.8, 71.0, 37.6, 29.8, 26.5, 25.7. HRMS-EI: $[M]^+$ $C_{14}H_{19}BO_3$ calcd 246.1427, found 246.1433. Mp: 126−127 °C. HPLC: purity 99.7% (220 nm, 14.1 min/20 min).

6-(2,2-Dimethoxyethoxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (17). Compound **17** was prepared following a similar procedure to compound **12**. Yield: 51.0%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.11 (s, 1H), 7.31 (d, 1H, J = 8.4 Hz), 7.25 (d, 1H, J = 2.4 Hz), 7.08 (dd, 1H, J = 8.4 and 2.4 Hz), 4.91 (s, 2H), 4.69 (t, 1H, J = 4.8 Hz), 3.98 (d, 2H, J = 5 Hz) and 3.35 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.0, 146.4, 122.1, 119.8, 114.2, 102.1, 70.9, 67.8, 54.1. HRMS-EI: $[M]^+$ $C_{11}H_{15}BO_5$ calcd 238.1013, found 238.1015. Mp: 102−104 °C. HPLC: purity 97.2% (220 nm, 10.9 min/15 min).

[2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]acetic Acid (18). To a solution of compound 26 (50 mg, 0.21 mmol) in THF (3 mL) was added lithium hydroxide monohydrate (16.8 mg, 0.4 mmol) in water (1 mL). The reaction mixture was stirred at room temperature for 40 h and then acidified with 1 M HCl (5 mL). The white solid was filtered and washed with water and dried to give compound **18** (36.6 mg, 88.0%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.99 (s, 1H), 9.15 (s, 1H), 7.32 (d, 1H, J = 8.1 Hz), 7.19 (d, 1H, J = 2.4 Hz), 7.05 (dd, 1H, J = 8.4 and 2.4 Hz), 4.92 (s, 2H), and 4.67 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 158.3, 147.8, 122.9, 119.5, 114.9, 70.7, 65.3. HRMS-EI: $[M]^+$ $C_9H_9BO_5$ calcd 208.0543, found 208.0544. Mp: 166−167 °C. HPLC: purity 97.8% (220 nm, 4.1 min/15 min).

[2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]acetaldehyde (19). To a solution of compound **17** (38 mg, 0.16 mmol) in acetone (2 mL) was added 6 M HCl (0.3 mL). The reaction mixture was stirred at 30 °C for 24 h. The residue after evaporation was purified by recrystallization (hexane/EtOAc) to give compound **19** (25.3 mg, 82.5%). ^{1}H NMR (400 MHz, acetone-d_6): δ 9.82 (s, 1H), 7.35 (d, 1H, J = 8.0 Hz), 7.23 (d, 1H, J = 2.4 Hz), 7.12 (dd, 1H, J = 8.1 and 2.4 Hz), 4.96 (s, 2H), and 4.79 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.7, 157.8, 147.5, 122.6, 118.9, 114.6, 73.1, 70.3. HRMS-EI: $[M]^+$ $C_9H_9BO_4$ calcd 192.0594, found 192.0595. Mp: 84−86 °C. HPLC: purity 96.8% (220 nm, 7.6 min/15 min).

***tert*-Butyl *N*-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yl)ethylcarbamate(20).** Compound **20** was prepared following a similar procedure to compound **12**. Yield: 26.0%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.13 (s, 1H), 7.30 (d, 1H, J = 8.4 Hz), 7.22 (d, 1H, J = 2.4 Hz), 7.04 (m, 2H), 4.91 (s, 2H), 3.95 (t, 2H, J = 6 Hz), 3.29 (q,2H, J = 5.6 and 10.4 Hz), and 1.38 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.1, 156.0, 146.5, 122.1, 119.4, 114.1, 79.7, 70.9, 67.4, 40.1, 28.4. HRMS-EI: $[M]^+$ $C_{14}H_{20}BNO_5$ calcd 293.1435, found 293.1442. Mp: 89−91 °C. HPLC: purity 97.3% (220 nm, 20.6 min/25 min).

6-Ethoxy-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (21). Compound **21** was prepared following a similar procedure to compound **12**. Yield: 65.0%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.13 (s, 1H), 7.29 (d, 1H, J = 8.1 Hz), 7.20 (d, 1H, J = 2.4 Hz), 7.03 (dd, 1H, J = 8.1 and 2.4 Hz), 4.91 (s, 2H), 4.02 (q, 2H, J = 7.0 Hz), and 1.33 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.5, 145.7, 122.0, 119.8, 113.9, 70.9, 63.7, 14.8. HRMS-EI: $[M]^+$ $C_9H_{11}BO_3$ calcd 178.0801, found 178.0802. Mp: 80−82 °C. HPLC: purity 95.8% (220 nm, 17.9 min/20 min).

6-Propoxy-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (22). Compound **22** was prepared following a similar procedure to compound **12**. Yield: 64.0%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 (s, 1H), 7.29 (d, 1H, J = 8.4 Hz), 7.23 (d, 1H, J = 2.4 Hz), 7.03 (dd, 1H, J = 8.1 and 2.4 Hz), 4.91 (s, 2H), 3.92 (t, 2H, J = 6.8 Hz), 1.78−1.68 (m, 2H), and 0.98 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.7, 145.6, 121.9, 119.7, 114.0, 70.9, 69.8, 22.6, 10.5. HRMS-EI: $[M]^+$ $C_{10}H_{13}BO_3$ calcd 192.0958, found 192.0959. Mp: 80−81 °C. HPLC: purity 99.2% (220 nm, 19.5 min/20 min).

6-Isopropoxy-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (23). Compound **23** was prepared following a similar procedure to compound **12**. Yield: 61.5%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.10 (s, 1H), 7.27 (d, 1H, J = 8.4 Hz), 7.23 (d, 1H, J = 2.4 Hz), 7.01 (dd, 1H, J = 8.1 and 2.4 Hz), 4.90 (s, 2H), 4.63−4.53 (m, 1H), and 1.27 (d, 6H, J = 6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.4, 145.6, 122.1, 121.1, 115.6, 70.9, 70.2, 22.0. HRMS-EI: $[M]^+$ $C_{10}H_{13}BO_3$ calcd 192.0958, found 192.0960. Mp: 62−65 °C. HPLC: purity 95.3% (220 nm, 14.4 min/15 min).

6-Butoxy-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (24). Compound **24** was prepared following a similar procedure to compound **12**. Yield: 22.0%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.09 (s, 1H), 7.29 (d, 1H, J = 8.4 Hz), 7.23 (d, 1H, J = 2.4 Hz), 7.03 (dd, 1H, J = 8.1 and 2.4 Hz), 4.91 (s, 2H), 3.90 (t, 2H, J = 6.6 Hz), 1.70 (m, 2H), 1.44(m, 2H), and 0.94 (t, 3H, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.7, 145.7, 122.0, 119.8, 113.9, 70.9, 67.9, 31.3, 19.2, 13.8. HRMS-EI: $[M]^+$ $C_{11}H_{15}BO_3$ calcd 206.1114, found 206.1115. Mp: 92−94 °C. HPLC: purity 96.3% (254 nm, 16.9 min/20 min).

6-(2-Methoxyethoxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (25). Compound **25** was prepared following a similar procedure to compound **12**. Yield: 43.3%. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.01 (s, 1H), 7.32 (d, 1H, J = 8.4 Hz), 7.26 (d, 1H, J = 2.4 Hz), 7.07 (dd, 1H, J = 8.4 and 2.4 Hz), 4.95 (s, 2H), 4.14 (t, 2H, J = 4.8 Hz), 3.71 (t, 2H, J = 4.8 Hz) and 3.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.1, 145.9, 121.9, 119.7, 113.9, 70.9, 70.8, 67.3, 59.0. HRMS-EI: $[M]^+$ $C_{10}H_{13}BO_4$ calcd 208.0907, found 208.0911. Mp: 68−70 °C. HPLC: purity 95.5% (220 nm, 13.3 min/20 min).

Ethyl [2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]acetate (26). It was prepared following a similar procedure to compound **12**. Yield: 61.0%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.14 (s, 1H), 7.32 (d, 1H, J = 8.1 Hz), 7.19 (d, 1H, J = 2.4 Hz), 7.06 (dd, 1H, J = 8.4 and 2.7 Hz), 4.92 (s, 2H), 4.77 (s, 2H), 4.17 (q, 2H, J = 7.2 Hz), and 1.21 (t, 3H, J = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.0, 157.3, 146.8, 122.2, 119.8, 114.0, 70.9, 65.5, 61.4, 14.1. HRMS-EI: $[M]^+$ $C_{11}H_{13}BO_5$ calcd 236.0856, found 236.0857. Mp: 96−97 °C. HPLC: purity 96.3% (254 nm, 13.3 min/20 min).

Methyl [2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]acetate (27). It was prepared following a similar procedure to compound **12**. Yield: 61.9%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.15 (s, 1H), 7.32 (d, 1H, J = 8.4 Hz), 7.19 (d, 1H, J = 2.4 Hz), 7.06 (dd, 1H, J = 8.4 and 2.7 Hz), 4.92 (s, 2H), 4.80 (s, 2H), and 3.70 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.5, 157.2, 146.9, 122.2, 119.5, 113.7, 70.9, 65.3, 52.2. HRMS-EI: $[M]^+$ $C_{10}H_{11}BO_5$ calcd 222.0699, found 222.0701. Mp: 102−104 °C. HPLC: purity 96.4% (220 nm, 11.9 min/20 min).

***tert*-Butyl [2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]acetate (28).** This compound was prepared following a similar procedure to compound **12**. Yield: 37.5%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.14 (s, 1H), 7.32 (d, 1H, J = 8.1 Hz), 7.18 (d, 1H, J = 2.4 Hz), 7.04 (dd, 1H, J = 8.4 and 2.7 Hz), 4.92 (s, 2H), 4.65 (s, 2H), and 1.42 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.1, 157.3, 146.7, 122.1, 119.4, 113.8, 82.4, 70.9, 66.7, 27.9. HRMS-EI: $[M]^+$ $C_{13}H_{17}BO_5$ calcd 264.1169, found 264.1170. Mp: 107−108 °C. HPLC: purity 98.3% (220 nm, 9.1 min/15 min).

1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yl *N*-Phenylcarbamate (29). Compound **11** (100 mg, 0.667 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C. To this solution under nitrogen were added in sequence Et_3N (0.28 mL, 2 mmol) and isocyanatobenzene (0.85 mL, 6.67 mmol). The reaction mixture was stirred for 2 d at room temperature and then treated with 1 M HCl (10 mL). After extraction with EtOAc, the organic layer was washed with water and brine and dried over Na_2SO_4. The residue after rotary evaporation was purified by column chromatography over silica gel to give compound **29** (32 mg, 18.0%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.23 (s, 1H), 9.25 (s, 1H), 7.53−7.02 (m, 8H), and 5.00 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.5, 149.9, 145.4, 137.7, 128.8, 124.6, 123.4, 122.9, 121.8, 118.7, 70.8. HRMS-ESI: $[M + Na]^+$ $C_{14}H_{12}BNO_4$Na calcd 292.0757, found 292.0802. Mp: 178−180 °C. HPLC: purity 95.2% (254 nm, 13.9 min/20 min).

1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yl *N*-Cyclohexylcarbamate (30). This compound was prepared following a similar procedure to compound **29**. Yield: 13.0%. ^{1}H NMR (300 MHz, acetone-d_6): δ 8.09 (s, 1H), 7.42−7.37 (m, 2H), 7.19 (dd, 1H, J = 8.4 and 2.1 Hz), 6.65 (m, 1H), 5.00 (s, 2H), 3.46 (m, 1H), and 1.98−1.15 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.7, 152.0, 147.2, 125.9, 123.9, 123.2, 72.1, 51.7, 34.1, 26.6, 26.2. HRMS-ESI: $[M + Na]^+$ $C_{14}H_{18}BNO_4$ calcd 298.1227, found 298.1249. Mp: 198−200 °C. HPLC: purity 99.8% (220 nm, 10.6 min/20 min).

Ethyl [2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]propanoate (31). This compound was prepared following a similar procedure to compound **12**. Yield: 57.0%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.18 (s, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.14 (d, J = 2.4 Hz, 1H), 7.02 (dd, J = 8.4 and 2.7 Hz, 1H), 4.95−4.87 (m, 3H), 4.19−4.08 (m, 2H), 1.51 (d, J = 6.6 Hz, 3H), and 1.17 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.3, 157.1, 146.6, 122.2, 120.2, 114.7, 72.7, 70.8, 61.4, 18.5, 14.1. HRMS-EI: $[M]^+$ $C_{12}H_{15}BO_5$ calcd 250.1012, found 250.1014. Mp: 65−67 °C. HPLC: purity 97.3% (220 nm, 16.6 min/20 min).

Ethyl [2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]butanoate (32). This compound was prepared following a similar procedure to compound **12**. Yield: 62.5%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.17 (s, 1H), 7.31 (d, 1H, J = 8.1 Hz), 7.16 (d, 1H, J = 2.4 Hz), 7.03 (dd, 1H, J = 8.4 and 2.7 Hz), 4.91 (s, 2H), 4.73 (t, 1H, J = 5.4 Hz, 1H), 4.20−4.09 (m, 2H), 1.96−1.83 (m, 2H), 1.17 (t, 3H, J = 6.9 Hz), and 1.00 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.8, 157.5, 146.6, 122.2, 120.2, 114.9, 77.9, 70.9, 61.2, 26.1, 14.1, 9.6. HRMS-EI: $[M]^+$ $C_{13}H_{17}BO_5$ calcd 264.1169, found 264.1168. Mp: 90−92 °C. HPLC: purity 97.8% (220 nm, 18.1 min/20 min).

Ethyl [2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]-2-methylpropanoate (33). This compound was prepared following a similar procedure to compound **12**. Yield: 61.0%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (d, 1H, J = 8.1 Hz), 7.16 (d, 1H, J = 2.4 Hz), 7.04 (dd, 1H, J = 8.4 and 2.7 Hz), 5.04 (s, 2H), 4.25 (q, 2H, J = 6.9 Hz), 1.60 (s, 6H), and 1.26 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.3, 154.7, 147.3, 123.6, 121.8, 120.1, 79.3, 70.9, 61.5, 25.3, 14.0. HRMS-EI: $[M]^+$ $C_{13}H_{17}BO_5$ calcd 264.1169, found 264.1170. Mp: 63−66 °C. HPLC: purity 97.7% (220 nm, 9.5 min/15 min).

Ethyl [2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)]-2-phenylacetate (34). This compound was prepared following a similar procedure to compound **12**. Yield: 60.0%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62−7.56 (m, 2H), 7.44−7.12 (m, 6H), 5.67 (s, 1H), 5.03 (s, 2H), 4.30−4.10 (m, 2H), and 1.20 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9, 156.8, 146.9, 135.4, 128.9, 128.7, 127.1, 122.3, 120.5, 115.3, 78.8, 70.9, 61.7, 13.9. HRMS-EI: $[M]^+$ $C_{17}H_{17}BO_5$ calcd 312.1169, found 312.1175. Mp: 94−97 °C. HPLC: purity 97.6% (220 nm, 13.9 min/15 min).

Ethyl-[2-(1,3-dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)] acetamide (35). Compound **11** (100 mg, 0.667 mmol) was dissolved in DMSO (10 mL). To this solution under nitrogen were added potassium carbonate (368 mg, 2.667 mmol), a catalytic amount of NaI, and 2-chloro-*N*-ethylacetamide (398 mg, 2.667 mmol). The reaction mixture was stirred for 36 h at 90 °C and then treated with 1 M HCl (10 mL). After extraction with EtOAc, the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. The residue after rotary evaporation was purified by column chromatography over silica gel and recrystallization (hexane/EtOAc) to give compound **35** (34 mg, 22.2%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.17 (s, 1H), 8.09 (t, 1H, J = 6 Hz), 7.33 (d, 1H, J = 8.4 Hz), 7.24 (d, 1H, J = 2.4 Hz), 7.10 (dd, 1H, J = 8.4 and 2.7 Hz), 4.92 (s, 2H), 4.45 (s, 2H), 3.19−3.11 (m, 2H), and 3.04 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.4, 166.7, 147.2, 122.3, 118.7, 114.8, 70.8, 67.5, 33.8, 14.4. HRMS-EI: $[M]^+$ $C_{11}H_{14}BNO_4$ calcd 235.1016, found 235.1017. Mp: 118−119 °C. HPLC: purity 96.1% (220 nm, 4.6 min/15 min).

***N*-*tert*-Butyl-2-(1,3-dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)acetamide (36).** This compound was prepared following a similar procedure to compound **35**. Yield: 35.4%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.13 (s, 1H), 7.41 (s, 1H), 7.32 (d, 1H, J = 8.4 Hz), 7.22 (s, 1H), 7.07 (d, 1H, J = 8.4 Hz), 4.92 (s, 2H), 4.39 (s, 2H), and 1.29 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.5, 156.6, 147.2, 122.2, 118.7, 114.9, 70.9, 67.8, 51.3, 28.6. HRMS-EI: $[M]^+$ $C_{13}H_{18}BNO_4$ calcd 263.1329, found 263.1330. Mp: 149−150 °C. HPLC: purity 97.8% (220 nm, 13.5 min/20 min).

1-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)pentan-2-one (37). This compound was prepared following a similar procedure to compound **12**. Yield: 33.6%. ^{1}H NMR (400 MHz, acetone-d_6): δ 9.14 (s, 1H), 7.33 (d, 1H, J = 8.1 Hz), 7.19 (d, 1H, J = 2.4 Hz), 7.08 (dd, 1H, J = 8.4 and 2.7 Hz), 4.96 (s, 2H), 4.73 (s, 2H), 2.57 (t, 2H, J = 7.2 Hz), 1.66−1.55 (m, 2H), and 0.92 (t, 3H, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.8, 157.4, 146.8, 122.4, 119.6, 114.1, 72.9, 70.9, 40.9, 16.6, 13.7. HRMS-ESI: $[M + H]^+$ $C_{12}H_{16}BO_4$ calcd 235.1141, found 235.1163. Mp: 107−109 °C. HPLC: purity 97.9% (220 nm, 11.2 min/15 min).

1-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)butan-2-one (38). This compound was prepared following a similar procedure to compound **12**. Yield: 30.0%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24 (d, 1H, J = 8.1 Hz), 7.09 (d, 1H, J = 2.4 Hz), 7.05 (dd, 1H, J = 8.4 and 2.7 Hz), 5.02 (s, 2H), 4.57 (s, 2H), 2.60 (q, 2H, J = 7.2 Hz), and 1.08 (t, 3H, J = 7.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 208.4, 157.4, 146.9, 122.4, 119.6, 113.9, 72.7, 70.9, 32.4, 7.1. HRMS-ESI: $[M + Na]^+$ $C_{11}H_{13}BO_4$Na calcd 243.0805, found 243.0839. Mp: 83−85 °C. HPLC: purity 95.4% (220 nm, 9.6 min/15 min).

2-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy) pentan-3-one (39). This compound was prepared following a similar procedure to compound **12**. Yield: 38.0%. ^{1}H NMR (400 MHz, $CDCl_3$):

δ 7.21 (d, 1H, J = 8.1 Hz), 7.06 (d, 1H, J = 2.4 Hz), 6.98 (dd, 1H, J = 8.4 and 2.7 Hz), 5.00 (s, 2H), 4.67 (q, 1H, J = 6.8 Hz), 2.73−2.61 (m, 1H), 2.49−2.39 (m, 1H), 1.47 (d, 3H, J = 6.8 Hz), and 0.99 (t, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 213.2, 157.3, 146.9, 122.7, 120.1, 114.9, 79.5, 71.1, 30.5, 18.1, 7.3. HRMS-EI: $[M]^+$ $C_{12}H_{15}BO_4$ calcd 234.1063, found 234.1065. Mp: 72−74 °C. HPLC: purity 96.4% (280 nm, 12.1 min/15 min).

3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy) heptan-4-one (40). This compound was prepared following a similar procedure to compound **12**. Yield: 45.0%, as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, 1H, J = 8.1 Hz), 7.07 (d, 1H, J = 2.4 Hz), 6.99 (dd, 1H, J = 8.4 and 2.7 Hz), 4.99 (s, 2H), 4.45−4.44 (m, 1H), 2.61−2.51 (m, 1H), 2.40−2.30 (m, 1H), 1.90−1.78 (m, 2H), 1.57−1.47 (m, 2H), 1.03−0.98 (m, 3H), and 0.85−0.79 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 212.8, 157.7, 146.8, 122.5, 119.7, 115.1, 84.7, 71.0, 39.6, 25.8, 16.5, 13.8, 9.8. HRMS-EI: $[M]^+$ $C_{14}H_{19}BO_4$ calcd 262.1376, found 262.1375. HPLC: purity 95.3% (220 nm, 10.2 min/15 min).

3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)butan-2-one (41). This compound was prepared following a similar procedure to compound **12**. Yield: 62.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (d, 1H, J = 8.1 Hz), 7.10 (d, 1H, J = 2.4 Hz), 7.02 (dd, 1H, J = 8.4 and 2.7 Hz), 5.04 (s, 2H), 4.66 (q, 1H, J = 6.8 Hz), 2.19 (s, 3H), and 1.51 (d, 3H, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 210.9, 157.2, 147.0, 122.7, 120.1, 114.9, 79.6, 71.1, 25.0, 17.8. HRMS-EI: $[M]^+$ $C_{11}H_{13}BO_4$ calcd 220.0907, found 220.0906. Mp: 90−92 °C. HPLC: purity 97.1% (220 nm, 9.9 min/15 min).

3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)-3-methylbutan-2-one (42). This compound was prepared following a similar procedure to compound **12**. Yield: 25.6%, as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (d, 1H, J = 8.1 Hz), 7.05 (d, 1H, J = 2.4 Hz), 6.93 (dd, 1H, J = 8.4 and 2.7 Hz), 5.00 (s, 2H), 2.27 (s, 3H), and 1.44 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 221.2, 154.4, 147.3, 122.7, 121.9, 119.4, 84.2, 70.9, 24.4, 23.7. HRMS-ESI: $[M + H]^+$ $C_{12}H_{16}BO_4$ calcd 235.1141, found 235.1171. HPLC: purity 96.3% (220 nm, 11.5 min/15 min).

3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)pentan-2-one (43). This compound was prepared following a similar procedure to compound **12**. Yield: 50.9%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.14 (s, 1H), 7.31 (d, 1H, J = 8.1 Hz), 7.12 (d, 1H, J = 2.4 Hz), 7.04 (dd, 1H, J = 8.4 and 2.7 Hz), 4.91 (s, 2H), 4.67−4.61 (m, 1H), 2.14 (s, 3H), 1.91−1.78 (m, 2H), and 0.96 (t, 3H, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 210.9, 157.4, 146.7, 122.4, 119.7, 114.6, 84.5, 70.9, 25.5, 25.4, 9.6. HRMS-ESI: $[M + H]^+$ $C_{12}H_{16}BO_4$ calcd 235.1141, found 235.1161. Mp: 68−70 °C. HPLC: purity 96.6% (220 nm, 11.5 min/15 min).

3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)hexan-2-one (44). This compound was prepared following a similar procedure to compound **12**. Yield: 50.4%, as a viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (d, 1H, J = 8.1 Hz), 7.07 (d, 1H, J = 2.4 Hz), 7.00 (dd, 1H, J = 8.4 and 2.7 Hz), 5.02 (s, 2H), 4.52−4.44 (m, 1H), 2.13 (s, 3H), 1.91−1.71 (m, 2H), 1.59−1.45 (m, 2H), and 0.94 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 210.9, 157.3, 146.6, 122.2, 119.2, 114.5, 83.1, 70.9, 34.1, 25.1, 18.4, 13.6. HRMS-ESI: $[M + Na]^+$ $C_{13}H_{17}BO_4Na$ calcd 271.1118, found 271.1156. HPLC: purity 95.4% (220 nm, 9.1 min/15 min).

3-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)-4-methylpentan-2-one (45). This compound was prepared following a similar procedure to compound **12**. Yield: 16.0%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, 1H, J = 8.1 Hz), 7.06 (d, 1H, J = 2.4 Hz), 6.99 (dd, 1H, J = 8.4 and 2.7 Hz), 5.00 (s, 2H), 4.19 (d, 1H, J = 6 Hz), 2.22−2.15 (m, 1H), 2.10 (s, 3H), 1.05 (d, 3H, J = 6.8 Hz), and 0.98 (d, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 210.7, 157.8, 146.8, 122.4, 119.6, 114.8, 88.4, 70.9, 31.3, 26.2, 18.7, 17.9. HRMS-ESI: $[M + Na]^+$ $C_{13}H_{17}BO_4Na$ calcd 271.1118, found 271.1155. Mp: 58−59 °C. HPLC: purity 96.6% (220 nm, 12.8 min/15 min).

1-(1,3-Dihydro-1-hydroxy-2,1-benzoxaborol-6-yloxy)-4-methylpentan-2-one (46). This compound was prepared following a similar procedure to compound **12**. Yield: 45.0%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.12 (s, 1H), 7.31 (d, 1H, J = 8.0 Hz), 7.15 (d, 1H, J = 2.8 Hz), 7.03 (dd, 1H, J = 8.4 and 2.4 Hz), 4.95 (s, 2H), 4.78 (s, 2H), 2.40 (d, 2H, J = 7.2 Hz), 2.12−2.02 (m, 1H), and 0.88 (d, 6H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 207.2, 157.3, 146.8, 122.3, 119.5, 113.9, 73.1, 70.9, 47.8, 24.1, 22.6. HRMS-ESI: $[M + H]^+$ $C_{13}H_{18}BO_4$ calcd 249.1298, found 249.1317. Mp: 107−108 °C. HPLC: purity 98.9% (220 nm, 12.3 min/15 min).

6-(2-Hydroxy-1-methylbutoxy)-1,3-dihydro-1-hydroxy-2,1-benzoxaborole (47). Compound **39** (75 mg, 0.32 mmol) was dissolved in MeOH (3 mL) and cooled to 0 °C. To this solution was added $NaBH_4$ (18.1 mg, 0.48 mmol). The reaction mixture was stirred for 2 h and then treated with saturated HCl (1 M). After evaporation, the residue was extracted with EtOAc and the organic layer was washed with water and brine. The residue after rotary evaporation was purified by preparative TLC to give compound **47** (45 mg, 59.0%) as a viscous oil. ^{1}H NMR (400 MHz, CD_3OD): δ 7.23 (d, 1H, J = 8.1 Hz), 7.16 (d, 1H, J = 2.4 Hz), 7.05 (dd, 1H, J = 8.4 and 2.7 Hz), 4.97 (s, 2H), 4.37−4.25 (m, 1H), 4.62−4.50 (m, 1H), 1.71−1.40 (m, 2H), 1.25 (t, 3H, J = 6 Hz), and 1.02−0.95 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.2, 146.5, 122.2, 121.0, 116.0, 75.9, 74.7, 70.9, 25.7, 15.7, 9.8. HRMS-ESI: $[M + Na]$ $C_{12}H_{17}BO_4Na$ calcd 259.1118, found 259.1123. HPLC: purity 96.9% (220 nm, 11.6 min/15 min).

Molecular Modeling. The sequence of *Trypanosoma brucei* LeuRS was retrieved from the protein knowledge base (Q386D9), and amino acids 250−511 corresponding to the editing domain were used in the modeling. Prime 2.0 (Schrödinger, LLC) was used to construct the homology model of *T. brucei* LeuRS editing domain using the *Candida albicans* LeuRS editing domain (PDB code 2WFG)[15] as the template. Secondary structures were predicted with SSPro, and sequence alignment was manually adjusted. The homology model containing amino acids 254−507 was further refined using the Refinement module with the emphasis on the loops and the active site residues. Hydrogen atoms and hydrogen bonds were optimized with Protein Preparation Wizard and checked with Procheck.[16]

The coordinates of benzoxaborole compounds in the binding site were generated based on the coordinates of (6-(ethylamino)-5-fluorobenzo-[*c*][1,2]oxaborol-1(3*H*)-ol) (AN3018) complexed in *C. albicans* LeuRS[15] followed by minimization in MacroModel using OPLS_2005 force field and Polak−Ribiere conjugate gradient (PRCG) method with termination threshold of 2000 steps or energy convergence gradient of 0.03 kJ/mol. The ligand and residues within 5 Å were set to be free. Residues between 5 and 10 Å were constrained by 200 kcal/(mol·Å^2) force, and residues between 10 and 15 Å were set to be frozen.

Expression Construct. *T. brucei* LeuRS coding region was amplified by polymerase chain reaction from genomic DNA isolated from *T. brucei* (TREU927) using the following primers: forward, 5′-GATAGTCATATGTCGACTGTACGACGTGAT; reverse, 5′-GATAGTGGATCCTGCCTTCTTGTCCGGAAAGGC. The PCR product was digested with NdeI/BamHI and cloned into NdeI/BamHI site of pET21a(+) (Novagen, Gibbstown, NJ) with fused His tag at the C-terminus. Correct construct was confirmed by sequencing and used to transform *E. coli* BL21(DE3)-RIPL (Stratagene, La Jolla, CA). The resulting colonies were selected for the ability of induced expression by 0.1 mM IPTG at 37 °C for 3 h as described by the Novagen manual.

Protein Expression. LeuRS was expressed in LB medium with 50 μg/mL ampicillin as described by the Novagen manual. The expression induction was performed at 20 °C overnight to increase soluble portion of the protein. The cell was collected and lysed by sonication and purified

by His-Bind (Novagen). The purity and concentration of LeuRS was determined by SDS−PAGE with BSA as concentration standard.

Aminoacylation Assay. All compounds were dissolved in DMSO. Experiments were performed in 70 μL reaction mixtures containing 50 mM HEPES-KOH (pH 7.8), 5 mM $MgCl_2$, 45 mM KCl, 1 mM DTT, 0.02% BSA (W/V), 0.4 mg/mL brewer's yeast tRNA (Roche), 4 mM ATP, 10 μM [^{14}C]Leu, 2 nM tbLeuRS, and compounds at varied concentrations. The reaction mixture without ATP was first preincubated at 37 °C for 20 min. Then the reaction was initiated by adding ATP. After 15 min, three aliquots of 20 μL of reaction solution were quenched on Whatman filter with 5% trichloroacetic acid (TCA) and washed three times with 95% ethanol. The filter was then dried under an infrared heat lamp, and the radioactivity of the precipitate was quantified using a scintillation counter. For each IC_{50} curve, nine data points at different concentrations were collected and the sigmoidal dose response curve fitting was done with GraphPad Prism. Compound **1c** was used as internal reference for each testing batch. Duplicates of IC_{50} curves at different time period over 3 years showed an average error range of 2-fold (for example, IC_{50} value of **1c** ranges from 15 to 30 μM), which is reasonable for aaRS assays.

In Vitro *Trypanosoma brucei* Assay. All in vitro antiparasite assays were conducted with the bloodstream-form *Trypanosoma brucei brucei* 427 strain. Parasites were cultured in T-25 vented cap flasks and kept in humidified incubators at 37 °C and 5% CO_2. The parasite culture medium was complete HMI-9 medium (Hirumi, H.; Hirumi, K. Continuous cultivation of *Trypanosoma brucei* bloodstream forms in a medium containing a low concentration of serum protein without feeder cell layers. *J. Parasitol.* **1989**, *75*, 985−989) containing 10% FBS, 10% Serum Plus medium, and penicillin/streptomycin. To ensure log growth phase, trypanosomes were subcultured at appropriate dilutions every 2−3 days. The log phase cultures were diluted 1:10 in HMI-9, and 10 μL was counted using hemocytometer to determine parasite concentration. Parasites were diluted to 2×10^5/mL in HMI-9 to generate a 2-fold working concentration for assay. Compounds to be tested were serially diluted in DMSO, and 0.5 μL was added to 49.5 μL HMI-9 in triplicate 96-well plates using a Biomek NX liquid handler. Parasites from the diluted stock were added to each well (50 μL) using a Multidrop 384 dispenser to give a final concentration of 1.0×10^5/mL parasites in 0.4% for DMSO. Trypanosomes were incubated with compounds for 72 h at 37 °C with 5% CO_2. Resazurin (20 μL of 12.5 mg/mL stock) from Sigma-Aldrich was added to each well, and plates were incubated for an additional 2−4 h. Assay plates were read using an EnVision plate reader at an excitation wavelength of 544 nm and emission of 590 nm. Triplicate data points were averaged to generate sigmoidal dose response curve and to determine IC_{50} values using XLfit curve fitting software from IDBS (Guildford, U.K.). IC_{50} values were measured in triplicate with an error range of ±0.2 μM. Suramin and pentamidine are used as positive control, and typical average IC_{50} values are 0.007 μg/mL (0.005 μM) and 0.009 μg/mL (0.026 μM), respectively.

In Vitro Mammalian Cell Cytotoxicity Assay. For evaluation of compound effects on mammalian cells, L929 mouse fibroblast cells were used. Cells were maintained as adherent cultures in T-25 vented cap flasks in a humidified incubator at 37 °C in the presence of 5% CO_2. Culture medium was D-MEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. L929 cells were maintained below confluent levels by subculturing at 1:10 dilution twice weekly using 0.05% trypsin for detachment. Subconfluent L929 cells were trypsinized, resuspended in fresh medium, and 10 μL was counted using hemocytometer to determine cell concentration. Cells were diluted to 1×10^4/mL in DMEM, dispensed (100 μL) into 96-well plates using a Multidrop 384 dispenser, and allowed to attach overnight. Spent medium was replaced with 99.5 μL of fresh D-MEM. Compounds to be tested were serially diluted in DMSO, and 0.5 μL was added using a Biomek NX liquid handler. Plates were incubated with compounds for 72 h at 37 °C with 5% CO_2. Resazurin (20 μL of 12.5 mg/mL stock) from Sigma-Aldrich was added to each well, and plates were incubated for an additional 3−4 h. Assay plates were read using an EnVision plate reader at an excitation wavelength of 544 nm and emission of 590 nm. Single data points were used to generate sigmoidal dose response curves and determine IC_{50} values using XLfit curve fitting software from IDBS (Guildford, U.K.).

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ACKNOWLEDGMENT

We thank National Science Foundation of China (Grant 20702031) and Ministry of Science and Technology of China (Grant 2009CB918404) for financial support of this work.

ABBREVIATIONS USED

T. brucei, *Trypanosoma brucei*; TbLeuRS, *T. brucei* leucyl-tRNA synthetase; LeuRS, leucyl-tRNA synthetase; aaRS, aminoacyl-tRNA synthetase; IleRS, isoleucyl-tRNA synthetase; MetRS, methionyl-tRNA synthetase; AsnRS, asparaginyl-tRNA synthetase; CaLeuRS, *Candida albicans* LeuRS

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