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Discovery of a Potent and Specific *M. tuberculosis* Leucyl-tRNA Synthetase Inhibitor: (*S*)-3-(Aminomethyl)-4-chloro-7-(2-hydroxyethoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol (GSK656)

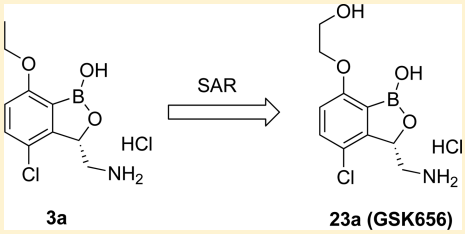
Xianfeng Li,[†] Vincent Hernandez,[†] Fernando L. Rock,[†] Wai Choi,[†] Yvonne S. L. Mak,[†] Manisha Mohan,[†] Weimin Mao,[†] Yasheen Zhou,[†] Eric E. Easom,[†] Jacob J. Plattner,[†] Wuxin Zou,[‡] Esther Pérez-Herrán,[§] Ilaria Giordano,[§] Alfonso Mendoza-Losana,[§] Carlos Alemparte,[§] Joaquín Rullas,[§] Iñigo Angulo-Barturen,[∥,§] Sabrinia Crouch,[§] Fátima Ortega,[§] David Barros,*[,§] and M. R. K. Alley[†]

[†]Anacor Pharmaceuticals, Inc., 1020 E. Meadow Circle, Palo Alto, California 94303, United States

[‡]BioDuro LLC, Building E, No. 29, Life Science Park Road, Beijing 102206, P. R. China

[§]GlaxoSmithKline, Tres Cantos Medicines Development Campus, Severo Ochoa 2, Tres Cantos, Madrid 28760, Spain

ABSTRACT: There is an urgent need to develop new and safer antitubercular agents that possess a novel mode of action. We synthesized and evaluated a novel series of 3-aminomethyl 4-halogen benzoxaboroles as *Mycobacterium tuberculosis* (*Mtb*) leucyl-tRNA synthetase (LeuRS) inhibitors. A number of *Mtb* LeuRS inhibitors were identified that demonstrated good antitubercular activity with high selectivity over human mitochondrial and cytoplasmic LeuRS. Further evaluation of these *Mtb* LeuRS inhibitors by in vivo pharmacokinetics (PK) and murine tuberculosis (TB) efficacy models led to the discovery of GSK3036656 (abbreviated as GSK656). This molecule shows potent inhibition of *Mtb* LeuRS (IC_{50} = 0.20 μM) and in vitro antitubercular activity (*Mtb* H37Rv MIC = 0.08 μM). Additionally, it is highly selective for the *Mtb* LeuRS enzyme with IC_{50} of >300 μM and 132 μM for human mitochondrial LeuRS and human cytoplasmic LeuRS, respectively. In addition, it exhibits remarkable PK profiles and efficacy against *Mtb* in mouse TB infection models with superior tolerability over initial leads. This compound has been progressed to clinical development for the treatment of tuberculosis.



INTRODUCTION

Tuberculosis (TB) is a life-threatening infectious disease caused by the bacteria *Mycobacterium tuberculosis* (*Mtb*). The World Health Organization (WHO) estimates that one-third of the world's population is infected with *Mtb*, resulting in 1.4 million deaths in 2015.[1] In the same year, there were additional 0.4 million people infected with HIV who also died of TB disease.[1] The current treatment for TB infection requires a combination therapy of four front-line drugs rifampin, isoniazid, pyrazinamide, and ethambutol for 6−9 months, often leading to significant side effects and poor patient compliance. In addition, emergence of multidrug-resistant TB (MDR-TB)[2] and extensively drug-resistant TB (XDR-TB)[3] has rendered many front-line and second-line drugs ineffective. Furthermore, totally drug-resistant TB (TDR-TB) has recently emerged that is resistant to all clinical drugs.[4] Therefore, there is an urgent need to develop new antitubercular agents that are effective against TB, which possess a novel mode of action that circumvents these resistances.[5−8]

Bedaquiline and delamanid have recently been approved by the Food and Drug Administration (FDA) or the European Medicines Agency (EMA) to treat MDR-TB, representing the first new TB drugs in more than 40 years.[9,10] Bedaquiline acts by the novel mechanism of mycobacterial adenosine 5′-triphosphate (ATP) synthase inhibition,[11] while delamanid is a nitroimidazole derivative that primarily inhibits synthesis of methoxymycolic and ketomycolic acid, which are components of the mycobacterial cell wall.[12] However, bedaquiline has a boxed warning related to possible cardiac toxicity[13] and delamanid (OPC-67683) induces QTc prolongation.[14] In addition there is cross-resistance between clofazimine and bedaquiline[15] as well as some of pre-existing resistance to delamanid.[16] There has been an increased activity toward the discovery of new antitubercular agents.[17−22] Other TB drug candidates that are in the clinical development include moxifloxacin, sutezolid, SQ109, and Q203[21,22] (Figure 1).

We recently identified a series of 3-aminomethyl-benzoxaboroles that target *Mtb* LeuRS (**1**−**4**, Figure 2).[23] These *Mtb* LeuRS inhibitors require the boron atom for *Mtb* LeuRS activity since it forms a bidentate covalent adduct with the terminal nucleotide of tRNA, Ade76. The resulting covalent adduct traps the 3′ end of $tRNA^{Leu}$ in the editing site in a nonproductive complex, inhibiting leucylation and thus protein synthesis. The amino group of the (*S*)-aminomethyl side chain at C-3 is critical for binding as it makes three hydrogen bonding interactions. An important finding from this work is that 4-halogen atom (especially Cl and Br) significantly improves *Mtb* LeuRS activity, antitubercular activity against *Mtb* H37Rv, and selectivity against other bacteria. This effort led to the discovery

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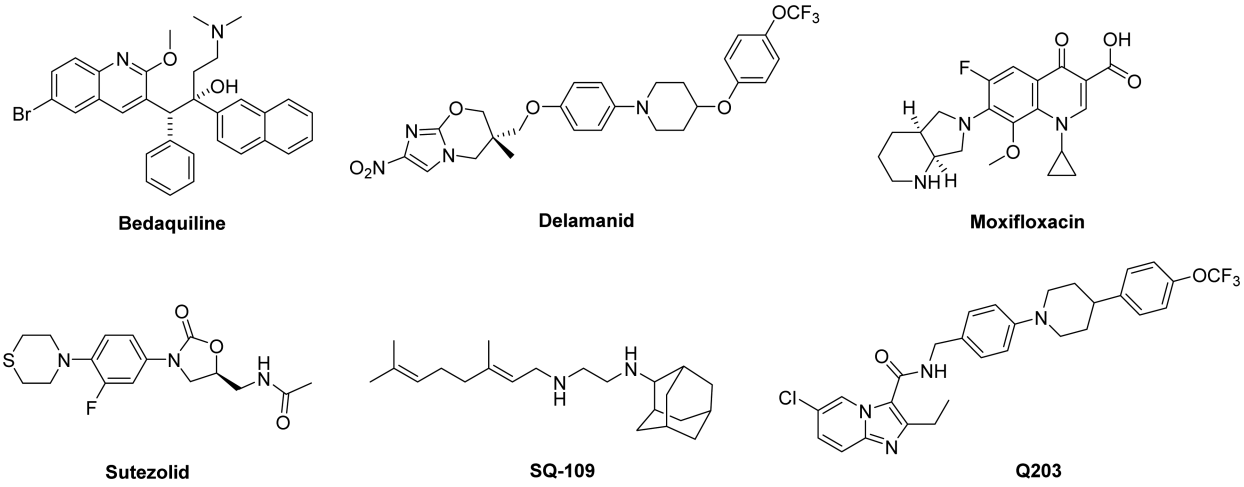

Figure 1. Bedaquiline, delamanid, and selected TB drug candidates in clinical trials.

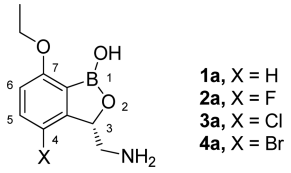

Figure 2. 3-Aminomethyl 4-halogen benzoxaborole inhibitors.

of potent *Mtb* LeuRS inhibitors **3a** and **4a** that are orally bioavailable and effective against *Mtb* in mouse TB infection models but with potential toxicity issues from inhibition of mammalian cytoplasmic LeuRS.[23] Furthermore, a once daily dose of 50 mg/kg **4a** in a mouse acute TB infection model was not tolerated and was a concern for an indication that involved long-term therapy.

Therefore, we set out to expand structure−activity relationship (SAR) of this 3-aminomethyl 4-halogen benzoxaborole series with the goal to improve the selectivity of the *Mtb* LeuRS inhibitors suitable for further development. From the binding pharmacophore revealed by the crystal structure of **3a** complexed with *Mtb* LeuRS,[23] the C-7 or C-6 position of the benzoxaborole appears to be solvent-exposed and may be open for structural modification. In the present work, we used **3a** and **4a** as lead compounds and focused on three modification sites which are C-7, C-6, and C-6/C-7 positions. We investigated various 7-substituted analogues represented by target compounds **5**−**24** (class A) and a number of 6-substituted compounds **25**−**32** (class B) as well as C-6/C-7 fused compounds **33**−**36** (class C). In this study, we intentionally made and evaluated both 4-Cl and 4-Br compounds in parallel, providing useful SAR information on their relative magnitudes in activity and selectivity. Further PK evaluation and efficacy studies of these *Mtb* LeuRS inhibitors led to the identification of a first-in-class boron-containing antitubercular agent **23a** (GSK656),[24] which had a much better safety profile than **3a** and **4a**.

RESULTS AND DISCUSSION

Chemistry. Throughout the article, compounds have been numbered using the format **23**, **23a**, and **23b** for the racemate, the (*S*)-isomer, and the (*R*)-isomer of compound **23**, respectively. The chemistry used to synthesize racemic 7-substituted compounds **11**−**20** and **23**−**24** (class A) is described in Scheme 1, starting from 2-bromo-3-hydroxybenzaldehyde **37**. Phenol **37** was converted to phenyl ether **38** by alkylation with alkyl bromide or alkyl iodide (when R is an alkyl group) or through copper-mediated arylation with arylboronic acid (when R is an aryl group). Incorporation of boron was accomplished by reaction of **38** with pinacol diborane in the presence of a palladium catalyst to give boronate **39** or by reaction of **41** with *n*-butyllithium and trimethyl borate followed by acidic workup to give boronic acid **42**. The Henry reaction of **39** or **42** with nitromethane afforded 3-nitromethylbenzoxaborole **40**.[25] Chlorination or bromination can be achieved through 3-nitromethylbenzoxaborole **40** or through *tert*-butoxycarbonyl (Boc) protected 3-aminomethylbenzoxaborole **43**. The removal of Boc group of compound **44** or the reduction of nitro group in compound **45** provided corresponding C-4 chloro or bromo analogues (**11**−**20**, **23**, **24**) as racemates. Compounds **23** and **24** were further resolved by chiral supercritical fluid chromatography (SFC) separation to provide active enantiomers **23a** and **24a**, respectively.

The chemistry used to synthesize enantiopure 7-substituted compounds **5a**−**10a**, **21a**, and **22a** (class A) is described in Scheme 2. This modular approach would introduce C-7 substitution in appropriate enantiopure benzoxaborole intermediates at a late stage of the synthesis and give active enantiomers, thus avoiding a lengthy multistep route and chiral separation. The enantiopure compound **46** with 3-hydroxypropoxy substitution at C-7 position was made according to literature procedure.[26] Dealkylation of **46** with BBr_3 followed by Boc protection provided the key intermediate **51** with 7-hydroxy substitution. Initial attempts to alkylate phenol **51** with an alkyl bromide under standard conditions (K_2CO_3, DMSO, room temperature) failed to give any desired 7-alkyoxy product, presumably due to the presence of the neighboring boron atom that forms an intramolecular complex and protects the phenol from further reaction. Finally we found that the reaction of **51** with an alkyl bromide in the presence of potassium *tert*-butoxide at 90 °C in DMSO afforded 7-substituted compound **52**. Subsequent chlorination or bromination of compound **52**, followed by Boc removal, provided the C-4 chloro or bromo analogs **7a**−**10a**. Compounds **21a** and **22a** were made by chlorination or bromination of Boc-protected intermediate **48**, followed by Boc removal. Compounds **5a** and **6a** were made similarly from the enantiopure material **47**.

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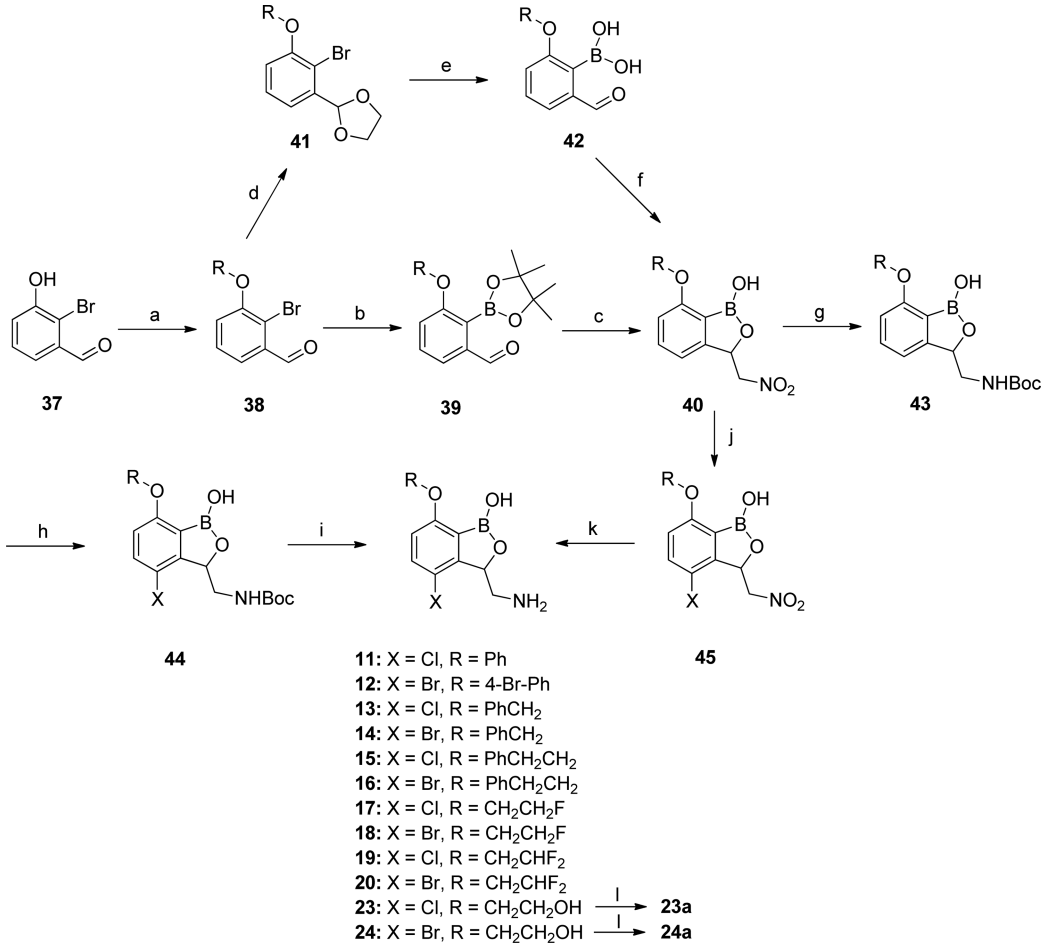
Scheme 1[a]



[a]Reagents and conditions: (a) when R = alkyl, alkyl bromide or alkyl iodide, K_2CO_3, DMF, heat; when R = aryl, arylboronic acid, $Cu(OAc)_2$, TEA, DCM; (b) $(Pin)_2B_2$, KOAc, $PdCl_2(dppf)$, THF, reflux; (c) CH_3NO_2, NaOH, water/THF; (d) ethylene glycol, TsOH, toluene, Dean−Stark apparatus, reflux; (e) (1) BuLi, $B(OMe)_3$, THF, −78 °C; (2) 2 N HCl, rt; (f) CH_3NO_2, NaOH, water/THF; (g) (1) Raney Ni, H_2, NH_3/EtOH, then HCl/Et_2O; (2) di-*tert*-butyl dicarbonate, TEA, DCM, 0 °C; (h) when X = Cl, NCS, DMF, heat or SO_2Cl_2, AcOH, rt; when X = Br, NBS, CH_3CN, heat; (i) TFA, DCM, then HCl; (j) when X = Cl, NCS, DMF, heat; when X = Br, NBS, CH_3CN, heat; (k) Raney Ni, H_2, NH_3/EtOH, then HCl/Et_2O; (l) chiral SFC separation.

The chemistry used to make 6-substituted analogues (class B) is described in Scheme 3 and Scheme 4. For this series of compounds, 4-halogen substituent needs to be incorporated at an early stage of the synthesis. As shown in Scheme 3, the 4-Cl analogues were synthesized starting from 2-chloro-4,6-dihydroxybenzaldehyde **53**. After selected protection of 4-hydroxy group, reaction of phenol **54** with triflic anhydride in the presence of pyridine gave triflate **55**. Borylation was accomplished by reaction of triflate **55** with pinacol diborane in the presence of a palladium catalyst. Subsequent multistep manipulation of boronate **56** including Henry reaction, nitro reduction, and alkylation provided the final 4-Cl compounds (**25**, **27**, **29**, and **31**). On the other hand, the 4-Br analogues were synthesized starting from 2,6-dibromo-4-hydroxy-benzaldehyde **60** as shown in Scheme 4. Alkylation of **60** followed by protection of aldehyde group provided symmetric dibromide **62**. Reaction of **62** with *n*-butyllithium and trimethyl borate followed by acidic workup converted one bromide to boronic acid **63**. Subsequent multistep manipulation of boronic acid **63** including Henry reaction and nitro reduction provided the final 4-Br compounds (**26**, **28**, **30**, and **32**).

The chemistry used to synthesize C-6/C-7 fused analogues **33**−**36** (class C) is described in Scheme 5. Reaction of 2-bromo-3,4-dihydroxybenzaldehyde **65** with dibromomethane provided five-membered dioxolane containing benzaldehyde **66** (n = 1). Similarly, reaction of **65** with 1,1,2,2-tetrabromoethane provided six-membered dioxolane-containing benzaldehyde **66** (n = 2). Incorporation of boron in compound **66** was accomplished by reaction with pinacol diborane in the presence of a palladium catalyst to give boronate **67**. The Henry reaction of boronate **67** with nitromethane provided 3-nitromethyl-benzoxaborole **68**. Chlorination or bromination of **68** gave corresponding C-4 chloro- or bromobenzoxaborole **69**. The reduction of nitro group in compound **69** gave the C-4 chloro or bromo C-6/C-7 fused analogs **33**−**36**. Compounds **35** and **36** were further separated into their active enantiomers **35a** and **36a** by chiral SFC separation.

Structure−Activity Relationships. The compounds were evaluated in the *Mtb* LeuRS aminoacylation assay as well as in a whole cell activity assay against *Mtb* H37Rv (Table 1). Since cell penetration can vary between series, the MIC values of these *Mtb* LeuRS inhibitors against *Mtb* H37Rv were used to optimize the potency and drive SAR, while the biochemical

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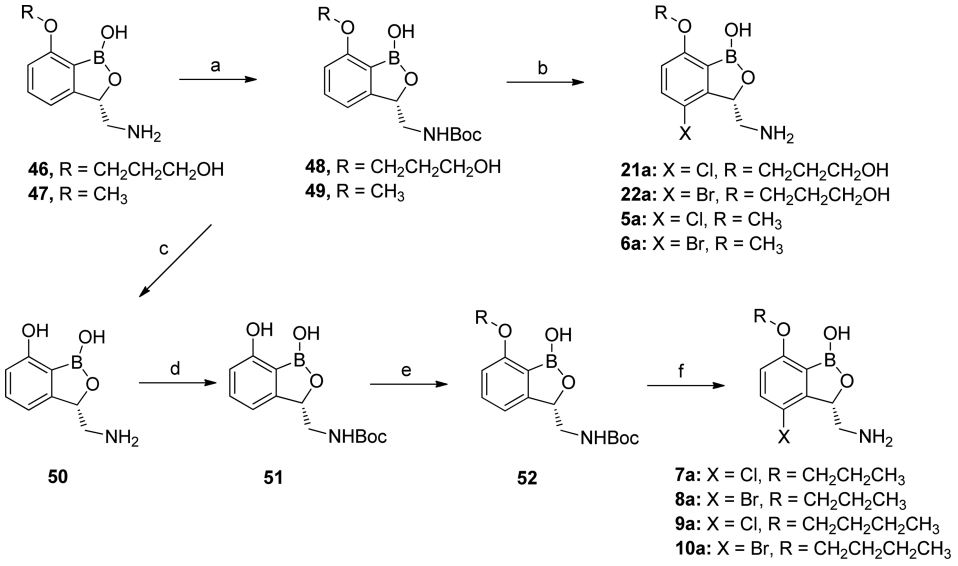
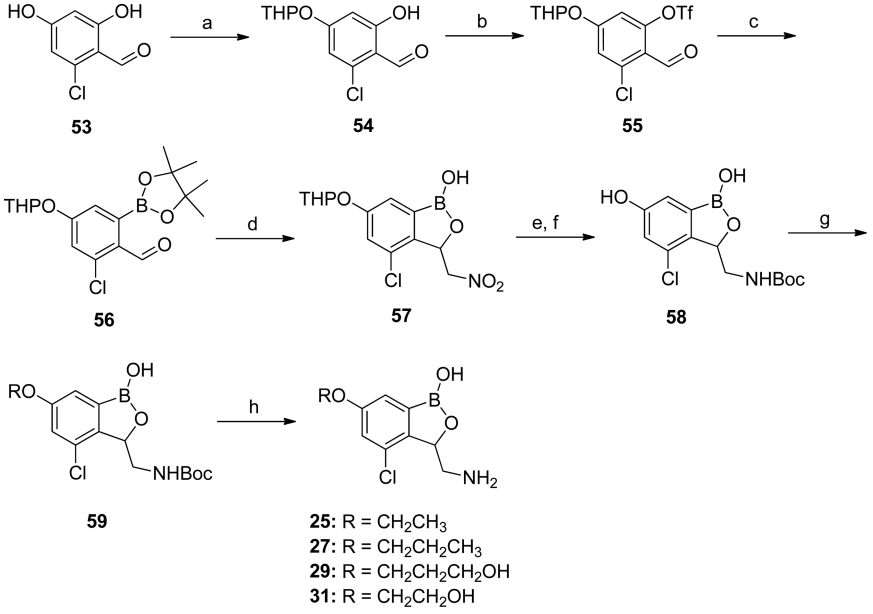
Scheme 2[a]



[a]Reagents and conditions: (a) di-*tert*-butyl dicarbonate, TEA, DCM, 0 °C; (b) (1) when X = Cl, NCS, DMF, heat; when X = Br, NBS, CH_3CN, heat; (2) TFA, DCM, then HCl; (c) BBr_3, DCM, −78 °C; (d) di-*tert*-butyl dicarbonate, TEA, DCM, 0 °C; (e) alkyl bromide, *t*-BuOK, DMSO, 90 °C; (f) (1) when X = Cl, NCS, DMF, heat or SO_2Cl_2, AcOH, rt; when X = Br, NBS, CH_3CN, heat; (2) TFA, DCM, then HCl.

Scheme 3[a]



[a]Reagents and conditions: (a) dihydropyran, PPTS (cat.), DCM; (b) Tf_2O, pyridine, DCM, 0 °C; (c) $(Pin)_2B_2$, $PdCl_2$(dppf), KOAc, THF, reflux; (d) CH_3NO_2, NaOH, THF; (e) conc HCl; (f) (1) Raney Ni, H_2, NH_3/EtOH, then HCl/EtOH; (2) di-*tert*-butyl dicarbonate, TEA, DCM, 0 °C; (g) alkyl bromide or alkyl iodide, K_2CO_3, DMF, heat; (h) TFA, DCM, then HCl.

inhibition was used to gauge whether the inhibitors were on target. Representative compounds with good antitubercular activity (*Mtb* H37Rv MIC < 0.2 μM) were selected and tested for selectivity in the human mitochondrial and cytoplasmic LeuRS biochemical assays as well as activity against cytoplasmic protein synthesis in intact human HepG2 cells (Table 2) to see if we could improve on the safety profile of the initial leads.

Most of compounds were made and tested as racemates first and separated into active (*S*)-isomers when racemates were very active (Table 1). Generally, the (*S*)-enantiomer is 2-fold more potent compared to the racemate and the (*R*)-enantiomer is completely inactive as exemplified by compounds **23**, **24**, **35**, and **36**. For these four compounds, the results from the racemate and both enantiomers were given for comparison.

We first explored the size of 7-alkoxyl side chain of the *Mtb* LeuRS inhibitors. The smaller 7-methoxy compounds **5a** and **6a** showed similar *Mtb* LeuRS potency and antitubercular activity, compared to 7-ethoxy leads **3a** and **4a**. Increasing the length of 7-alkoxy group provided 7-propoxy compounds **7a**/**8a** and butoxy compounds **9a**/**10a**. These analogues with larger 7-alkoxy substitutions exhibited decreased *Mtb* LeuRS potency and antitubercular activity by 2- to 5-fold, suggesting that

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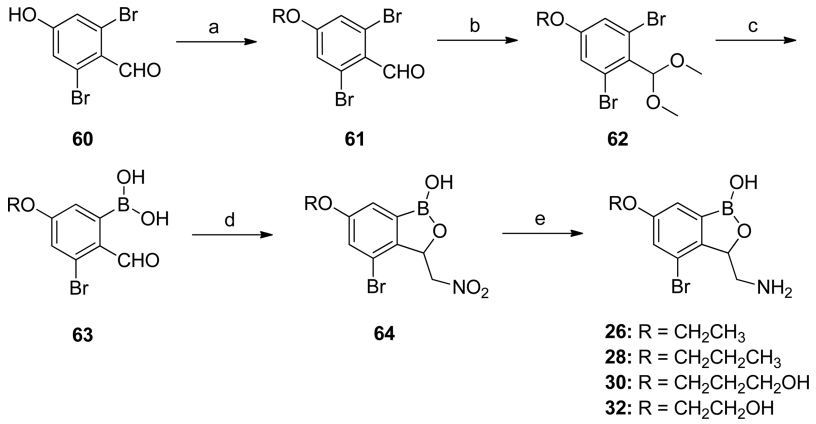
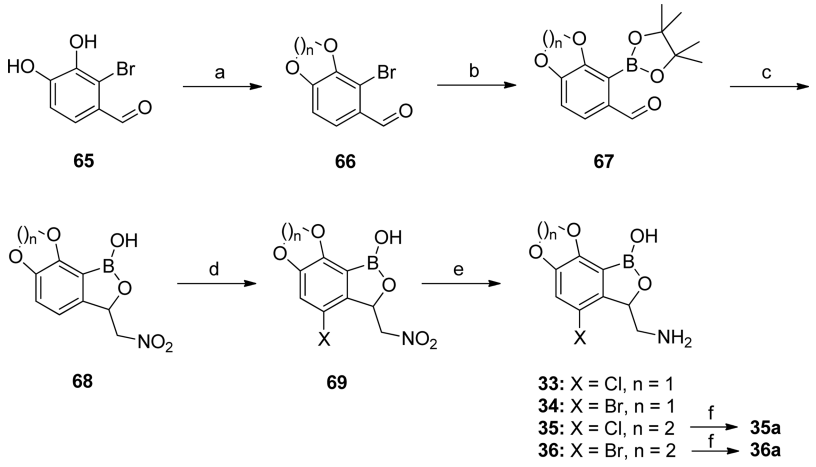
Scheme 4[a]



[a]Reagents and conditions: (a) alkyl bromide, K_2CO_3, DMF, heat; (b) $CH(OMe)_3$, H_2SO_4, MeOH, reflux; (c) (1) BuLi, $B(OMe)_3$, THF, −78 °C; (2) 2 N HCl, rt; (d) CH_3NO_2, NaOH, water/THF; (e) Raney Ni, H_2, NH_3/EtOH, then HCl/EtOH.

Scheme 5[a]



[a]Reagents and conditions: (a) CH_2Br_2 (n = 1) or $BrCH_2CH_2Br$ (n = 2), K_2CO_3, DMF, heat; (b) $(Pin)_2B_2$, KOAc, $PdCl_2$(dppf), dioxane, reflux; (c) CH_3NO_2, NaOH, THF; (d) when X = Cl, NCS, DMF, heat or SO_2Cl_2, AcOH, rt; when X = Br, NBS, CH_3CN; (e) Raney Ni, H_2, NH_3/EtOH, then HCl/EtOH; (f) chiral SFC separation.

adding size and lipophilicity at 7-position is not beneficial for biochemical and antitubercular activity. We also incorporated aromatic moieties in 7-substitution and made six analogues with one-, two, or three-atom linker between the two aromatics (**11**−**16**). These large compounds showed decent *Mtb* LeuRS activity; however, all of them lost antitubercular activity, suggesting that adding hydrophobic aromatics in the molecule was detrimental to their antitubercular activity. **5a** and **6a** were selected for in vitro selectivity evaluation.

Since the addition of fluorine atoms to drugs is well-known to have the potential to acquire additional interactions, modulate potency and selectivity of bioactive compounds, we did a fluorine scan of the 7-ethoxy side chain and explored the effects of C-7 fluorine substitution on *Mtb* LeuRS and antitubercular activity. Introduction of one fluorine atom in the 7-ethoxy group (**17** and **18**) resulted in approximately equivalent MIC values (considering compounds **17** and **18** are racemates). Introduction of two fluorine atoms in the 7-ethoxy group of compounds **19** and **20** resulted in slight decrease in antitubercular activity.

We also explored the impacts of a hydrophilic side chain at C-7 position on the potency of the *Mtb* LeuRS inhibitors. **21a** and **22a** with 3-hydroxypropoxy group at C-7 position maintained *Mtb* LeuRS and antitubercular activity. To further reduce the lipophilicity, the size of the three-carbon linker was reduced to a two-carbon linker giving **23** and **24**, both of which were very active. After chiral separation, their corresponding *S* enantiomers **23a** and **24a** were found to exhibit excellent antitubercular activity (MIC = 0.08 μM and 0.08 μM, respectively). Both **23a** and **24a** were selected for in vitro selectivity determinations. It is interesting to note that **23a** or **24a** is present as a seven-membered tricyclic ring form based on ^{1}H NMR in deuterated DMSO (Figure 3), suggesting that an equilibrium exists between open and close forms depending on solvent and environment. This dynamic equilibrium between open and closed forms of the benzoxaborole pharmacophore and its six- and seven-membered analogues has been studied by Hall and his group.[27]

We also expanded chemical space and synthesized a number of 6-substituted compounds **25**−**32** (class B), as described in Schemes 3 and 4. These 6-substituted analogs showed decent *Mtb* LeuRS activity in the range of 0.25−1.11 μM. However, they exhibited poor antitubercular activity compared to the corresponding 7-substituted analogs. Although **27** and **28**

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Table 1. In Vitro Activity of Compounds 1−36[a]

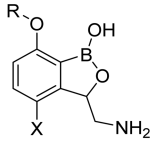
Compound	Structure			IC_{50} (μM) *Mtb* LeuRS	MIC (μM) *Mtb* H37Rv
	Configuration	R (or n)	X		
Class A					
1a	(*S*)	CH_2CH_3	H	0.13	0.6
2a	(*S*)	CH_2CH_3	F	0.08	0.2
3a	(*S*)	CH_2CH_3	Cl	0.056	0.1
4a	(*S*)	CH_2CH_3	Br	0.075	0.1
5a	(*S*)	CH_3	Cl	0.15	0.1
6a	(*S*)	CH_3	Br	0.074	0.1
7a	(*S*)	$CH_2CH_2CH_3$	Cl	0.31	0.16
8a	(*S*)	$CH_2CH_2CH_3$	Br	0.24	0.38
9a	(*S*)	$CH_2CH_2CH_2CH_3$	Cl	0.27	0.6
10a	(*S*)	$CH_2CH_2CH_2CH_3$	Br	0.20	0.45
11	racemate	Ph	Cl	0.34	5
12[b]	racemate	4-Br-Ph	Br	0.69	>5
13	racemate	CH_2Ph	Cl	0.32	5
14	racemate	CH_2Ph	Br	0.44	5
15	racemate	CH_2CH_2Ph	Cl	0.72	5
16	racemate	CH_2CH_2Ph	Br	0.47	>5
17	racemate	CH_2CH_2F	Cl	0.076	0.2
18	racemate	CH_2CH_2F	Br	0.26	0.2
19	racemate	CH_2CHF_2	Cl	0.29	0.3
20	racemate	CH_2CHF_2	Br	0.24	0.31
21a	(*S*)	$CH_2CH_2CH_2OH$	Cl	0.47	0.14
22a	(*S*)	$CH_2CH_2CH_2OH$	Br	0.16	0.3
23	racemate	CH_2CH_2OH	Cl	0.24	0.63
23a	(*S*)			0.20	0.08
23b	(*R*)			10	5
24	racemate	CH_2CH_2OH	Br	0.15	0.16
24a	(*S*)			0.12	0.08
24b	(*R*)			14.2	10

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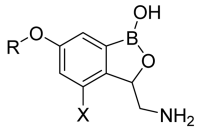
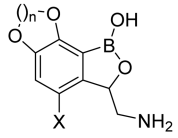
Table 1. continued

Compound	Structure			IC_{50} (μM) *Mtb* LeuRS	MIC (μM) *Mtb* H37Rv
	Configuration	R (or n)	X		
Class B					
25	racemate	CH_2CH_3	Cl	0.63	>5
26	racemate	CH_2CH_3	Br	0.26	3.75
27	racemate	$CH_2CH_2CH_3$	Cl	0.25	1.3
28	racemate	$CH_2CH_2CH_3$	Br	0.37	0.6
29	racemate	$CH_2CH_2CH_2OH$	Cl	0.44	>5
30	racemate	$CH_2CH_2CH_2OH$	Br	0.37	>5
31	racemate	CH_2CH_2OH	Cl	1.11	>5
32	racemate	CH_2CH_2OH	Br	0.89	>5
Class C					
33	racemate	n = 1	Cl	0.25	>5
34	racemate	n = 1	Br	0.61	>5
35	racemate	n = 2	Cl	0.13	0.31
35a	(*S*)			0.046	0.08
35b	(*R*)			12.6	80
36	racemate	n = 2	Br	0.092	0.5
36a	(*S*)			0.12	0.03
36b	(*R*)			4.2	40

[a]All compounds were prepared as HCl or TFA salts. Experimental procedures are described in the Experimental Section. Values are the mean of duplicate or more experiments. [b]Desired product with 7-phenoxy group (R = Ph) was not formed during the bromination step, and dibrominated product **12** with 7-(4-bromophenoxy) group was isolated instead.

showed some MIC against *Mtb* H37Rv (0.6−1.3 μM), further investigation of this subseries of *Mtb* LeuRS inhibitors was discontinued due its poor antitubercular activity.

In another subseries to expand chemical space (class C), we investigated a C-6/C-7 fused ring in the scaffold to determine the structure−activity relationship by making compounds **33**−**36**, as described in Schemes 5. While **33** and **34** with a five-membered dioxolane ring failed to demonstrate antitubercular activity (MIC > 5 μM), compounds **35** and **36** with a six-membered dioxane ring exhibited potent *Mtb* LeuRS and antitubercular activity. Presumably the dioxolane-containing compound **33** or **34** has an unfavorable conformation and/or electronics that resulted in their poor cellular activity. The dioxane-containing compounds **35** and **36** were separated into their respective enantiomers **35a** and **36a**, both of which exhibited potent antitubercular activity (MIC = 0.08 μM and 0.03 μM, respectively). **35a** and **36a** were selected for in vitro selectivity evaluation.

In Vitro Selectivity. Although in vitro potency against *Mtb* was not significantly improved over the initial analogs **3a** and **4a**, we evaluated the inhibitors against several different in vitro selectivity assays in order to improve their safety profile including a cell-based protein synthesis assay (Table 2). Fortunately these series were effectively inactive against human mitochondrial LeuRS with IC_{50} > 300 μM, which is presumably due to this enzyme being editing defective.[28] Compared to the leads **3a** and **4a**, the new inhibitors **5a**, **6a**, **23a**, **24a**, **35a** all exhibited improved selectivity against human cytoplasmic LeuRS and HepG2 protein synthesis assay by 2- to 9-fold, suggesting that decreasing lipophilicity or adding polarity in the molecule could translate into improved selectivity profiles. These results were consistent with and further supported by our HepG2 cell toxicity EC_{50} data (48 h)

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Table 2. In Vitro Selectivity Data for Representative Compounds[a]

compd	human mito LeuRS IC_{50} (μM)	human cytoplasm LeuRS IC_{50} (μM)	HepG2 protein synthesis EC_{50} (μM)	selectivity index[b]	HepG2 cell 48 h EC_{50} (μM)[c]
3a	>300	38.8	19.6	196	65.8
4a	>300	66.9	30.5	305	47.1
5a	>300	260	105	1050	327
6a	>300	>300	97	970	74.5
23a	>300	132	137	1713	381
24a	>300	118	116	1450	292
35a	NT[d]	96.6	82.8	1035	69.5
36a	NT[d]	118	39.5	1317	43.2

[a]The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents. [b]Selectivity index is defined as a ratio of HepG2 protein synthesis EC_{50} over MIC against *Mtb* H37Rv. Values are the mean of duplicate or more experiments. [c]Readout performed with CellTiter Glo luminescent viability assay kit (Promega). [d]NT = not tested.

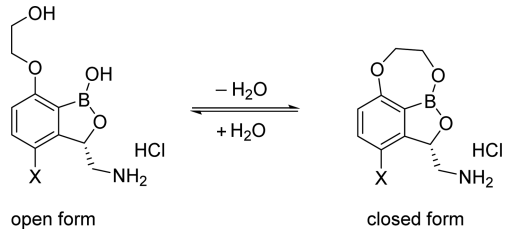

Figure 3. 3-Aminomethyl 4-halogen benzoxaborole inhibitors **23a** and **24a**.

obtained with these *Mtb* LeuRS inhibitors. The compounds **5a**, **23a**, and **24a** exhibited the least activity against HepG2 cells and thus were advanced further for in vivo testing. Also, it is worthy to note that little difference exists in activity and selectivity profiles between 4-Cl compound and 4-Br counterpart, as exemplified by **3a** vs **4a** and **23a** vs **24a**. Compounds **6a**, **23a**, and **35a** with different structural features (7-methoxy, 7-hydroxyethoxy, and 6-/7-fused analog) were chosen for drug metabolism and pharmacokinetics (DMPK) studies.

DMPK. The PK of new compounds **6a**, **23a**, and **35a** were evaluated in mice (Table 3). All three new compounds exhibited low clearance and excellent exposure, which are consistent with previous PK results of **4a**. The dose-normalized (DN) AUC values for **6a**, **23a**, and **35a** were 1.17, 2.03, and 2.54 h·μg/mL, respectively. After oral administration, the DNAUC values of **6a**, **23a**, and **35a** were 0.84, 2.96, and 1.26 h·μg/mL, corresponding to bioavailability of 72%, 100%, and 50%, respectively. These *Mtb* LeuRS inhibitors show low molecular weight, low polar surface area (PSA), and $clogD_{7.4}$ value similar to frontline TB drugs isoniazid, pyrazinamide, and ethambutol. We believe that the physicochemical properties of these compounds (such as low molecular weight, low PSA, and favorable $clogD_{7.4}$) contribute to their remarkable exposure and bioavailability in the in vivo PK assays.

In Vivo Efficacy. To evaluate the ability of these *Mtb* LeuRS inhibitors to treat tuberculosis, we examined **6a**, **23a**, and **35a** in an animal TB efficacy model. The results from a murine acute TB infection model are shown in Table 4. In this TB infection model, **23a** showed the best efficacy with ED_{99} (efficacious dose that gives 2 log colony-forming units (CFU) reduction compared to the untreated control) of 0.4 mg/kg among the four compounds evaluated. **6a** is 10-fold less potent

Table 4. Efficacy in a Mouse Model of Acute TB Infection[a]

	4a[b]	**6a**	**23a**	**35a**
ED_{99} (mg/kg)	<1.0	3.8	0.4	70

[a]Efficacy of compounds in a mouse model of acute TB infection under QD dosing regimen (once a day). C57BL/6J mice were infected with *M. tuberculosis* H37Rv intratracheally (~10^5 CFU) and were dosed starting on the following day after infection for 8 days. Mice were sacrificed at least 24 h after the last drug administration. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals. [b]Efficacy data for **4a** was reported previously in ref 23.

Table 3. Physicochemical Properties and PK Parameters[a]

parameter	**4a**[b]	**4a**[c]	**6a**[b]	**23a**[c]	**35a**[c]
MW	285.9		271.9	239.5	255.5
$clogD_{7.4}$	0.95		0.60	−0.4	−0.10
PSA	64.71		64.71	53.71	73.94
dose (mg/kg), iv	30	28.5	5.0	5.5	30.0
V_{ss} (L/kg)	3.2	1.7	2.5	2.3	1.5
Cl (mL min^{-1} kg^{-1}), iv	11.45	6.6	14.2	8.5	6.6
$t_{1/2}$ (h)	3.4	3.1	2.4	3.6	2.9
AUC_{0-inf} (h·μg/mL), iv	43.4	72.3	5.84	11.12	76.32
$DNAUC_{0-inf}$ (h·μg/mL per mg/kg), iv	1.45	2.54	1.17	2.03	2.54
dose (mg/kg), po	30	26.1	5.0	30.0	30.0
C_{max} (μg/mL)	6.3	11.14	0.98	17.76	6.21
T_{max} (h)	0.5	2.0	0.25	0.9	1.0
AUC_{0-24h} (h·μg/mL), po	57.6	77.86	4.21	88.24	37.67
$DNAUC_{0-24h}$ (h·μg/mL), po	1.92	2.98	0.84	2.94	1.26
F (%)	~100	~100	72	~100	50

[a]All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals. [b]Mouse PK studies were conducted by using CD-1 mice as described in ref 23. PK values for **4a** by using CD-1 mice were from ref 23 and are presented for comparison. [c]Mouse PK studies were conducted by using C57 mice as described in the Experimental Section.

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Table 5. Efficacy in a Mouse Model of Chronic TB Infection[a]

	no treatment		linezolid	23a					
dose (mg/kg)			100	0.1	0.3	1	3	10	30
$\log_{10}$ CFU	5.8[b] (0.3)	6.4[c] (0.3)	3.9[d] (0.2)	6.4 (0.6)	6.0 (0.1)	4.7[d] (0.2)	4.6[d] (0.2)	4.3[d] (0.1)	4.4[d] (0.2)

[a]Efficacy of compounds in a mouse model of chronic TB infection under QD dosing regimen (once a day). C57BL/6J mice were infected with *M. tuberculosis* H37Rv intratracheally (~10^2 CFU) and were dosed once daily for 8 weeks starting 6 weeks after infection. Mice were sacrificed at least 24 h after the last drug administration. Every data point represents the mean values (±standard deviations) from 7 mice per group for untreated and linezolid-treated groups and from 3 mice for **23a**-treated groups. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals. [b]6 weeks after infection. [c]14 weeks after infection. [d]Statistically significant difference compared to 14-week untreated mice ($p < 0.05$, ANOVA, Dunnett's multiple comparison test).

in terms of dose with ED_{99} of 3.8 mg/kg, compared to **23a**. **35a** showed a significant decrease in efficacy with ED_{99} of 70 mg/kg (>100-fold). The reason for an in vitro and in vivo disconnect of **35a** is not clear and is apparently not related to a lack of in vitro potency or in vivo exposure. Unlike with **4a**, which was not tolerated at 50 mg/kg QD, **23a** was well tolerated at the maximum dose tested of 100 mg/kg. In a maximum single tolerated dose study in healthy mice, **23a** was well tolerated at 300 mg/kg and **4a** was not tolerated at the lowest dose tested of 150 mg/kg. Therefore, further evaluations of **23a** in a murine chronic TB infection model were performed in parallel with the protein synthesis inhibitor linezolid (Table 5). **23a** exhibited a dose-dependent reduction in CFU from 0.1 mg/kg to 10 mg/kg QD. Importantly, 10 mg/kg QD resulted in 2.1 $\log_{10}$ reduction in CFU compared with a 2.5 $\log_{10}$ reduction in CFU for 100 mg/kg of linezolid QD. From these studies, **23a** was identified as having the best overall profiles, with remarkable oral bioavailability and in vivo efficacy at low doses in acute and chronic mouse TB infection models.

CONCLUSIONS

In this work, we designed and synthesized a new series of *Mtb* LeuRS inhibitors by investigating various substituents in the aromatic ring of the 3-aminomethyl 4-halogen benzoxaborole scaffold. The SARs which include the influence of substitution size, nature, and pattern at positions 6 and 7 were explored, leading to the identification of a number of *Mtb* LeuRS inhibitors with excellent antitubercular potency in the mid-nanomolar range. These *Mtb* LeuRS inhibitors exhibited improved selectivity over human cytoplasmic LeuRS as well as protein synthesis inhibition in intact human cells over the initial leads. Moreover, the superior tolerability of the new compounds has enabled their further progression and ultimately the selection of a clinical candidate. These *Mtb* LeuRS inhibitors exhibited remarkable oral exposure, with small molecular weight and low clogD similar to frontline TB drugs isoniazid, pyrazinamide, and ethambutol. From these compounds, **23a** was identified as having the best overall profiles, with excellent in vivo efficacy at low doses in acute and chronic mouse TB infection models. As a result, **23a** has been progressed to clinical development for the treatment of tuberculosis (ClinicalTrials.gov identifier NCT03075410). Future publications will report other preclinical studies and clinical trial results.

EXPERIMENTAL SECTION

LeuRS Aminoacylation Assay. An N-terminal six histidine-tagged LeuRS from *M. tuberculosis* H37Rv and humans (cytoplasmic and mitochondrial) were codon-optimized for *E. coli* (GenScript, Piscataway, NJ, USA) and overexpressed and purified according to Novagen (Madison, WI, USA) using an *E. coli* BL21(DE3) T7 RNA polymerase overexpression strain. Aminoacylation assay was performed in 96-well microtiter plates, using 80 μL of reaction mixtures containing 50 mM HEPES–KOH (pH 8.0), 30 mM $MgCl_2$ 30 mM KCl, 13 μM L-[^{14}C]leucine (306 mCi/mmol, PerkinElmer), 15 μM total *E. coli* tRNA (Roche, Switzerland), 0.02% (w/v) BSA, 1 mM DTT, 0.2 pM LeuRS, and 4 mM ATP at 30 °C. Reactions were started by the addition of 4 mM ATP. After 7 min, reactions were quenched and tRNA was precipitated by the addition of 50 μL of 10% (w/v) TCA and transferred to 96-well nitrocellulose membrane filter plates (Millipore Multiscreen HTS, MSHAN4B50). Each well was then washed three times with 100 μL of 5% TCA. Filter plates were then dried under a heat lamp, and the precipitated L-[^{14}C]leucine $tRNA^{Leu}$ was quantified by liquid scintillation counting using a Wallac MicroBeta Trilux model 1450 liquid scintillation counter (PerkinElmer, Waltham, MA, USA). To determine the inhibitor concentration, which reduces enzyme activity by 50% (IC_{50}), increasing concentrations of compound inhibitors that covered the IC_{50} value were incubated with LeuRS enzyme, tRNA, and L-leucine for 20 min. Reactions were initiated by the addition of 4 mM ATP. Reactions were stopped after 7 min and then precipitated and counted to quantify radioactivity. IC_{50} values were determined using a four-parameter logistic nonlinear regression model (GraphPad Software Inc. (La Jolla, CA, USA). The only difference was with the human cytoplasmic LeuRS aminoacylation assay when we used tRNA isolated from Brewer's yeast (Roche Diagnostics GmbH).

Determination of MIC for *M. tuberculosis*. *Mycobacterium tuberculosis* H37Rv was grown at 37 °C in Middlebrook 7H9 broth (Difco) supplemented with 0.025% Tween 80 and 10% albumin-dextrose-catalase (ADC) or on Middlebrook 7H10 plates supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC). The antitubercular activity against extracellular of intracellular *Mycobacterium* strains was performed as previously described.[29]

HepG2 Protein Synthesis EC_{50} Determination. Human liver carcinoma HepG2 cells were grown in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 50 units/mL penicillin–streptomycin at 37 °C with 5% CO_2. HepG2 cells were seeded in 96-well plates at 3000 cells/200 μL/well in cell culture medium. Stock solutions of compounds were made in DMSO. Increasing concentrations of compounds were added with the final DMSO concentration being 0.5%. Vehicle-treated samples had DMSO added at 0.5% with no compound. Cells were then grown in the presence of the compounds at 37 °C in 10% CO_2 for 2 days. After 48 h, the medium was removed and then replaced by fresh medium containing 0.5 μCi of L-[^{14}C]leucine and the same concentrations of compounds. After incubation for 3 h, the medium was removed and 50 μL of trypsin–EDTA solution was added to each well. After 15 min, the trypsinized cells were transferred to a 96-well plate containing ice-cold 20% (w/v) TCA. After 60 min on ice the samples were collected over vacuum on 96-well glass fiber filter plate (Millipore, MSFBNB50) and washed three times with 150 μL of ice-cold 10% (w/v) TCA. To the dried filter plate 40 μL of scintillation cocktail was added, and counts were obtained in Wallac MicroBeta Trilux model 1450 liquid scintillation counter (PerkinElmer, Waltham, MA). IC_{50} values were determined by curve-fitting using the Prism by GraphPad (San Diego, CA, USA).

HepG2 Cytotoxicity Assay. HepG2 (HB-8065) cells were cultured with fresh medium (essential minimum Eagle medium,

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EMEM, supplemented with 5% fetal calf serum and 2 mM L-glutamine) the day before subculturing the plates. On the day of the assay, cells (10 000 cells/well) were seeded in a black 96-well collagen coated microplate with clear bottom, (Becton Dickinson) except in column 11, which was dispensed only 100 mL of culture medium. Stock solution from each test substances was prepared in 100% DMSO. Ten serial 1:2 dilutions were prepared of each test compound, and finally, a 1:200 dilution was made, in medium, to achieve a final concentration of 0.5% of DMSO. Resazurin tablets (Merck) were dissolved in phosphate buffer saline at a concentration of 0.0042%. After 24 h of incubation of the cells (37 °C, 5% CO_2, 95% relative humidity), a volume of 150 μL of culture medium containing the appropriate test concentrations of the compounds dilutions was added to cells in two replicates. In column 12, only 150 μL of 0.5% DMSO was added (blank control). Then, cells were exposed to test compounds for 48 h. After that, medium was removed and resazurin solution was added to each well and incubated for further 1.5 h. Fluorescence was measured at an excitation wavelength of 515 nm and an emission wavelength of 590 nm in a Microplate reader 1420 Multilabel HTS counter, Victor 2 (Wallac). Data were processed using an Excel spreadsheet and GraphPad software analysis. Tox50 values were calculated from the Sigmoidal dose−response (variable slope) curves by nonlinear regression analysis.

Procedure for PK Studies. For pharmacokinetic studies, C57BL/6 female mice (18−20 g) were used, and compound concentrations were determined using peripheral whole blood. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals.

4a was administered by intravenous route at 30 mg/kg single dose in saline and by oral gavage at 30 mg/kg single dose in 1% methylcellulose (1% MC). For iv route aliquots of 25 μL of blood were taken from the lateral tail vein by puncture from each mouse (n = 5) at 5, 15, and 30 min and 1, 2, 4, and 8 h postdose; for oral route aliquots of 25 μL of blood were taken by cardiac puncture for each mouse (euthanized by CO_2, n = 3 mice per time point) at 15, 30, and 45 min and 1, 1.5, 2, 3, 4, 8, and 24 h postdose.

23a was administered by intravenous route at 5 mg/kg single dose in saline and by oral gavage at 30 mg/kg single dose in 1% methylcellulose (1% MC). For iv route aliquots of 15 μL of blood were taken from the lateral tail vein by puncture from each mouse (n = 3) at 5, 15, and 30 min and 1, 2, 4, 8, and 24 h postdose; for oral route aliquots of 15 μL of blood were taken from the lateral tail vein by puncture from each mouse (n = 3) at 15, 30, and 45 min and 1, 2, 4, 8, and 24 h postdose.

35a was administered by intravenous route at 30 mg/kg single dose in saline and by oral gavage at 30 mg/kg single dose in 1% methylcellulose (1% MC). For iv route aliquots of 15 μL of blood were taken from the lateral tail vein by puncture from each mouse (n = 3) at 5, 15, and 30 min and 1, 2, 4, 8, and 24 h postdose; for oral route aliquots of 15 μL of blood were taken from the lateral tail vein by puncture from each mouse (n = 3) at 15, 30, and 45 min and 1, 2, 4, 8, and 24 h postdose.

LC−MS/MS was used as the analytical method for the establishment of compound concentration in blood. Pharmacokinetic analysis was performed by noncompartmental data analysis (NCA) with Phoenix WinNonlin 6.3 (Pharsight, Certara L.P.), and supplementary analysis was performed with GraphPad Prism 6 (GraphPad Software, Inc.).

Murine Model of Acute and Chronic TB Infections. Specific pathogen-free, 8- to 10-week-old female C57BL/6 mice were purchased from Harlan Laboratories and were allowed to acclimate for 1 week. The experimental design for the acute assay has been previously described.[30] In brief, mice were intratracheally infected with 100 000 CFU/mouse of *M. tuberculosis* H37Rv. Compounds were administered for 8 consecutive days starting 1 day after infection. For the chronic assay, mice were intratracheally infected with 100 CFU/mouse and the products administered daily (7 days a week) for 8 consecutive weeks starting 6 weeks after infection. Lungs were harvested 24 h after the last administration, and all lung lobes were aseptically removed, homogenized, and frozen. Homogenates were plated onto 10% OADC−Middlebrook 7H11 medium and incubated for 21 days at 37 °C. All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals. The viable CFU were converted to logarithms, which were then evaluated by one-way analysis of variance, followed by multiple-comparison analysis of variance by a one-way Tukey test (SigmaStat software program). Differences were considered significant at the 95% level of confidence.

Chemical Synthesis. Starting materials used were either commercially available or prepared according to literature procedures and had experimental data in accordance with those reported. High performance liquid chromatography (HPLC) was used to determine the purity of the compounds synthesized. The data confirmed that the target compounds generally had ≥95% of purity with the exception of compounds **8** and **30**. ^{1}H NMR spectra were recorded on a Varian or Bruker 300 or 400 MHz spectrometer. Chemical shifts were reported in ppm and were referenced to the appropriate residual solvent signals, such as 2.49 ppm for DMSO-d_6 and 7.26 ppm for $CDCl_3$. LC−MS data were obtained using an Agilent LC−MS 1200 with 6110 MS detector. The mass spectrometer was equipped with an electrospray ion source (ESI) operated in a positive or negative mode. Flash column chromatography was typically preformed using silica gel 60 (230−400 mesh). Typical solvents used for flash chromatography or thin layer chromatography (TLC) were mixtures of MeOH/DCM and EtOAc/petroleum ether. HPLC analysis was performed on a Gilson HPLC system using a Gemini 5 μm C18 column (150 mm × 4.6 mm i.d.) or a Venusil MP C18 3 μm C18 column (100 mm × 4.6 mm i.d.). Purification using prep-HPLC was accomplished using a Gilson HPLC system with a Gemini 5 μm C18 column (150 mm × 21.2 mm i.d.). Typically, the mobile phase used for HPLC was a linear gradient of water (A) and acetonitrile (B). The water and acetonitrile were mixed with 0.1% TFA or 0.1% HCOOH. The flow rate was maintained at 0.8−1.2 mL/min for analytic HPLC and 20 mL/min for prep-HPLC, and the eluent was monitored with UV detector at 220 nm. Chiral separation was performed on a Thar preparative SFC 80 system with a Daicel Chiralpak AD-H 5 μm (250 mm × 20 mm i.d.), eluted with carbon dioxide and methanol mobile phase. The flow rate was maintained at 65−70 mL/min, and eluent was monitored with UV detector at 220 nm. For enantiomeric excess determination, chiral HPLC analysis was performed on a Thar analytical SFC system with a Chiralpak AD-H 5 μm (150 mm × 4.6 mm i.d.) or a ChiralCel OJ-H 5 μm (150 mm × 4.6 mm i.d.) or a ChiralPak AY-H 5 μm (150 mm × 4.6 mm i.d.), eluted with carbon dioxide and methanol mobile phase at a flow rate of approximately 2.4−4 mL/min. The ee of all tested enantiomers was >97%.

(*S*)-3-(Aminomethyl)-4-chloro-7-methoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (5a). This compound was prepared by the similar method described below for the synthesis of **21a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (b, 1H), 8.20 (b, 3H), 7.54 (d, 1H, J = 9.0 Hz), 7.01 (d, 1H, J = 9.0 Hz), 5.33 (m, 1H), 3.82 (s, 3H), 3.56 (m, 1H), 2.92 (m, 1H). MS (ESI) m/z = 228 $[M + H]^+$. HPLC purity: 99.9% (220 nm). **5a** had >99% ee based on starting material **47**.

(*S*)-3-(Aminomethyl)-4-bromo-7-methoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (6a). This compound was prepared by the similar method described below for the synthesis of **22a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (b, 1H), 8.41 (b, 3H), 7.66 (d, 1H, J = 8.8 Hz), 6.94 (d, 1H, J = 8.4 Hz), 5.34 (m, 1H), 3.81 (s, 3H), 3.62 (m, 1H), 2.85 (m, 1H). MS (ESI) m/z = 272 $[M + H]^+$. HPLC purity: 99.7% (220 nm). **6a** had >99% ee based on starting material **47**.

(*S*)-3-(Aminomethyl)-4-chloro-7-propoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (7a). To a solution of (*S*)-3-(aminomethyl)-7-(3-hydroxypropoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol hydrochloride **46** (30 g, 0.11 mol) and triethylamine (33.3 g, 0.33 mol) in DCM (300 mL) at 0 °C was added di-*tert*-butyl dicarbonate (36 g, 0.165 mol). The mixture was stirred at rt for overnight. The solvent was removed to afford (*S*)-*tert*-butyl (1-hydroxy-7-(3-hydroxy-

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propoxy)-1,3-dihydrobenzo[*c*][1,2]oxaborol-3-yl)methylcarbamate as a white solid (38 g, yield 100%). To a stirred solution of the above compound (10 g, 29.7 mmol) in CH_2Cl_2 (30 mL) at −78 °C was added BBr_3 (8.3 mL, 89.1 mmol) dropwise. The reaction was stirred at −78 °C for 2 h and stirred at rt overnight. The mixture was poured into ice−water and extracted with DCM. The aqueous layer was adjusted to pH = 8 with sat. $NaHCO_3$, and di-*tert*-butyl dicarbonate (6.5 g, 29.7 mmol) was added. The reaction mixture was stirred at rt overnight and then extracted with EtOAc (3 × 50 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give (*S*)-*tert*-butyl (1,7-dihydroxy-1,3-dihydrobenzo[*c*][1,2]-oxaborol-3-yl)methylcarbamate (3 g, 36%). To a solution of the above compound (600 mg, 2.15 mmol) in 10 mL of DMSO at 0 °C under N_2 was added *t*-BuOK (362 mg, 3.2 mmol). The reaction mixture was stirred at rt for 30 min, followed by the addition of 1-bromopropane (1.1 mL, 1.4 g, 10.7 mmol). The reaction mixture was stirred at rt for 12 h. After the reaction was quenched with ice−water, the mixture was extracted with EtOAc (50 mL). The organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography, eluting with EtOAc and petroleum ether (1:3) to give (*S*)-*tert*-butyl (1-hydroxy-7-propoxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-3-yl)-methylcarbamate as colorless oil (110 mg, 16%). A solution of the above compound (440 mg, 1.37 mmol) and *N*-chlorosuccinimide (183 mg, 1.37 mmol) in 20 mL of CH_3CN was stirred at 90 °C for 1 h. The mixture was concentrated in vacuo. The residue was purified by prep-HPLC, eluting with water/acetonitrile gradient to give (*S*)-*tert*-butyl (4-chloro-1-hydroxy-7-propoxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-3-yl)methylcarbamate as a white solid (89 mg, 18%). To a solution of the above compound (89 mg, 0.25 mmol) in 10 mL of DCM was added TFA (1 mL). The reaction mixture was stirred at rt for 1 h and then concentrated in vacuo. After 1 M HCl in Et_2O (10 mL) was added, it was stirred for 1 h. The residue was filtered and dried in vacuo to give (*S*)-7 as a white solid (71 mg, 97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (b, 1H), 8.19 (b, 3H), 7.50 (d, 1H, J = 8.6 Hz), 6.99 (d, 1H, J = 8.7 Hz), 5.38 (m, 1H), 4.00 (t, 2H), 3.57 (m, 1H), 2.94 (m, 1H), 1.75 (m, 2H), 0.99 (t, 3H). MS (ESI) m/z = 256 $[M + H]^+$. HPLC purity: 96.3% (220 nm). **7a** had >99% ee based on the use of starting material **46**.

(*S*)-3-(Aminomethyl)-4-bromo-7-propoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (8a). This compound was prepared by the similar method described above for the synthesis of **7a**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.09 (b, 1H), 8.03 (b, 3H), 7.63 (d, 1H, J = 8.6 Hz), 6.93 (d, 1H, J = 8.7 Hz), 5.26 (m, 1H), 3.98 (t, 2H), 3.63 (m, 1H), 3.00 (m, 1H), 1.75 (m, 2H), 0.98 (t, 3H). MS (ESI) m/z = 300 $[M + H]^+$. HPLC purity: 93.1% (220 nm). **8a** had >99% ee based on starting material **46**.

(*S*)-3-(Aminomethyl)-7-butoxy-4-chlorobenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (9a). This compound was prepared by the similar method described above for the synthesis of **7a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (b, 1H), 8.11 (b, 3H), 7.50 (d, 1H, J = 8.4 Hz), 6.99 (d, 1H, J = 8.4 Hz), 5.36 (m, 1H), 4.04 (t, 2H), 3.57 (m, 1H), 2.96 (m, 1H), 1.72 (m, 2H), 1.47 (m, 2H), 0.94 (t, 3H). MS (ESI) m/z = 270 $[M + H]^+$. HPLC purity: 97.2% (220 nm). **9a** had >99% ee based on starting material **46**.

(*S*)-3-(Aminomethyl)-7-butoxy-4-bromobenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (10a). This compound was prepared by the similar method described above for the synthesis of **7a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (b, 1H), 8.11 (b, 3H), 7.64 (d, 1H, J = 8.8 Hz), 6.96 (d, 1H, J = 8.4 Hz), 5.28 (m, 1H), 4.04 (t, 2H), 3.61 (m, 1H), 2.96 (m, 1H), 1.71 (m, 2H), 1.45 (m, 2H), 0.94 (t, 3H). MS (ESI) m/z = 314 $[M + H]^+$. HPLC purity: 99.2% (220 nm). **10** had >99% ee based on starting material **46**.

3-(Aminomethyl)-4-chloro-7-phenoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (11). This compound was prepared by the similar method described below for the synthesis of **23**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.57−7.29 (m, 3H), 7.17 (t, 1H, J = 7.4 Hz,), 7.06−6.97 (m, 2H), 6.79 (d, 1H, J = 8.6 Hz,), 5.51 (m, 1H), 3.88 (m, 1H), 3.08 (m, 1H). MS (ESI) m/z = 290 $[M + H]^+$. HPLC purity: 98.0% (220 nm).

3-(Aminomethyl)-4-bromo-7-(4-bromophenoxy)benzo[*c*]-[1,2]oxaborol-1(3*H*)-ol Hydrochloride (12). This compound was prepared by the similar method described below for the synthesis of **24**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.66−7.63 (d, 1H, J = 8.5 Hz), 7.51−7.46 (t, 2H, J = 7.9 Hz), 6.94−6.91 (d, 2H, J = 8.8 Hz), 6.85−6.80 (t, 1H, J = 9.0 Hz), 5.44 (m, 1H), 3.94 (m, 1H), 3.09 (m, 1H). MS (ESI) m/z = 414 $[M + H]^+$. HPLC purity: 98.0% (220 nm).

3-(Aminomethyl)-7-(benzyloxy)-4-chlorobenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (13). This compound was prepared by the similar method described below for the synthesis of **23**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.20 (*b*, 1H), 8.29 (*b*, 3H), 7.49−7.31 (m, 6H), 7.04 (d, 1H, J = 8.8 Hz), 5.41 (m, 1H), 5.23 (s, 2H), 3.57 (m, 1H), 2.94 (m, 1H). MS (ESI) m/z = 304 $[M + H]^+$. HPLC purity: 96.8% (220 nm).

3-(Aminomethyl)-7-(benzyloxy)-4-bromobenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (14). This compound was prepared by the similar method described below for the synthesis of **24**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.62 (d, 1H, J = 8.0 Hz), 7.52−7.32 (m, 5H), 7.00 (d, 1H, J = 8.0 Hz), 5.41 (m, 1H), 5.21 (s, 2H), 3.94 (m, 1H), 3.07 (m, 1H). MS (ESI) m/z = 348 $[M + H]^+$. HPLC purity: 99.6% (220 nm).

3-(Aminomethyl)-4-chloro-7-phenethoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (15). This compound was prepared by the similar method described below for the synthesis of **23**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.45 (d, 1H, J = 8.0 Hz), 7.38 (d, 2H, J = 8.0 Hz), 7.31 (*m*, 2H), 7.22 (*m*, 1H), 6.97 (d, 1H, J = 8.0 Hz), 5.43 (*m*, 1H), 4.27 (t, 2H), 3.83 (*m*, 1H), 3.11 (t, 3H), 3.03 (m, 1H). MS (ESI) m/z = 318 $[M + H]^+$. HPLC purity: 99.6% (220 nm).

3-(Aminomethyl)-4-bromo-7-phenethoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (16). This compound was prepared by the similar method described below for the synthesis of **24**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.60 (d, 1H, J = 8.0 Hz), 7.37 (d, 2H, J = 8.0 Hz), 7.30 (*m*, 2H), 7.22 (*m*, 1H), 6.91 (d, 1H, J = 8.0 Hz), 5.37 (*m*, 1H), 4.25 (t, 2H), 3.91 (*m*, 1H), 3.10 (t, 3H), 3.01 (m, 1H). MS (ESI) m/z = 362 $[M + H]^+$. HPLC purity: 100% (220 nm).

3-(Aminomethyl)-4-chloro-7-(2-fluoroethoxy)benzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (17). This compound was prepared by the similar method described below for the synthesis of **23**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.46 (d, 1H, J = 9.0 Hz), 7.00 (d, 1H, J = 9.0 Hz), 5.43 (m, 1H), 4.81 (m, 1H), 4.65 (m, 1H), 4.35 (m, 1H), 4.28 (m, 1H), 3.81 (m, 1H), 3.04 (m, 1H). MS (ESI) m/z = 260 $[M + H]^+$. HPLC purity: 100% (220 nm).

3-(Aminomethyl)-4-bromo-7-(2-fluoroethoxy)benzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (18). This compound was prepared by the similar method described below for the synthesis of **24**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.61 (d, 1H, J = 9.0 Hz), 6.94 (d, 1H, J = 9.0 Hz), 5.36 (m, 1H), 4.81 (m, 1H), 4.65 (m, 1H), 4.37 (m, 1H), 4.27 (m, 1H), 3.89 (m, 1H), 3.04 (m, 1H). MS (ESI) m/z = 304 $[M + H]^+$. HPLC purity: 97.8% (220 nm).

3-(Aminomethyl)-4-chloro-7-(2,2-difluoroethoxy)benzo[*c*]-[1,2]oxaborol-1(3*H*)-ol Hydrochloride (19). This compound was prepared by the similar method described below for the synthesis of **23**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.48 (d, 1H, J = 9.0 Hz), 7.02 (d, 1H, J = 9.0 Hz), 6.35 (m, 1H), 5.44 (m, 1H), 4.35 (m, 2H), 3.80 (m, 1H), 3.05 (m, 1H). MS (ESI) m/z = 278 $[M + H]^+$. HPLC purity: 99.4% (220 nm).

3-(Aminomethyl)-4-bromo-7-(2,2-difluoroethoxy)benzo[*c*]-[1,2]oxaborol-1(3*H*)-ol Hydrochloride (20). This compound was prepared by the similar method described below for the synthesis of **24**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.27 (b, 1H), 8.32 (b, 3H), 7.65 (d, 1H, J = 9.0 Hz), 7.00 (d, 1H, J = 9.0 Hz), 6.40 (m, 1H), 5.33 (m, 1H), 4.40 (m, 2H), 3.58 (m, 1H), 2.92 (m, 1H). MS (ESI) m/z = 322 $[M + H]^+$. HPLC purity: 98.3% (220 nm).

(*S*)-3-(Aminomethyl)-4-chloro-7-(3-hydroxypropoxy)benzo-[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (21a). This compound was prepared by the similar method described above for the synthesis of **7a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (b, 3H), 7.43−7.41 (d, 1H, J = 8 Hz), 6.96−6.94 (d, 1H, J = 8 Hz), 5.45 (m, 1H), 4.39 (m, 2H), 4.16 (m, 2H), 3.60 (m, 1H), 3.10−2.80 (m, 1H), 2.00 (m, 2H). MS (ESI) m/z = 272 $[M + H]^+$. HPLC purity: 98.3% (220 nm). **21a** had >99% ee based on starting material **46**.

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(*S*)-3-(Aminomethyl)-4-bromo-7-(3-hydroxypropoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (22a). This compound was prepared by the similar method described above for the synthesis of **8a**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (b, 3H), 7.65−7.63 (d, 1H, J = 8.0 Hz), 6.96−6.93 (d, 1H, J = 8.4 Hz), 5.29 (m, 1H), 4.11 (m, 2H), 3.70−3.50 (m, 3H), 2.91 (m, 1H), 1.89 (m, 2H). MS (ESI) m/z = 316 $[M + H]^+$. HPLC purity: 97.3% (220 nm). **22a** had >99% ee based on starting material **46**.

3-(Aminomethyl)-4-chloro-7-(2-hydroxyethoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (23). To 2-bromoethanol (1.88 g, 15 mmol) was added dihydropyran (1.26 g, 15 mmol) dropwise at 0 °C. The mixture was stirred for 30 min at 0 °C and then 2 h at rt. Subsequently, 2-bromo-3-hydroxybenzaldehyde (2.0 g, 10 mmol) was added, followed by potassium carbonate (1.52 g, 11 mmol), potassium iodide (332 mg, 2 mmol), and dry DMF (20 mL). The reaction was stirred at 70 °C overnight. The mixture was cooled to rt and diluted with diethyl ether (100 mL). After the inorganic salts were removed by filtration, the filtrate was diluted with hexane (100 mL). The organic layer was washed with water (3 × 50 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography, eluting with EtOAc and petroleum ether (1:3) to give 2-bromo-3-(2-(tetrahydro-2*H*-pyran-2-yloxy)ethoxy)benzaldehyde as yellow oil (3.0 g, yield 91%). A mixture of the above compound (160 g, 0.49 mol), bis(pinacolato)diborane (249 g, 0.98 mol), $PdCl_2$(dppf) (20 g, 24.5 mmol), and KOAc (144 g, 1.47 mol) in DMF (2.0 L) was degassed and stirred at 90 °C overnight. After the reaction was quenched with ice−water (4 L), the reaction mixture was extracted with EtOAc (3 × 1.5 L). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography, eluting with EtOAc and petroleum ether (1:10 to 1:2) to give 3-(2-(tetrahydro-2*H*-pyran-2-yloxy)ethoxy)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde as yellow oil (88 g, yield 48%). To a solution of NaOH (4.8 g, 0.12 mol) in water (100 mL) was added nitromethane (18.3 g, 0.3 mol) at 5−10 °C. After stirring for 15 min at 5−10 °C, cetyltrimethylammonium bromide (2.2 g, 6.0 mmol) was added. After the above compound (45 g, 0.12 mol) was added slowly at 5−10 °C, the reaction mixture was stirred at rt for 5 h. Subsequently, the reaction mixture was acidified to pH = 1 using diluted hydrochloric acid and stirred at rt overnight. The residue was filtered and dried in vacuo to give 7-(2-hydroxyethoxy)-3-(nitromethyl)benzo[*c*][1,2]oxaborol-1(3*H*)-ol as a white solid (14.5 g, yield 48%). To a solution of the above compound (29 g, 115 mmol) in DMF (250 mL) at 80 °C was added a solution of *N*-chlorosuccinimide (16.5 g, 123 mmol) in DMF (100 mL). The mixture was stirred for 30 min at 80 °C. After cooling down to rt, the reaction mixture was poured into ice−water and extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by recrystallization from EtOAc and petroleum ether (1:10) to give crude product 4-chloro-7-(2-hydroxyethoxy)-3-(nitromethyl)benzo[*c*][1,2]oxaborol-1(3*H*)-ol (24 g). A solution of the above compound (24 g, 83.5 mmol), Raney Ni (4.0 g), and 7 M NH_3 in MeOH (20 mL) in MeOH (300 mL) was shaken under an atmosphere of hydrogen for 2 h at rt. The mixture was filtered through a bed of Celite, and the filtrate was concentrated in vacuo. The crude amine was dissolved in MeOH (20 mL), and 1 M HCl in Et_2O (30 mL) was added. After stirring for 1 h, the residue was filtered, washed with acetonitrile/hexane (2:1, 2 × 200 mL), and dried in vacuo to give **23** as a white solid (12 g, yield 49%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19 (b, 3H), 7.51−7.48 (d, 1H, J = 9 Hz), 6.99−6.96 (d, 1H, J = 9 Hz), 5.54 (m, 1H), 4.69 (m, 1H), 4.36−4.23 (m, 3H), 3.58 (m, 1H), 3.03 (m, 1H). MS (ESI) m/z = 258 $[M + H]^+$. HPLC purity: 99.7% (220 nm).

(*S*)-3-(Aminomethyl)-4-chloro-7-(2-hydroxyethoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (23a) and (*R*)-3-(Aminomethyl)-4-chloro-7-(2-hydroxyethoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (23b). To a mixture of **23** (8.0 g, 27.2 mmol) and triethylamine (10.2 g, 101 mmol) in dichloromethane (250 mL) at 0 °C was added di-*tert*-butyl dicarbonate (11 g, 50.6 mmol). The reaction mixture was stirred for 2 h at rt. After the reaction was quenched with sat. $NaHCO_3$ (150 mL), the resulting mixture was extracted with EtOAc (2 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by prep-HPLC, eluting with water/acetonitrile gradient to give the Boc-protected **23** (4.6 g, yield 47%). The racemic Boc-protected **23** was separated on a Thar preparative SFC 80 system with a Daicel Chiralpak AD-H 5 μm (250 mm × 20 mm i.d.), eluting with carbon dioxide and methanol mobile phase. The two isomers were obtained. Each compound (4.6 g, 12.9 mmol) was dissolved in 1 M HCl/Et_2O (80 mL) and stirred at rt for 1 h and concentrated in vacuo. The residue was washed with acetonitrile (2 × 5 mL) and dried in vacuo to give the desired product as a white solid. **23a** (1.2 g, yield 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.31 (b, 3H), 7.51−7.48 (d, 1H, J = 9 Hz), 6.98−6.95 (d, 1H, J = 9 Hz), 5.59 (m, 1H), 4.70 (m, 1H), 4.36−4.23 (m, 3H), 3.57 (m, 1H), 3.02 (m, 1H). MS (ESI) m/z = 258 $[M + H]^+$. HPLC purity: 99.7% (220 nm). Analysis of the Boc-derivatized material using a ChiralCel OJ-H analytical column showed the (*S*) enantiomer with a retention time of 2.94 min and 99.9% ee. **23b** (1.3 g, yield 34%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (b, 3H), 7.51−7.48 (d, 1H, J = 9 Hz), 6.98−6.95 (d, 1H, J = 9 Hz), 5.58 (m, 1H), 4.69 (m, 1H), 4.36−4.23 (m, 3H), 3.59 (m, 1H), 3.02 (m, 1H). MS (ESI) m/z = 258 $[M + H]^+$. HPLC purity: 100% (220 nm). Analysis of the Boc-derivatized material using a ChiralCel OJ-H analytical column showed the (*R*) enantiomer with a retention time of 2.67 min and 97.8% ee.

3-(Aminomethyl)-4-bromo-7-(2-hydroxyethoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (24). A solution of 7-(2-hydroxyethoxy)-3-(nitromethyl)benzo[*c*][1,2]oxaborol-1(3*H*)-ol (1.5 g, 5.9 mmol), Raney Ni (200 mg), and 2 M NH_3 in EtOH (5 mL) in ethanol (40 mL) was shaken under an atmosphere of hydrogen for 2 h at rt. The mixture was filtered through a bed of Celite, and the filtrate was concentrated in vacuo. The crude amine was dissolved in EtOH (20 mL), and 1 M HCl in Et_2O (30 mL) was added. After stirring for 1 h, the residue was filtered, washed with acetonitrile/hexane (2:1, 2 × 20 mL), and dried in vacuo to give 3-(aminomethyl)-7-(2-hydroxyethoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol hydrochloride as a white solid (700 mg, yield 46%). To a mixture of the above compound (700 mg, 2.7 mmol) and triethylamine (879 mg, 8.7 mmol) in dichloromethane (10 mL) at 0 °C was added di-*tert*-butyl dicarbonate (948 mg, 4.4 mmol). The mixture was stirred at rt for 2 h. The reaction was quenched with sat. $NaHCO_3$ (15 mL), and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash-column chromatography to give *tert*-butyl (1-hydroxy-7-(2-hydroxyethoxy)-1,3-dihydrobenzo[*c*][1,2]oxaborol-3-yl)methylcarbamate (500 mg, yield 57%). To a solution of the above compound (0.5 mg, 1.5 mmol) and *N*-bromosuccinimide (354 mg, 2.0 mmol) in CH_3CN (15 mL) was added 2,2′-azobis(2-methylpropionitrile) (27 mg). The mixture was stirred for 1 h at 90 °C. The reaction mixture was concentrated in vacuo, and the residue was purified by prep-HPLC, eluting with water/acetonitrile gradient to give *tert*-butyl (4-bromo-1-hydroxy-7-(2-hydroxyethoxy)-1,3-dihydrobenzo[*c*][1,2]oxaborol-3-yl)-methylcarbamate (300 mg, yield 50%). A mixture of the above compound (0.2 g, 0.5 mmol) in 1 M HCl/Et_2O (10 mL) was stirred at rt for 1 h and then concentrated in vacuo. The residue was washed with acetonitrile (2 × 5 mL) and dried in vacuo to give **24** as a white solid (140 mg, yield 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (b, 3H), 7.64−7.61 (d, 1H, J = 9 Hz), 6.93−6.60 (d, 1H, J = 9 Hz), 5.51 (m, 1H), 4.69 (m, 1H), 4.36−4.23 (m, 3H), 3.62 (m, 1H), 3.01 (m, 1H). MS (ESI) m/z = 302 $[M + H]^+$. HPLC purity: 99.5% (220 nm).

(*S*)-3-(Aminomethyl)-4-bromo-7-(2-hydroxyethoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (24a) and (*R*)-3-(Aminomethyl)-4-bromo-7-(2-hydroxyethoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (24b). The racemic *tert*-butyl (4-bromo-1-hydroxy-7-(2-hydroxyethoxy)-1,3-dihydrobenzo[*c*][1,2]-oxaborol-3-yl)methylcarbamate was separated on a Thar preparative SFC 80 system with a Daicel Chiralpak AD-H 5 μm (250 mm × 20 mm i.d.), eluted with carbon dioxide and methanol mobile phase. Two

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isomers were obtained. Each compound (1.2 g, 3.1 mmol) in 1 M HCl/Et_2O (20 mL) was stirred at rt for 1 h and concentrated in vacuo. The residue was washed with acetonitrile (2 × 5 mL) and dried in vacuo to give the desired product as a white solid. **24a** (900 mg, yield 90%). ^1H NMR (300 MHz, DMSO-d_6) δ 8.37 (b, 3H), 7.63−7.60 (d, 1H, J = 9 Hz), 6.92−6.89 (d, 1H, J = 9 Hz), 5.51 (m, 1H), 4.68 (m, 1H), 4.36−4.23 (m, 3H), 3.61 (m, 1H), 3.01 (m, 1H). MS (ESI) m/z = 302 $[M + H]^+$. HPLC purity: 98.4% (220 nm). Analysis of the Boc-derivatized material using a ChiralCel AD-H analytical column showed the (*S*) enantiomer with a retention time of 3.79 min and 98.0% ee. **24b** (900 mg, yield 90%). ^1H NMR (300 MHz, DMSO-d_6) δ 8.38 (b, 3H), 7.63−7.60 (d, 1H, J = 9 Hz), 6.92−6.89 (d, 1H, J = 9 Hz), 5.50 (m, 1H), 4.68 (m, 1H), 4.35−4.23 (m, 3H), 3.61 (m, 1H), 3.00 (m, 1H). MS (ESI) m/z = 302 $[M + H]^+$. HPLC purity: 99.5% (220 nm). Analysis of the Boc-derivatized material using a ChiralCel AD-H analytical column showed the (*R*) enantiomer with a retention time of 3.34 min and 98.1% ee.

3-(Aminomethyl)-4-chloro-6-ethoxybenzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (25). A solution of 2-chloro-4,6-dihydroxybenzaldehyde (6.2 g, 35.9 mmol), DHP (6.02 g, 71.7 mmol), and PPTS (0.9 g) in 150 mL of CH_2Cl_2 was stirred at rt for 1.5 h. After the reaction was quenched with sat. $NaHCO_3$ solution (50 mL) at 0 °C, the mixture was washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography, eluting with EtOAc and petroleum ether (1:100) to give 2-chloro-6-hydroxy-4-(tetrahydro-2*H*-pyran-2-yloxy)-benzaldehyde as colorless oil (7.8 g, 85%). To a stirred solution of the above compound (7.8 g, 30.4 mmol) and pyridine (10.9 mL) in 150 mL of CH_2Cl_2 at −10 °C was added Tf_2O (7.66 mL). The reaction was stirred at 0 °C for 2 h. After the reaction was quenched with brine (35 mL), the mixture was extracted with EtOAc (100 mL). The solvent was evaporated in vacuo. The residue was purified by silica gel chromatography, eluting with EtOAc and petroleum ether (1:200) to give 3-chloro-2-formyl-5-(tetrahydro-2*H*-pyran-2-yloxy)phenyl trifluoromethanesulfonate as yellow oil (7.0 g, 59%). A mixture of the above compound (3.4 g, 8.7 mmol), bis(pinacolato)diborane (4.0 g, 15.8 mmol), KOAc (2.3 g, 23.7 mmol), and $PdCl_2$(dppf) (0.58 g) in 120 mL of 1,4-dioxane was degassed with N_2 six times. The reaction was stirred at 80 °C for 1 h. The solvent was evaporated in vacuo. The residue was purified by silica gel chromatography, eluting with EtOAc and petroleum ether (1:10) to give 2-chloro-4-(tetrahydro-2*H*-pyran-2-yloxy)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde as a white solid (3.1 g, 97%). To a stirred solution of the above compound (2.2 g, 6.0 mmol) and CH_3NO_2 (1.1 g, 18.0 mmol) in 40 mL of THF was added a solution of NaOH (0.24 g, 6.0 mmol) in 40 mL of water. The reaction was stirred at rt for 3 h. The reaction mixture was treated with citric acid to pH = 6 and was then extracted with EtOAc (120 mL). The organic phase was washed with brine (15 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo to give crude 4-chloro-3-(nitromethyl)-6-(tetrahydro-2*H*-pyran-2-yloxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol. A solution of the above compound (3.3 g, 10.1 mmol) in 33 mL of conc HCl and 66 mL of MeOH was stirred at reflux for 1 h. The solvent was evaporated in vacuo to give 4-chloro-3-(nitromethyl)benzo[*c*][1,2]oxaborole-1,6(3*H*)-diol (3 g, crude). It was used directly in the next step without further purification. A mixture of the above compound (1.4 g, 5.8 mmol) and Raney Ni (0.5 g) in 5 mL of ammonium in ethanol (2 M, 5 mL) and 20 mL of ethanol was hydrogenated at an atmosphere of hydrogen for 6 h. The mixture was filtered through a bed of Celite, and the filtrate was concentrated in vacuo. The residue was dissolved in 30 mL of ethanol and treated with 1 mL of conc HCl. The solvent was evaporated in vacuo to give 3-(aminomethyl)-4-chlorobenzo[*c*]-[1,2]oxaborole-1,6(3*H*)-diol hydrochloride as solid (1.3 g, 90%). To a stirred solution of the above compound (1.3 g, 5.2 mmol) and Et_3N (1.16 g, 11.5 mmol) in 200 mL of CH_2Cl_2 at 0 °C was added Boc_2O (2.5 g, 11.5 mmol). The reaction was stirred at rt for 4 h. The mixture was washed with water (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography, eluting with MeOH and CH_2Cl_2 (1:10) to give *tert*-butyl (4-chloro-1,6-dihydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-3-yl)methylcarbamate (0.4 g, 25%) as oil. A mixture of the above compound (0.4 g, 1.3 mmol), EtI (0.6 g, 3.8 mmol), and K_2CO_3 (0.5 g, 3.8 mmol) in 30 mL of acetone was stirred at reflux for 16 h. The solvent was evaporated in vacuo. The residue was purified by silica gel chromatography, eluting with MeOH and CH_2Cl_2 (1:20) to give *tert*-butyl (4-chloro-6-ethoxy-1-hydroxy-1,3-dihydrobenzo[*c*][1,2]-oxaborol-3-yl)methylcarbamate (0.43 g, 99%). To a stirred solution of the above compound (0.43 g, 1.3 mmol) in 4 mL of CH_2Cl_2 was added TFA (2 mL). The reaction was stirred at rt for 1 h. The solvent was evaporated in vacuo. The residue was purified by prep-HPLC followed by treating with conc HCl to give **25** as a white solid (20 mg, 6%). ^1H NMR (400 MHz, CD_3OD) δ 7.17 (s, 1H), 7.14 (s, 1H), 5.47 (m, 1H), 4.10 (q, 2H), 3.82 (m, 1H), 3.04 (m, 1H), 1.42 (t, 3H). MS (ESI) m/z = 242 $[M + H]^+$. HPLC purity: 97.0% (220 nm).

3-(Aminomethyl)-4-bromo-6-ethoxybenzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (26). To a solution of 2,6-dibromo-4-hydroxybenzaldehyde (2 g, 7.1 mmol) and potassium carbonate (2.96 g, 21.4 mmol) in DMF (50 mL) was added CH_3CH_2Br (2.3 g, 21.4 mmol). The mixture was stirred at rt for 3 h. After water was added, the mixture was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo to give 2,6-dibromo-4-ethoxybenzaldehyde as a white solid (2.1 g, 95%). To a stirred solution of the above compound (2 g, 6.5 mmol) and $HC(OMe)_3$ (1.38 g, 13 mmol) in MeOH (30 mL) was added conc H_2SO_4 (1 drop) at rt. The reaction was stirred at reflux for 1 h. MeONa was added until pH = 7. After the solvent was removed, the residue was washed with petroleum ether. The organic layer was concentrated in vacuo to afford 1,3-dibromo-2-(dimethoxymethyl)-5-ethoxybenzene as oil (2.0 g, 87%). To a solution of the above compound (500 mg, 1.4 mmol) in anhydrous ether (20 mL) was added *n*-BuLi (2.5 M, 0.56 mL, 1.4 mmol) dropwise at −78 °C under N_2. After the mixture was stirred at −78 °C for 15 min, trimethyl borate (176 mg, 1.7 mmol) was added dropwise. The resulting mixture was stirred at −78 °C for 30 min. The reaction was quenched by adding 2 N HCl solution until pH = 1, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo to give 3-bromo-5-ethoxy-2-formylphenylboronic acid (500 mg, crude). A mixture of the above compound (500 mg, 1.8 mmol), NaOH (73 mg, 1.8 mmol) in H_2O (10 mL) and THF (20 mL) was stirred for 30 min. Nitromethane (558 mg, 9.2 mmol) was added, and the reaction was stirred at rt for 3 h. The mixture was adjusted to pH = 1 by adding diluted HCl (2 N) and then extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo to give 4-bromo-6-ethoxy-3-(nitromethyl)benzo[*c*][1,2]oxaborol-1(3*H*)-ol as oil (500 mg, crude). A mixture of the above compound (500 mg, 1.6 mmol), NH_3 in MeOH (7 N, 0.2 mL), and Raney Ni (100 mg) in MeOH (40 mL) was stirred under an atmosphere of hydrogen for 4 h at rt. The mixture was filtered through a bed of Celite, and the filtrate was concentrated in vacuo. The residue was purified by prep-HPLC followed by treating with conc HCl to give **26** as a white solid (120 mg, 24%). ^1H NMR (300 MHz, DMSO-d_6) δ 9.77 (b, 1H), 8.19 (b, 3H), 7.41 (s, 1H), 7.32 (s, 1H), 5.31 (m, 1H), 4.10 (m, 2H), 3.64 (m, 1H), 2.87 (m, 1H), 1.36 (m, 3H). MS (ESI) m/z = 286 $[M + H]^+$. HPLC purity: 95.3% (220 nm).

3-(Aminomethyl)-4-chloro-6-propoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (27). This compound was prepared by the similar method described above for the synthesis of **25**. ^1H NMR (400 MHz, CD_3OD) δ 7.16 (s, 1H), 7.12 (s, 1H), 5.45 (m, 1H), 3.99 (t, 2H), 3.79 (m, 1H), 3.06 (m, 1H), 1.82 (m, 2H), 1.07 (t, 3H). MS (ESI) m/z = 256 $[M + H]^+$. HPLC purity: 96.2% (220 nm).

3-(Aminomethyl)-4-bromo-6-propoxybenzo[*c*][1,2]-oxaborol-1(3*H*)-ol Hydrochloride (28). This compound was prepared by the similar method described above for the synthesis of **26**. ^1H NMR (300 MHz, DMSO-d_6) δ 9.71 (b, 1H), 8.09 (b, 3H), 7.36 (s, 1H), 7.31 (s, 1H), 5.28 (m, 1H), 3.97 (m, 2H), 3.60 (m, 1H),

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2.86 (m, 1H), 1.74 (m, 2H), 0.98 (t, 3H). MS (ESI) m/z = 300 $[M + H]^+$. HPLC purity: 96.5% (220 nm).

3-(Aminomethyl)-4-chloro-6-(3-hydroxypropoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (29). This compound was prepared by the similar method described above for the synthesis of **25**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.36 (s, 1H), 7.18 (s, 1H), 5.48 (m, 1H), 4.13 (t, 2H), 3.80 (m, 1H), 3.75 (t, 2H), 3.05 (m, 1H), 2.02 (m, 2H). MS (ESI) m/z = 272 $[M + H]^+$. HPLC purity: 95.3% (220 nm).

3-(Aminomethyl)-4-bromo-6-(3-hydroxypropoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (30). This compound was prepared by the similar method described above for the synthesis of **26**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.72 (b, 1H), 8.13 (b, 3H), 7.39 (s, 1H), 7.33 (s, 1H), 5.29 (m, 1H), 4.08 (t, 2H), 3.63 (m, 1H), 3.57 (m, 2H), 2.86 (m, 1H), 1.87 (m, 2H). MS (ESI) m/z = 316 $[M + H]^+$. HPLC purity: 91.3% (220 nm).

3-(Aminomethyl)-4-chloro-6-(3-hydroxypropoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (31). This compound was prepared by the similar method described above for the synthesis of **25**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.22 (s, 1H), 7.20 (s, 1H), 5.48 (m, 1H), 4.11 (t, 2H), 3.91 (t, 2H), 3.85 (m, 1H), 3.05 (m, 1H). MS (ESI) m/z = 258 $[M + H]^+$. HPLC purity: 98.4% (220 nm).

3-(Aminomethyl)-4-bromo-6-(3-hydroxypropoxy)benzo[*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (32). This compound was prepared by the similar method described above for the synthesis of **26**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.72 (b, 1H), 8.11 (b, 3H), 7.39 (s, 1H), 7.32 (s, 1H), 5.28 (m, 1H), 4.91 (t, 1H), 4.04 (t, 2H), 3.75 (t, 2H), 3.70 (m, 1H), 2.86 (m, 1H). MS (ESI) m/z = 302 $[M + H]^+$. HPLC purity: 98.9% (220 nm).

3-(Aminomethyl)-4-chloro[1,3]dioxolo[4′,5′:5,6]benzo[1,2-*c*][1,2]oxaborol-1(3*H*)-ol Hydrochloride (33). This compound was prepared by the similar method described below for the synthesis of **35**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.65 (b, 1H), 7.98 (b, 3H), 7.24 (s, 1H), 6.17 (ds, 2H), 5.33 (m, 1H), 3.54 (m, 1H), 2.99 (m, 1H). MS (ESI) m/z = 242 $[M + H]^+$. HPLC purity: 98.9% (220 nm).

3-(Aminomethyl)-4-bromo[1,3]dioxolo[4′,5′:5,6]benzo[1,2-*c*][1,2]oxaborol-1(3*H*)-ol Trifluoroacetate (34). This compound was prepared by the similar method described below for the synthesis of **35** and obtained as TFA salt after prep-HPLC purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (b, 1H), 7.96 (b, 3H), 7.29 (s, 1H), 6.09 (ds, 2H), 5.18 (m, 1H), 3.52 (m, 1H), 2.90 (m, 1H). MS (ESI) m/z = 286 $[M + H]^+$. HPLC purity: 95.1% (220 nm).

3-(Aminomethyl)-4-chloro-7,8-dihydro[1,2]oxaborolo-[3′,4′:3,4]benzo[1,2-*b*][1,4]dioxin-1(3*H*)-ol Hydrochloride (35). A mixture of 2-bromo-3,4-dihydroxybenzaldehyde (4.5 g, 20.7 mmol), 1,2-dibromoethane (4.65 g, 24.8 mmol), and K_2CO_3 (8.9 g, 64.5 mmol) in DMF (40 mL) was stirred at 50 °C overnight. After H_2O (80 mL) was added, the mixture was extracted with EtOAc (2 × 80 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography to give 5-bromo-2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde (1.5 g, yield 30%). A mixture of the above compound (2.68 g, 11.0 mmol), bis(pinacolato)diborane (4.0 g, 16.3 mmol), KOAc (4.1 g, 41.8 mmol), and $PdCl_2$(dppf) (310 mg, 4.2 mmol) in dioxane (60 mL) was degassed for 15 min with N_2. The reaction was stirred at 100 °C for 3 h. After the reaction was quenched with ice–water (25 mL), the mixture was extracted with EtOAc (3 × 70 mL). The combined organic layers were washed with water (35 mL) and brine (40 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography to give 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydrobenzo[*b*][1,4]dioxine-6-carbaldehyde (1.5 g, yield 47%). A mixture of the above compound (1.52 g, 5.2 mmol), 10% NaOH solution (6 mL), and THF (30 mL) was stirred for 0.5 h at rt. After nitromethane (980 mg 16.1 mmol) was added, the reaction was stirred at rt for 2 h. The mixture was adjusted to pH = 2 by adding diluted HCl (2 N) and extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by prep-HPLC, eluting with water/acetonitrile gradient to give the 6,7-dixoane-3-(nitromethyl)benzo[*c*][1,2]oxaborol-1(3*H*)-ol (1.3 g, yield 99%). To a mixture of the above compound (200 mg, 0.8 mmol) in glacial acetic acid (15 mL) was added sulfuryl dichloride (64 μL, 0.8 mmol) dropwise. The reaction was stirred at rt for 1.5 h. After the reaction was quenched with water (15 mL), the mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue 6,7-dixoane-4-chloro-3-(nitromethyl)benzo[*c*][1,2]oxaborol-1(3*H*)-ol was used directly for the next step without purification. A mixture of the above compound, Raney Ni (~50 mg), and 2 M NH_3 in EtOH (2 mL) in EtOH (10 mL) was shaken under an atmosphere of hydrogen for 3 h. The mixture was filtered through a bed of Celite, and the filtrate was concentrated in vacuo. The crude amine was dissolved in EtOAc (1 mL), and 1 M HCl in Et_2O (8 mL) was added. After stirring for 1 h, the residue was filtered, washed with hexane, and dried in vacuo to give **35** as a white solid (90 mg, yield 38%, two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (b, 1H), 8.25 (b, 3H), 7.08 (s, 1H), 5.30 (m, 1H), 4.27–4.23 (m, 4H), 3.48 (m, 1H), 2.84 (m, 1H). MS (ESI) m/z = 256 $[M + H]^+$. HPLC purity: 98.9% (220 nm).

(*S*)-3-(Aminomethyl)-4-chloro-7,8-dihydro[1,2]oxaborolo-[3′,4′:3,4]benzo[1,2-*b*][1,4]dioxin-1(3*H*)-ol Hydrochloride (35a) and (*R*)-3-(Aminomethyl)-4-chloro-7,8-dihydro[1,2]oxaborolo-[3′,4′:3,4]benzo[1,2-*b*][1,4]dioxin-1(3*H*)-ol Hydrochloride (35b). These compounds were prepared by the similar method described above for the synthesis of **23a** and **23b**. **35a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (b, 1H), 8.26 (b, 3H), 7.12 (s, 1H), 5.33 (m, 1H), 4.30–4.26 (m, 4H), 3.52 (m, 1H), 2.86 (m, 1H). MS (ESI) m/z = 256 $[M + H]^+$. HPLC purity: 98.1% (220 nm). Analysis of the Boc-derivatized material using a ChiralPak AY-H analytical column showed the (*S*) enantiomer with a retention time of 1.64 min and >99% ee. **35b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (b, 1H), 8.17 (b, 3H), 7.13 (s, 1H), 5.31 (m, 1H), 4.30–4.26 (m, 4H), 3.54 (m, 1H), 2.90 (m, 1H). MS (ESI) m/z = 256 $[M + H]^+$. HPLC purity: 95.7% (220 nm). Analysis of the Boc-derivatized material using a ChiralPak AY-H analytical column showed the (*R*) enantiomer with a retention time of 1.95 min and >99% ee.

3-(Aminomethyl)-4-bromo-7,8-dihydro[1,2]oxaborolo-[3′,4′:3,4]benzo[1,2-*b*][1,4]dioxin-1(3*H*)-ol Trifluoroacetate (36). This compound was prepared by the similar method described above for the synthesis of **35** and obtained as TFA salt after prep-HPLC purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (b, 1H), 8.05 (b, 3H), 7.25 (s, 1H), 5.20 (m, 1H), 4.30–4.23 (m, 4H), 3.50 (m, 1H), 2.93 (m, 1H). MS (ESI) m/z = 300 $[M + H]^+$. HPLC purity: 98.8% (220 nm).

(*S*)-3-(Aminomethyl)-4-bromo-7,8-dihydro[1,2]oxaborolo-[3′,4′:3,4]benzo[1,2-*b*][1,4]dioxin-1(3*H*)-ol Hydrochloride (36a) and (*R*)-3-(Aminomethyl)-4-bromo-7,8-dihydro[1,2]oxaborolo-[3′,4′:3,4]benzo[1,2-*b*][1,4]dioxin-1(3*H*)-ol Hydrochloride (36b). These compounds were resolved by the similar method described above for the synthesis of **23a** and **23b**. **36a**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.28 (b, 1H), 8.19 (b, 3H), 7.23 (s, 1H), 5.22 (m, 1H), 4.29 (m, 4H), 3.56 (m, 1H), 2.88 (m, 1H). MS (ESI) m/z = 300 $[M + H]^+$. HPLC purity: 97.9% (220 nm). Analysis of the Boc-derivatized material using a ChiralCel AD-H analytical column showed the (*S*) enantiomer with a retention time of 2.79 min and 97.6% ee. **36b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.28 (b, 1H), 8.15 (b, 3H), 7.23 (s, 1H), 5.23 (m, 1H), 4.29 (m, 4H), 3.54 (m, 1H), 2.89 (m, 1H). MS (ESI) m/z = 300 $[M + H]^+$. HPLC purity: 96.5% (220 nm). Analysis of the Boc-derivatized material using a ChiralCel AD-H analytical column showed the (*R*) enantiomer with a retention time of 2.62 min and 99.6% ee.

AUTHOR INFORMATION

Corresponding Author

*Phone: (+34) 629170293. Fax: (+34) 918070550. E-mail: david.a.barros@gsk.com.

ORCID

David Barros: 0000-0002-4099-0438

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Present Address

[‖]I.A.-B.: The Art of Discovery, SL, Biscay Science and Technology Park, Astondo Bidea, BIC Bizkaia Building, No. 612, Derio 48160, Bizkaia, Basque Country, Spain.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

CFU, colony-forming units; DNAUC, dose-normalized area under the curve; dppf, 1,1′-bis(diphenylphosphino)ferrocene; i.d., inside diameter; LeuRS, leucyl-tRNA synthetase; *Mtb*, *Mycobacterium tuberculosis*; $(pin)_2$, bis(pinacolato); TEA, triethylamine; TDR-TB, totally drug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis

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