Epetraborole Is Active against *Mycobacterium abscessus*

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ABSTRACT Benzoxaboroles are a new class of leucyl-tRNA synthetase inhibitors. We recently reported that the antitubercular 4-halogenated benzoxaboroles are active against *Mycobacterium abscessus*. Here, we find that the nonhalogenated benzoxaborole epetraborole, a clinical candidate developed for Gram-negative infections, is also active against *M. abscessus in vitro* and in a mouse model of infection. This expands the repertoire of advanced lead compounds for the discovery of a benzoxaborole-based candidate to treat *M. abscessus* lung disease.

KEYWORDS epetraborole, *Mycobacterium abscessus*, NTM, nontuberculous mycobacteria, benzoxaborole

*M*ycobacterium abscessus lung disease is notoriously difficult to treat due to the bacterium's high intrinsic drug resistance (1, 2). In addition to resistance to all first-line tuberculosis (TB) drugs, *M. abscessus* displays resistance to macrolides (3, 4), threatening the current macrolide-based treatment regimens (2, 5). Therefore, new antibiotics with novel targets and mechanisms of action are needed to treat this disease (6).

Benzoxaboroles are a class of boron-heterocyclic antimicrobials that target leucyl-tRNA synthetase (LeuRS) (7). Acting through the oxaborole tRNA-trapping (OBORT) mechanism (8), these compounds form adducts with uncharged tRNA^{Leu} molecules that subsequently bind to the LeuRS editing domain, blocking protein synthesis. Following the discovery of tavaborole (7, 8), a benzoxaborole with antifungal activity, this compound class was optimized for antibacterial activity. Addition of a 3-aminomethyl group to the benzoxaborole core improved interactions with the editing domain of *Escherichia coli* LeuRS, while a 7-*O*-propanol substituent added a novel interaction with the phosphate backbone of tRNA^{Leu} (9). Combining these modifications yielded epetraborole (Fig. 1), a clinical candidate with potent activity against a broad range of Gram-negative bacteria (9, 10). The subsequent addition of a 4-halogen group (particularly Cl or Br) improved antituberculosis activity (11–13).

Recently, we reported that the antituberculosis 4-halogen benzoxaborole EC/11770 (Fig. 1) is active against *M. abscessus in vitro* and *in vivo* in a mouse infection model (14). Here, we asked whether the anti-Gram-negative, nonhalogenated benzoxaborole epetraborole (Fig. 1) is active against *M. abscessus*. We first measured the MIC of this compound against our screening strain *M. abscessus* subsp. *abscessus* Bamboo (15) in Middlebrook 7H9 medium using 96-well plates, as previously described (14). Surprisingly, epetraborole showed activity similar to that of the antitubercular EC/11770 (Table 1). Epetraborole retained activity against culture collection reference strains for each of the three subspecies of the *M. abscessus* complex and a panel of *M. abscessus* clinical isolates (16, 17) (Table 1). Taken together, the anti-Gram-negative, nonhalogenated benzoxaborole epetraborole was active against the *M. abscessus* complex *in vitro*.

To confirm that epetraborole indeed exerts its antimycobacterial activity by targeting *M. abscessus* LeuRS, we selected for epetraborole-resistant *M. abscessus* mutants (Table 2).
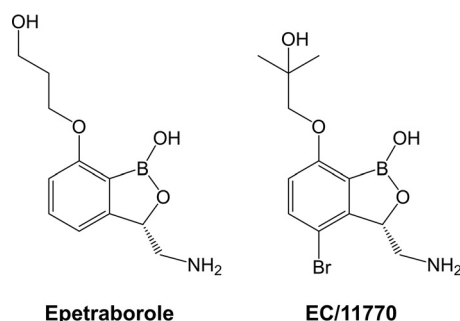
FIG 1 Structures of epetraborole and EC/11770.

Adapting our previously described method (14), *M. abscessus* ATCC 19977 culture was plated on Middlebrook 7H10 agar containing 16.5 μM epetraborole, the lowest concentration suppressing the emergence of wild-type colonies. After 5 days of incubation, apparent resistant colonies were confirmed by restreaking on epetraborole-containing agar. Based on two independent selections, we calculated the frequency of resistance to epetraborole to be 5.4×10^{-8}/CFU. This frequency of resistance was on the lower end of a range determined for epetraborole in several Gram-negative bacterial species (3.8×10^{-8}/CFU to 8.1×10^{-7}/CFU) (9) and was comparable to what we reported for EC/11770 in *M. abscessus* (3.9×10^{-8}/CFU) (14). MIC profiling of nine epetraborole-resistant mutants (RM1 to -9) showed high-level resistance to epetraborole (Table 2). Sequencing of *leuS* (*MAB_4923c*) showed that RM1 to -9 all had missense mutations in the LeuRS editing domain (residues V292 to K502) (Table 2). These results suggest that epetraborole retains LeuRS as its target to exert its anti-*M. abscessus* activity (8, 9).

Development of epetraborole for the treatment of complicated urinary tract infections caused by Gram-negative bacteria was discontinued after rapid emergence of drug resistance in a phase II clinical trial (18). Determination of spontaneous resistance frequencies for epetraborole in the current study, and for EC/11770 previously (14), suggest low propensity for the development of resistance against benzoxaboroles in *M. abscessus*. However, it is to note that we needed to carry out selection of resistant mutants on agar containing high concentrations of the drugs (50 to 100$\times$ broth MIC), as lower concentrations did not suppress outgrowth of wild-type bacteria. Thus, it cannot be excluded that the spontaneous resistance frequency of *M. abscessus* against the benzoxaboroles would be higher than the observed 4×10^{-8} to 5×10^{-8}/CFU when lower drug concentrations could be used. Such resistant strains, presumably displaying

TABLE 1 Activity of epetraborole against members of the *M. abscessus* complex

Strain	Strain type	MIC (μM)[a]		
		CLR	EC/11770[b]	EPB
M. abscessus Bamboo	Clinical isolate, screening strain	0.30	1.2	0.28
M. abscessus subsp. *abscessus* ATCC 19977	Culture collection reference strain	0.90	0.70	0.33
M. abscessus subsp. *massiliense* CCUG 48898[T]	Culture collection reference strain	0.22	0.71	0.32
M. abscessus subsp. *bolletii* CCUG 50184[T]	Culture collection reference strain	1.3	1.3	0.49
M. abscessus subsp. *abscessus* M9	Clinical isolate	1.4	0.49	0.42
M. abscessus subsp. *abscessus* M199	Clinical isolate	3.3	0.93	0.56
M. abscessus subsp. *abscessus* M337	Clinical isolate	1.6	0.50	0.44
M. abscessus subsp. *abscessus* M404	Clinical isolate	0.2	0.52	0.3
M. abscessus subsp. *abscessus* M422	Clinical isolate	0.68	0.33	0.34
M. abscessus subsp. *bolletii* M232	Clinical isolate	1.6	0.67	0.37
M. abscessus subsp. *bolletii* M506	Clinical isolate	0.28	0.48	0.28
M. abscessus subsp. *massiliense* M111	Clinical isolate	0.25	0.95	0.44
M. abscessus subsp. *abscessus* K21	Clinical isolate, infection model	0.78	0.60	0.40

[a]MIC values are the means from two independent experiments. CLR, clarithromycin; EPB, epetraborole.
[b]EC/11770 MIC values are from published literature (14) and are included for comparison.

TABLE 2 Characterization of *M. abscessus* epetraborole-resistant mutants

| Strain | Batch | MIC (μM)[a] | | LeuS mutation | Other bacteria with LeuS mutation (reference)[b] |
		CLR	EPB		
M. abscessus ATCC 19977		1.3	0.48	None	
RM1	1	1.5	>100	LeuS G393V	None
RM2	1	2.7	>100	LeuS T322I	*E. coli, Proteus mirabilis* (18)
RM3	1	1.3	>100	LeuS T323P	None
RM4	2	1.5	>100	LeuS S303L	*M. tuberculosis* (11)
RM5	2	1.8	>100	LeuS S303L	*M. tuberculosis* (11)
RM6	2	1.6	>100	LeuS S303L	*M. tuberculosis* (11)
RM7	2	1.4	>100	LeuS Y421D	*M. tuberculosis* (Y421C) (11)
RM8	2	0.9	>100	LeuS T322I	*E. coli, P. mirabilis* (18)
RM9	2	2.2	>100	LeuS F321V	None

[a]MIC values are the means from two independent experiments. CLR, clarithromycin; EPB, epetraborole.
[b]Corresponding benzoxaborole resistance-conferring LeuS mutations reported for other bacteria.

low level resistance, would have been missed in our selection experiments. In any case, given the use of multidrug chemotherapy in *M. abscessus* treatment (2, 5), the risk of benzoxaborole resistance emerging in this bacterium would be reduced significantly.

To determine whether epetraborole is active against *M. abscessus in vivo*, we evaluated the efficacy of this compound in a previously established murine model of *M. abscessus* infection (17). All experiments involving live animals were approved by the Institutional Animal Care and Use Committee of the Center for Discovery and Innovation, Hackensack Meridian Health. NOD SCID mice were infected intranasally with *M. abscessus* K21. At day 1 postinfection, the lung bacterial burden of the mice reached ~10^6 CFU (Fig. 2A). Beginning on day 1, clarithromycin (formulated in 0.5% carboxymethyl cellulose-0.5% Tween 80-sterile water), epetraborole (formulated in sterile phosphate-buffered saline [PBS]), or vehicle (sterile PBS) was administered by oral gavage once per day for 10 days. Based on a previous efficacy study using a *Pseudomonas aeruginosa* mouse infection model (9), epetraborole was administered at 150 and 300 mg/kg body weight. The lung bacterial burden remained unchanged in mice that received the drug-free vehicle control (Fig. 2A, day 11). Mice that received epetraborole at 300 mg/kg showed a statistically significant 1-log reduction in lung CFU that was comparable to that after treatment with clarithromycin at 250 mg/kg (Fig. 2A). A similar pattern of CFU reduction was observed in the spleen (Fig. 2B). Thus, epetraborole was active against *M. abscessus in vivo*. It is interesting to note that epetraborole, despite having similar *in vitro* activity as the previously characterized benzoxaborole EC/11770 (Table 1) (14), required with 300 mg/kg a 30-fold higher dosing to achieve a similar (~10-fold) reduction in bacterial lung burden. The basis for this difference remains to be determined but may be due to differences in the pharmacokinetic properties of the two compounds, including oral bioavailability (9, 14).
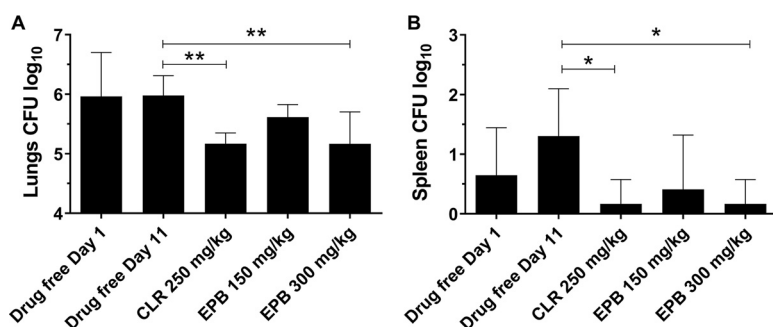

FIG 2 Epetraborole is active against *M. abscessus in vivo*. Lung CFU (A) and spleen CFU (B) from NOD SCID mice 1 day after intranasal infection with *Mab* (drug-free day 1) and following daily oral administration of drug-free vehicle, clarithromycin (CLR), or epetraborole (EPB) for 10 days (day 11). Data represent the means plus standard deviations from six mice per treatment group. Statistical significance of the results was analyzed by one-way analysis of variance (ANOVA) multiple-comparison and Tukey's posttests. *, $P < 0.05$; **, $P < 0.01$.

In conclusion, we show that epetraborole, an advanced nonhalogenated 3-amino-methyl benzoxaborole developed for Gram-negative infections, is also active against *M. abscessus in vitro* and in a mouse model of infection. This agrees with a recent publication that identified epetraborole in a screen of the MMV pandemic response box for anti-*M. abscessus* activity and reported this compound's efficacy against *M. abscessus* in a zebrafish infection model (19). Our findings reaffirm leucyl-tRNA synthetase as an attractive target against *M. abscessus* and expand the repertoire of advanced lead compounds for the discovery of a benzoxaborole-based candidate for the treatment of *M. abscessus* lung disease.

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