Mycobacterium tuberculosis DprE1 Inhibitor OPC-167832 Is Active against Mycobacterium abscessus In Vitro

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ABSTRACT The antituberculosis candidate OPC-167832, an inhibitor of DprE1, was active against Mycobacterium abscessus. Resistance mapped to \( M. \) abscessus \( dprE1 \), suggesting target retention. OPC-167832 was bactericidal and did not antagonize activity of clinical anti-M. abscessus antibiotics. Due to its moderate potency compared to that against \( M. \) tuberculosis, the compound lacked efficacy in a mouse model and is thus not a repurposing candidate. These results identify OPC-167832–DprE1 as a lead-target couple for a \( M. \) abscessus-specific optimization program.

KEYWORDS OPC-167832, DprE1, SigA, Mycobacterium abscessus, NTM, nontuberculous mycobacteria

The nontuberculous mycobacterium (NTM) and opportunistic pathogen \( M. \) abscessus can establish extremely difficult to treat lung infections (1–3). Complex antibiotic regimens, typically consisting a macrolide (clarithromycin or azithromycin), the aminoglycoside amikacin, and a \( \beta \)-lactam (cefotixin or imipenem), are administered often for years and show low cure rates (4–8). In brief, there is no reliable cure for \( M. \) abscessus lung disease. The \( M. \) abscessus drug pipeline is thinly populated (9), and new repurposing candidates and lead-target couples are sorely needed (10).

Decaprenylphosphoryl-\( \beta \)-D-ribose oxidase (DprE1) has emerged as an attractive target for antituberculosis (anti-TB) drug development (11–13). The enzyme, catalyzing the formation of decaprenyl-phospho-arabinose (DPA), is essential for growth and viability of \( M. \) tuberculosis (14–16). DPA serves as a precursor for the synthesis of arabinogalactan, a critical component of the mycobacterial cell wall (14). Inhibitors of \( M. \) tuberculosis DprE1 have been identified from various structural scaffolds and show potent activity in vitro and in mouse models (13, 17, 18). BTZ-043, PBTZ-169, OPC-167832, and TBA-7371 have progressed to phase I or II clinical trials for TB (19).

Transposon mutagenesis studies have shown that \( M. \) abscessus \( dprE1 \) (\( mab_0192c \)) is genetically essential (20). Whether \( M. \) abscessus DprE1 is a vulnerable target whose inhibition would translate into whole-cell antimicrobial activity has not been established. BTZ-043 and its analog PBTZ-169 have been tested for activity against \( M. \) abscessus, and both were found to be inactive (21, 22). This is likely due to an amino acid polymorphism in \( M. \) abscessus DprE1. BTZ-043 and PBTZ-169 form covalent adducts with cysteine 387 in \( M. \) tuberculosis DprE1 as their on-target mechanism of action (22–24). \( M. \) abscessus DprE1 has alanine at the corresponding amino acid residue position, thus preventing covalent adduct formation and enzyme inhibition by the covalent inhibitors (22–24).

Here, we tested the growth inhibitory activity of the DprE1 inhibitors OPC-167832 and TBA-7371, which do not form covalent adducts with their target (25, 26). The MIC of the compounds against the type strain \( M. \) abscessus subsp. \( abscessus \) ATCC 19977 (American Type Culture Collection) was determined in Middlebrook 7H9 broth (BD) using the broth microdilution method with optical density at 600 nm (OD\(_{600}\)) as the readout as described.

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Previously (27), the MIC was defined as 90% growth inhibition compared to the drug-free culture. While TBA-7371 (MedChem Express) was inactive (MIC = 100 µM), the dihydrocarbostyril OPC-167832 (MedChem Express) was found to be active (MIC = 5.2 µM). To determine whether the activity of OPC-167832 against the type strain was retained against the broader *M. abscessus* complex (28), MICs were measured against the reference strains of the two other subspecies, *M. abscessus* subsp. *bolletii* CCUG 50184T and *M. abscessus* subsp. *massiliense* CCUG 48898T (Culture Collection University of Goteborg), and against a panel of clinical isolates which include *erm41*-harboring macrolide-resistant strains (29, 30).

Potency was largely consistent across the members of the *M. abscessus* complex, with MICs ranging from 5.2 to 15 µM (Table 1).

The micromolar concentration activity against the NTM is in stark contrast to the nanomolar concentration activities of OPC-167832 reported for *M. tuberculosis* (25). The dramatic in vitro potency difference of the TB drug candidate against *M. abscessus* suggests that OPC-167832 is likely not a repurposing candidate for the treatment of this lung disease. This was confirmed by in vivo pharmacokinetic-pharmacodynamic analyses. 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 (no. 269.030 and no. 265.015).

The plasma concentration-time profile upon oral administration of OPC-167832 in uninfected CD-1 mice (Charles River Laboratories) was determined by measuring the plasma concentrations of the compound via high-pressure liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) as described previously (31). Dosing at 20 or 100 mg/kg of body weight resulted in a plasma concentration versus time curve above the MIC for *M. tuberculosis* (25) for most of a 24-h interval; however, the MIC for *M. abscessus* was not reached (Fig. 1A). As increasing the dose from 100 to 200 mg/kg did not result in a significant increase of exposure (Fig. 1A), 100 mg/kg was chosen as the highest dose for an efficacy study in a *M. abscessus* mouse model. NOD.CB17-Prkdcscid/NCrCrl mice (NOD SCID; Charles River Laboratories) were infected with *M. abscessus* K21 as described previously (32) and treated once daily for 10 days with orally administered OPC-167832 (50 or 100 mg/kg), clarithromycin (250 mg/kg) as the positive control, or drug-free vehicle. As expected, OPC-167832 treatment did not result in a statistically significant reduction of the lung bacterial burden (Fig. 1B).

| *M. abscessus* strain | *erm41* sequevar | Clarithromycin susceptibility | MIC (µM) of4: | OPC-167832 | Clarithromycin |
|-----------------------|------------------|-------------------------------|--------------|------------|---------------|
| Reference strains     |                  |                               |              |            |               |
| *M. abscessus* subsp. *abscessus* ATCC 19977 | T28 | Resistant | 5.2 | 1.2 |
| *M. abscessus* subsp. *bolletii* CCUG 50184-T | T28 | Resistant | 5.4 | 3.2 |
| *M. abscessus* subsp. *massiliense* CCUG 48898-T | Deletion | Sensitive | 6 | 0.6 |
| Clinical isolates5 |                          |                               |              |            |               |
| *M. abscessus* subsp. *abscessus* Bamboo | C28 | Sensitive | 10 | 0.8 |
| *M. abscessus* subsp. *abscessus* K21 | C28 | Sensitive | 15 | 2 |
| *M. abscessus* subsp. *abscessus* M9 | T28 | Resistant | 10 | 5.4 |
| *M. abscessus* subsp. *abscessus* M199 | T28 | Resistant | 10 | 4.8 |
| *M. abscessus* subsp. *abscessus* M337 | T28 | Resistant | 7 | 2.9 |
| *M. abscessus* subsp. *abscessus* M404 | C28 | Sensitive | 8.5 | 0.8 |
| *M. abscessus* subsp. *abscessus* M421 | T28 | Resistant | 9.3 | 1.3 |
| *M. abscessus* subsp. *bolletii* M232 | T28 | Resistant | 8.8 | 5.4 |
| *M. abscessus* subsp. *bolletii* M506 | C28 | Sensitive | 6 | 0.6 |
| *M. abscessus* subsp. *massiliense* M111 | Deletion | Sensitive | 11 | 0.7 |

4 *erm41* is the methylase gene responsible for inducible clarithromycin resistance. The C28 and deletion sequevars are inactive *erm41* alleles and result in susceptibility to clarithromycin, while the T28 sequevar is functional and confers inducible resistance against clarithromycin (29, 30).

5 MIC determination was carried out three times independently, and the results are presented as mean values. Clarithromycin (Sigma-Aldrich) was used as the assay control (34).

5 *M. abscessus* Bamboo, *M. abscessus* K21, and the other clinical isolates were characterized and reported previously (32, 39, 40).
in vivo analyses suggest that OPC-167832 is not a repurposing candidate for *M. abscessus* lung disease due to its moderate micromolar concentration in vitro potency compared to its nanomolar concentration activity against *M. tuberculosis*. It is interesting to note that OPC-167832 at 100 mg/kg showed a weak effect on the bacterial burden in the spleen, similar to the positive control, clarithromycin, at 250 mg/kg (Fig. 1B). The reason for this apparent organ-specific effect remains to be determined and may involve differential drug penetration and/or differences in the pathophysiology of the bacteria.

To determine whether OPC-167832 retains DprE1 as its target in *M. abscessus* and inform future lead optimization efforts, spontaneously resistant mutants were isolated using the type strain *M. abscessus* ATCC 19977 on Middlebrook 7H10 agar as described previously (31). A total of $2 \times 10^6$ CFU were plated on agar medium containing $16 \times$ MIC (MIC = 5.2 μM) (Table 1), the lowest OPC-167832 concentration that suppressed growth of wild-type colonies, resulting in a frequency of resistance of $10^{-9}$/CFU. The experiment was repeated with another independently grown culture, yielding a similar frequency of resistance. Twelve randomly selected OPC-167832-resistant strains (OPC_RM1 to OPC_RM12) from the two selection experiments achieved pronounced resistance with an ~30- to 100-fold-higher MIC than the parent strain (Table 2). Whole-genome sequencing (Novogene Corporation, Inc.), followed by Sanger sequencing (Geneviz, Inc.), revealed that the 12 OPC-167832-resistant strains comprised two genotypic classes. Six strains (OPC_RM1 to OPC_RM6) harbored four different missense mutations in the *M. abscessus* homolog of *dprE1*, while the other six resistant strains (OPC_RM7 to OPC_RM12) harbored five different missense mutations in the homolog of *sigA* (mab_3009), which encodes the essential sigma factor A that assists the RNA polymerase in recognizing promoters of target genes (Table 2) (33).

To confirm that the observed polymorphisms detected in *dprE1* and *sigA* are indeed responsible for resistance to OPC-167832, merodiploid strains were engineered using custom-synthesized pMV262-hsp60-based expression systems (Geneviz, Inc.) as described previously (34). To confirm involvement of *dprE1* missense mutations, a copy of the mutant *dprE1* allele from a representative resistant strain (OPC_RM1) (Table 2) was constitutively expressed under the control of the hsp60 promoter in wild-type *M. abscessus* ATCC 19977. As expected, the strain expressing the mutant *dprE1* allele displayed high-level resistance to OPC-167832 (Table 2). To exclude the possibility that the observed resistance was caused by

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**FIG 1** Pharmacokinetic profile and activity of OPC-167832 in mice. (A) Plasma concentration versus time profile of OPC-167832. Female CD-1 mice received a single dose of 20, 100, or 200 mg/kg of OPC-167832 formulated in 5% (wt/vol) gum arabic solution (Sigma-Aldrich) by oral gavage. Blood samples were collected from the tail vein at 0.5, 1, 3, 5, 7, and 24 h after drug administration, and the plasma concentration of OPC-167832 was measured by high-pressure liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The MIC of OPC-167832 against *M. abscessus* K21 (15 μM [6,852.6 ng/mL]) (Table 1) is indicated by the red dotted line. The reported MIC of OPC-167832 against *M. tuberculosis* H37Rv (1.1 nM [0.5 ng/mL]) is indicated by the green dotted line (25). (B) *In vivo* efficacy of OPC-167832 against *M. abscessus* in a NOD SCID mouse model. NOD SCID mice were infected intranasally with *M. abscessus* K21. Starting 1 day postinfection (D1), OPC-167832 (50 or 100 mg/kg, formulated in 5% gum arabic solution), the positive control, clarithromycin (250 mg/kg, formulated in 0.5% carboxymethyl cellulose–0.5% Tween 80–sterile water), or drug-free OPC-167832 vehicle was orally administered to infected mice for 10 consecutive days via oral gavage. Twenty-four hours after the last dose (11 days postinfection), all mice were euthanized, and their lungs and spleen were aseptically removed prior to homogenization. Serial dilutions of organ homogenates were plated onto Middlebrook 7H11 agar (BD) to quantify lung and spleen bacterial load on day 1 postinfection and after administration of drug-free vehicle (D11 Vehicle), OPC-167832 (OPC), and clarithromycin (CLR). The mean and standard deviation are shown for each treatment group (n = 6). Statistical significance was determined using one-way analysis of variance (ANOVA) for multiple comparisons and Dunnett’s posttest (*, P < 0.05; **, P < 0.01). The experiment was carried out twice, showing similar results, and one representative data set is shown. (C) Plasma concentrations of OPC-167832 in infected NOD SCID mice 3 and 24 h after the last dose in the efficacy experiment shown in panel B. The graphs were generated using GraphPad Prism 9 software.
DprE1 Inhibitor Active against M. abscessus

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TABLE 2 Characterization of spontaneous and engineered OPC-167832-resistant M. abscessus ATCC 19977 strains

| Strain                  | MIC (µM) of OPC-167832 | Clarithromycin | Candidate resistance gene | Polymorphisms in candidate resistance gene | Polymorphisms in other genes |
|-------------------------|-------------------------|----------------|---------------------------|-------------------------------------------|-------------------------------|
| Wild type (WT)          | 5.2                     | 1.2            | NA                        | NA                                        | NA                            |
| **Spontaneous mutants** |                         |                |                           |                                           |                              |
| OPC_RM1 (1)             | >500                    | 1.5            | dprE1                     | G745A/Gly249Arg                           | None                          |
| OPC_RM2 (1)             | 230                     | 1.6            | dprE1                     | G103A/Ala33Thr                            | mab_0020 A104C/Gly249Arg     |
| OPC_RM3 (1)             | 220                     | 1.8            | dprE1                     | T872G/Val291Gly                           | None                          |
| OPC_RM4 (2)             | >500                    | 1.6            | dprE1                     | G745A/Gly249Arg                           | None                          |
| OPC_RM5 (2)             | 180                     | 1.2            | dprE1                     | C484T/Pro162Ser                           | None                          |
| OPC_RM6 (2)             | 380                     | 1.2            | dprE1                     | G103A/Ala33Thr                            | mab_0341 C38T/Ala13Val       |
| OPC_RM7 (1)             | >500                    | 2              | sigA                      | G789A/Gly263Ser                           | mab_1186c Δ29G               |
| OPC_RM8 (1)             | >500                    | 2.3            | sigA                      | A509G/Tyr170Cys                           | mab_1612 G901A/Glu301Lys     |
| OPC_RM9 (2)             | >500                    | 1.8            | sigA                      | C822A/Phe274Leu                           | mab_2152 Δ−14G               |
| OPC_RM10 (2)            | >500                    | 1.6            | sigA                      | T589G/Tyr197Asp                           | None                          |
| OPC_RM11 (2)            | >500                    | 1.8            | sigA                      | G789A/Gly263Ser                           | mab_4225ΔΔ1Δ67CT             |
| OPC_RM12 (2)            | >500                    | 2              | sigA                      | G895A/Ala299Thr                           | mab_0938c Δ752T; mab_0389c   |
|                         |                         |                |                           |                                           | A223C/Lys75Glu               |

*MIC determination was carried out three times independently and the results are presented as mean values. Clarithromycin was used as the assay control.
*Non-dprE1 and non-sigA polymorphisms detected by whole-genome sequencing. Consistent polymorphism in other genes were not observed. The identified mutations in the resistant strains are detailed by the changes in the DNA/amino acid sequence of the affected genes and proteins, respectively.
*NA, not applicable.
*Twelve spontaneous OPC-167832-resistant strains (OPC_RM1 to OPC_RM12), isolated from two independent culture batches, were randomly selected for characterization.
*The spontaneously resistant strains OPC_RM1 to OPC_RM12 were subjected to whole-genome sequencing, followed by confirmation by Sanger sequencing. The primers used for Sanger sequencing are described in Table S1. The identified mutations in the resistant strains are detailed by the changes in the DNA/amino acid sequence of the affected genes and proteins, respectively.

To further evaluate the attractiveness of OPC-167832-DprE1 as a lead-target couple, in vitro bactericidal activity and in vitro drug-drug potency interactions with anti-M. abscessus antibiotics were determined using M. abscessus ATCC 19977 as described previously (27).
OPC-167832 was bactericidal, with a 3-log kill at 4× MIC (Fig. 2). The absence of antagonism with clarithromycin, amikacin (Sigma-Aldrich), cefoxitin (MedChem Express), or imipenem (Cayman Chemical) (Table 3), together with the clean drug-drug interaction profile of OPC-167832 as required under multidrug TB therapy (25, 37, 38), suggests that dihydrocarbostyril analogs are compatible with the current standard of care for M. abscessus lung disease.

In conclusion, we identified OPC-167832 as the first whole-cell active inhibitor of M. abscessus DprE1, thus validating DprE1 as a vulnerable target in the opportunistic pathogen. The 1,000-fold-weaker activity of OPC-167832 against M. abscessus compared to M. tuberculosis results in unfavorable pharmacokinetic-pharmacodynamic parameters and lack of efficacy in a mouse model of M. abscessus infection. Thus, the TB drug candidate is unlikely to present a repurposing candidate for the treatment of M. abscessus lung disease. The reason for the pronounced potency difference against the two mycobacterial species remains to be determined and may involve target binding, uptake/excretion, or intrabacterial metabolism (10). If the basis for the differential potency can be elucidated, OPC-167832 may present an attractive chemical starting point for a rational, pathogen-specific lead optimization program.

**TABLE 3** In vitro drug-drug potency interaction between OPC-167832 and selected clinically used drugs against M. abscessus ATCC 19977

| Drug | Class | Target | MIC (µM) | Outcome |
|------|-------|--------|----------|---------|
| OPC-167832 | 3,4-Dihydrocarbostyril | DprE1 | 5.2 | Additivity |
| Clarithromycin | Macrolide | 50S ribosomal subunit | 1.2 | |
| OPC-167832 | 3,4-Dihydrocarbostyril | DprE1 | 5.2 | Additivity |
| Amikacin | Aminoglycoside | 30S ribosomal subunit | 30 | |
| OPC-167832 | 3,4-Dihydrocarbostyril | DprE1 | 5.2 | Additivity |
| Cefoxitin | β-Lactam | Peptidoglycan biosynthesis transpeptidases | 26 | |
| OPC-167832 | 3,4-Dihydrocarbostyril | DprE1 | 5.2 | Synergy |
| Imipenem | β-Lactam | Peptidoglycan biosynthesis transpeptidases | 25 | |

*To determine possible antagonisms between OPC-167832 and clinically used drugs, checkerboard analyses were carried using a 96-well plate format (41, 42). The effect of serially diluted OPC-167832 ranging from 0.39 µM to 25 µM was tested against the partner drugs ranging from 0.49 µM to 250 µM.

*The fractional inhibitory concentration index (FICI) was calculated as [(MIC of partner drug in combination/MIC of partner drug alone) + (MIC of OPC-167832 in combination/MIC of OPC-167832 alone)]. An FICI value of ≤0.5 indicates synergy, an FICI value of 0.5 to 4 indicates additivity (no interaction), and an FICI value of >4 indicates antagonism (43).
SUPPLEMENTAL MATERIAL

Supplemental material is available only online.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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