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MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS

Anti-*Mycobacterium abscessus* Activity of Tuberculosis F-ATP Synthase Inhibitor GaMF1

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ABSTRACT New drug targets and molecules with bactericidal activity are needed against the respiratory mycobacterial pathogen *Mycobacterium abscessus*. Employing a lead repurposing strategy, the antituberculosis compound GaMF1 was tested against *M. abscessus*. Whole-cell and ATP synthesis assays demonstrated that GaMF1 inhibits growth and kills *M. abscessus* by targeting the F-ATP synthase. GaMF1's anti-*M. abscessus* activity increased in combination with clofazimine, rifabutin, or amikacin. The study expands the repertoire of anti-*M. abscessus* compounds targeting oxidative phosphorylation.

KEYWORDS *Mycobacterium abscessus*, nontuberculous mycobacteria, bioenergetics, F-ATP synthase, γ subunit inhibitor, multidrug resistance

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The treatment of diseases caused by nontuberculosis mycobacteria (NTM), including the fast-growing bacterium *Mycobacterium abscessus*, is problematic because of low permeability of the cell wall, biofilm formation, deficient drug-activating enzymes, numerous enzymes that neutralize drugs or modify their specific targets, naturally occurring polymorphism, and induction of drug efflux pumps (1, 2). Such pumps use either ATP- or proton-motive force-driven energy forms generated by the electron transport chain (ETC), which generates the proton-motive force for condensing ADP plus P_i to form ATP in the process of oxidative phosphorylation by the F_1F_0-ATP synthase (3). Oxidative phosphorylation is the major process for ATP synthesis in the pathogen, making the enzymes of the ETC and the F_1F_0-ATP synthase attractive as drug targets, as shown for clofazimine (CFZ), affecting NADH dehydrogenase (NDH-2) of the ETC (4), or the repurposed antituberculosis (TB) drug bedaquiline (BDQ) and its derivative, TBAJ876, targeting *M. abscessus*'s F_1F_0 ATP synthase (5–7). However, despite being potent growth inhibitors, these compounds are mostly bacteriostatic against *M. abscessus*.

The mycobacterial F_1F_0-ATP synthase consists of its proton translocation F_0 sector (a:c_9), which is connected by the central, rotating γ:ε and the peripheral stalk subunits b:b':δ to the catalytic α_3:β_3 sector, in which ATP is formed (8–10). In contrast to non-mycobacterial F_1F_0-ATP synthase, the mycobacterial F-ATP synthase subunits α, δ, and γ contain a C-terminal elongation (11), inserted domain (8), or an extra loop of 12 to 14 amino acids (12), respectively. These add-ons are essential for regulation (9–11, 13) or ATP synthesis (11, 12, 14). For example, the mycobacterial extra loop of subunit γ provides a failsafe device (15) by which one of its aspartate residues forms a salt bridge with an arginine residue of the peripheral stalk subunit b' during rotation, when subunit γ comes into proximity of subunit b' (15). These novel mycobacterial features of subunits α and γ are targets for specific and novel inhibitors (14, 16). GaMF1, a bactericidal anti-TB compound that targets the extra loop of the *M. tuberculosis* γ subunit, represents such a novel mycobacterial F-ATP synthase inhibitor, which has a clog*P* of 4.37

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The authors declare a conflict of interest. G.G., P.R. are inventors on the patent 10202104853U which are related to the inhibitor described in this article.

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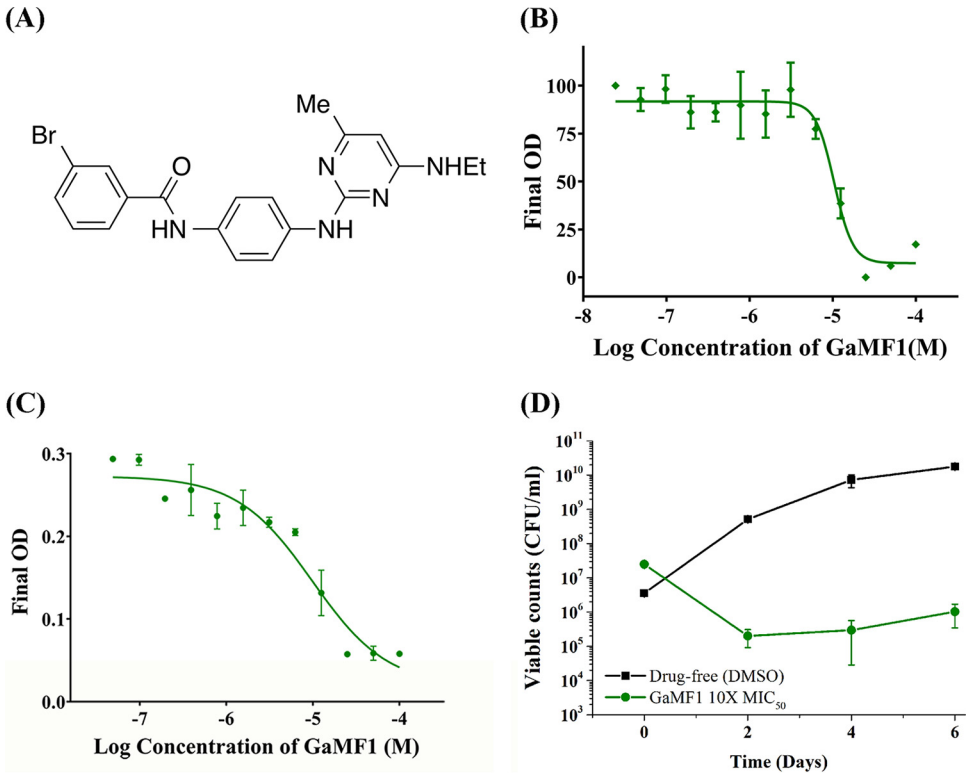

FIG 1 GaMF1 susceptibility testing. (A) Structure of GaMF1. (B) Effects of GaMF1 of *M. abscessus* subsp. *abscessus* ATCC19977 (*M. abscessus*) growth when grown on CAMH medium. The experiments were performed in triplicate. (C) Effect of GaMF1 against the clinical isolate *M. abscessus* Bamboo in CAMH broth. The experiment was performed in triplicate. $P < 0.0001$. Statistical analysis was carried out for the experiments in panels B to D using one-sample *t* and Wilcoxon tests (29). (D) Six days of GaMF1 kill kinetics against *M. abscessus*. The bacteria were grown in liquid culture (CAMH medium) in the presence of the indicated concentrations of GaMF1. The experiments were repeated twice, and the profiles were similar. $P < 0.001$. Statistical analysis was carried out for the experiment using ordinary one-way analysis of variance (ANOVA) with Bartlett's test.

and good metabolic stability in mouse liver microsomes and does not inhibit growth of Gram-positive and Gram-negative bacteria like *Staphylococcus aureus* and *Escherichia coli* (16).

Here, we have used a repurposing approach for the GaMF1 anti-TB compound (Fig. 1A) and studied *M. abscessus*'s susceptibility to the compound in cation-adjusted Mueller-Hinton (CAMH) medium (17, 18) and in complete Middlebrook 7H9 broth (19) using the type strain *M. abscessus* subsp. *abscessus* ATCC 19977 as the test organism. GaMF1 displayed potency with a MIC required to inhibit the growth of 50% of *M. abscessus* (MIC_{50}) of 13 $\pm$ 2.1 μM (Fig. 1B) and 33 $\pm$ 4.7 μM (see Fig. S1A in the supplemental material). These values are comparable to the ones determined for GaMF1 in *M. tuberculosis* (33 μM) and *M. bovis* Calmette-Guérin (BCG) (17 μM) (16), and are in the range of the clinically used anti-*M. abscessus* drugs cefoxitin (38 μM [20]), amikacin (55 μM [20]), and imipenem (14 μM [21]). While the F-ATP synthase inhibitors BDQ and TBAJ876 reveal a 100$\times$ higher potency against *M. tuberculosis* than *M. abscessus* (7), GaMF1 retains similar efficacy in cell growth inhibition and killing over a wide spectrum of mycobacteria.

Next, we determined whether GaMF1 susceptibility is retained against the clinical isolate *M. abscessus* subsp. *abscessus* Bamboo (22). The MIC_{50} determined in CAMH medium and Middlebrook 7H9 broth was 10 $\pm$ 1.9 μM (Fig. 1C) and 33 $\pm$ 3.2 μM (Fig. S1B), respectively, similar to the values determined for the *M. abscessus* type strain *M. abscessus* subsp. *abscessus* ATCC 19977.

To determine whether GaMF1 is bactericidal against *M. abscessus*, we measured the

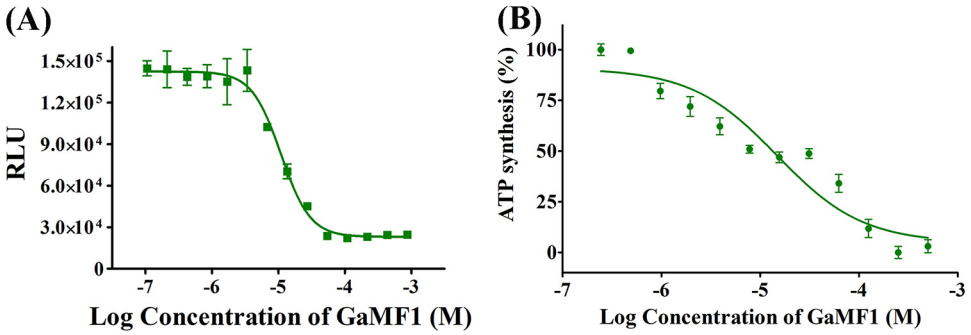

FIG 2 GaMF1 inhibits oxidative phosphorylation. (A) Inhibition of intracellular ATP synthesis of *M. abscessus* cells by GaMF1. The ATP content of the cells was measured by adding BacTiter-Glo (Promega) to the cells. The total ATP content is directly proportional to number of relative luminescence units (RLU). The experiments were performed in triplicate. (B) Inhibition of ATP synthesis by GaMF1 in *M. abscessus* IMVs using the electron donor NADH. ATP synthesis by *M. abscessus* IMVs was measured as luminescence upon addition of CellTiter-Glo (Promega). The experiments were performed in triplicate. $P < 0.0001$. Statistical analysis was carried out for both experiments in panels A and B using one-sample *t* and Wilcoxon tests (29).

survival of the type strain upon drug exposure. GaMF1 was bactericidal against *M. abscessus* at 10-fold its MIC_{50} in both CAMH medium (Fig. 1D) and 7H9 (Fig. S1C).

The ability of GaMF1 to inhibit mycobacterial F-ATP synthase was evaluated using an intracellular ATP synthesis assay (23) and inside-out membrane vesicles (IMVs) of *M. abscessus* (16), which were prepared according to Hotra et al. (16). As displayed in Fig. 2A, compound GaMF1 depleted ATP synthesis within the cell with a half-maximal inhibitory concentration (IC_{50}) of 10 $\pm$ 0.9 μM and depleted NADH-driven ATP synthesis of *M. abscessus* IMVs with an IC_{50} of 13 $\pm$ 2.5 μM (Fig. 2B), suggesting that growth inhibition of the pathogen is caused by the inhibition of the F-ATP synthase.

Since treatment of *M. abscessus* infections requires drug combinations, we measured the growth inhibition activity of GaMF1 combined with CFZ, proposed to compete with the mycobacterial specific electron acceptor menaquinone for its reduction

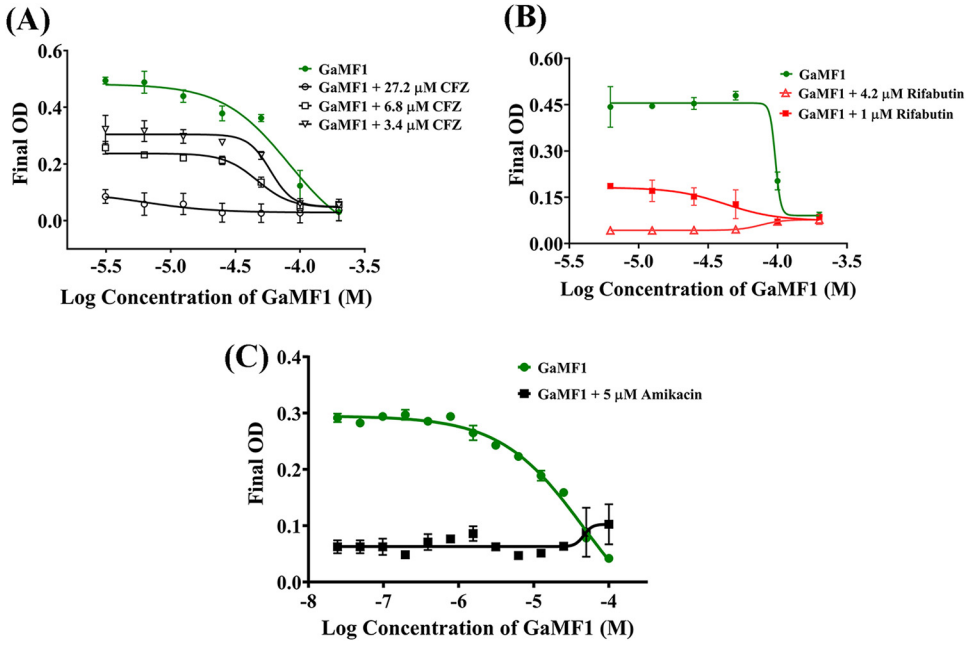

FIG 3 Increased potency of GaMF1 in combination with the antibiotics CFZ, rifabutin, and amikacin in 7H9 medium. (A) *M. abscessus* growth inhibition by GaMF1 (green circle) in combination with 3.4 μM (inverted triangle), 6.8 μM (open square), or 27.2 μM (open circle) CFZ. (B) GaMF1 (green circle) susceptibility of *M. abscessus* in combination with 1 μM (red square) or 4.2 μM (red triangle) rifabutin. (C) Amikacin (5 μM [black square]) increases the potency of GaMF1 growth inhibition of *M. abscessus* subsp. *abscessus*. $P < 0.0001$. Statistical analysis was carried out using two-way ANOVA for all the experiments presented.

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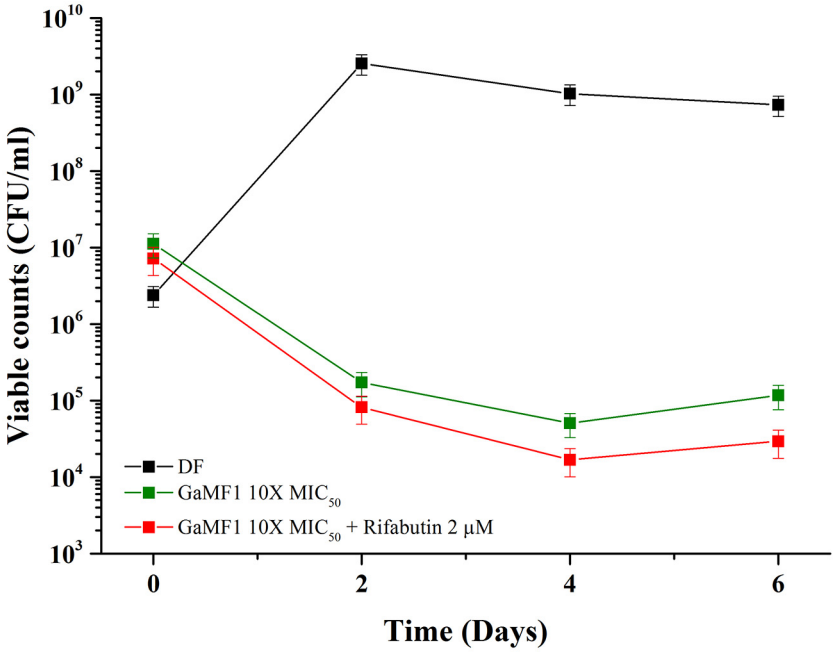

FIG 4 GaMF1 and rifabutin killing potency. Initial six days of GaMF1 and GaMF1 plus rifabutin kill kinetics against *M. abscessus* subsp. *abscessus* growth. The bacteria were grown in liquid culture (7H9) in the presence of the indicated concentrations of GaMF1 and rifabutin. The experiments were repeated twice, and the profiles were similar. $P < 0.0001$. Statistical analysis was carried out for the experiment using ordinary one-way ANOVA with Bartlett's test.

by the NDH-2 complex (23). CFZ has been recently shown to be active against *M. abscessus* and is used clinically as a repurposed drug (23–26). As revealed in Fig. 3, the combination of 3 μM CFZ or 6.8 μM (MIC_{50} of CFZ [25]) with GaMF1 increased the potency of *M. abscessus* cell inhibition in 7H9 medium (Fig. 3A). When GaMF1 was combined with *M. abscessus*'s RNA polymerase inhibitor rifabutin (MIC of 1 to 4 μM) (Fig. 3B) or the anti-*M. abscessus* antibiotic amikacin (5 μM) (Fig. 3C) (27), a drastic reduction of cell growth was observed.

Considering the increased anti-*M. abscessus* activity in the GaMF1-rifabutin combination and rifabutin's bactericidal activity (27, 28), the combinatory GaMF1-rifabution effect in *M. abscessus* killing was shown to be only marginal (Fig. 4).

In conclusion, this study has shown that GaMF1 is active in cell growth inhibition and killing of *M. abscessus*, similar to the compound's activity against *M. tuberculosis*. The identification of GaMF1 as a novel *M. abscessus* inhibitor expands the poorly populated compound pipeline against this difficult-to-cure opportunistic NTM pathogen. By targeting *M. abscessus*'s F-ATP synthase, GaMF1 depletes cellular ATP, essential for cell wall formation, replication, ana- and catabolic processes, and ATP-dependent efflux pumps. Inhibition of the latter could impede drug flux, which has emerged as an important determinant of drug resistance of BDQ and CFZ (25) in *M. abscessus*. Finally, *M. abscessus* revealed an increased susceptibility of GaMF1 in combination with CFZ, rifabutin, or amikacin, which is a prerequisite for combinatory approaches to treat *M. abscessus* infections.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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Conceptualization: G.G. Investigation: P.R. Writing—original draft: P.R. and G.G. Writing—review and editing: P.R., T.D., and G.G. Funding acquisition: T.D. and G.G. Supervision: G.G.

G.G. and P.R. are inventors on Singapore (SG) "Provisional" Patent 10202112053Y, Discovery of a F-ATP synthase inhibitor for the treatment Of *Mycobacterium abscessus* diseases filed on 29th October 2021, related to the inhibitor described in this article.

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