REVIEW ARTICLE

World Health Organization Group 5 Drugs for the Treatment of Drug-Resistant Tuberculosis: Unclear Efficacy or Untapped Potential?

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Background. Treatment of multidrug-resistant or extensively drug-resistant tuberculosis (DR-tuberculosis) is challenging because commonly used second-line drugs are poorly efficacious and highly toxic. Although World Health Organization group 5 drugs are not recommended for routine use because of unclear activity, some may have untapped potential as more efficacious or better tolerated alternatives.

Methods. We conducted an exhaustive review of in vitro, animal, and clinical studies of group 5 drugs to identify critical research questions that may inform their use in current treatment of DR-tuberculosis and clinical trials of new DR-tuberculosis regimens.

Results. Clofazimine may contribute to new short-course DR-tuberculosis regimens. Beta-lactams merit further evaluation—specifically optimization of dose and schedule. Linezolid appears to be effective but is frequently discontinued due to toxicity. Thiacetazone is too toxic to warrant further evaluation. *Mycobacterium tuberculosis* has intrinsic inducible resistance to clarithromycin.

Conclusions. Clofazimine and beta-lactams may have unrealized potential in the treatment of DR-tuberculosis and warrant further study. Serious toxicities or intrinsic resistance limit the utility of other group 5 drugs. For several group 5 compounds, better understanding of structure-toxicity relationships may lead to better-tolerated analogs.

Keywords. tuberculosis; second-line drugs; drug resistance; multidrug-resistant tuberculosis; clofazimine; linezolid; amoxicillin/clavulanate; carbapenems; thiacetazone; clarithromycin; extensively drug-resistant tuberculosis.

The World Health Organization (WHO) estimates that more than 1.3 million people with multidrug-resistant (MDR) tuberculosis caused by *Mycobacterium tuberculosis* resistant to isoniazid and rifampin will require treatment in the 27 countries with the highest MDR-tuberculosis burden between 2010 and 2015 [1]. Extensively drug-resistant tuberculosis (XDR-tuberculosis; caused by *M. tuberculosis* resistant to isoniazid, rifampin, fluoroquinolones, and at least one injectable agent), a more difficult-to-treat form of tuberculosis, is widespread [2]. Second-line drugs used to treat drug-resistant (DR) tuberculosis have undesirable toxicity profiles and/or lack potency, and current MDR-tuberculosis treatment requires at least 18 months of multidrug therapy. To improve treatment of DR-tuberculosis with existing drugs and identify optimized background regimens for trials with new compounds, the Drug Efficacy Subgroup of Research Excellence to Stop tuberculosis Resistance (RESIST-tuberculosis; http://www.resisttb.org) reviewed the extant literature on second-line tuberculosis drugs to ascertain the contribution of individual agents to DR-tuberculosis treatment. This review summarizes the evidence and gaps in knowledge for drugs that are classified by WHO as "group 5"—not recommended for routine use for treatment of DR-tuberculosis because of unclear efficacy (Table 1) [3]. This group includes clofazimine, linezolid, amoxicillin-clavulanate, carbapenems, thiacetazone, and clarithromycin. We highlight and prioritize key research questions about these drugs.

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Table 1. Grouping of Drugs for Tuberculosis by the World Health Organization

Group	Description	Drugs
1	First-line oral agents	Isoniazid, rifampin, ethambutol, pyrazinamide, and rifabutin
2	Injectable agents	Kanamycin, amikacin, capreomycin, and streptomycin
3	Fluoroquinolones	Moxifloxacin, levofloxacin, gatifloxacin, and ofloxacin
4	Oral bacteriostatic second-line agents	Ethionamide, protionamide, cycloserine, terizidone, and para-aminosalicylic acid
5	Agents with unclear efficacy	Clofazimine, linezolid, amoxicillin-clavulanate, thiacetazone, clarithromycin, and carbapenems

Modified from Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis 2008 (and 2011 update) from the World Health Organization, available at http://whqlibdob.who.int//publications/2011/9789241501583_eng.pdf; http://whqlibdob.who.int//publications/2008/9789241547581_eng.pdf.

SEARCH STRATEGY AND SELECTION CRITERIA

In vitro studies were included if they used *M. tuberculosis* laboratory or clinical strains and reported minimum inhibitory concentration (MIC) or bactericidal activity as outcomes. Animal studies involving mice or guinea pigs infected with laboratory or clinical *M. tuberculosis* strains describing pharmacokinetic (PK), lung or spleen colony-forming units (CFUs), or mortality as outcomes were included. Clinical studies were included if they had relevant PK, safety, and tolerability endpoints; bacteriologic endpoints such as log decrease in CFUs per day (early bactericidal activity [EBA]) or sputum culture conversion; or clinical endpoints such as cure without relapse, failure, 1-year favorable status, or death.

A search strategy using the MeSH terms "tuberculosis" and the drug being evaluated for the period January 2008 through September 2011 was employed in PubMed and Embase. References at the end of included articles were hand-searched. References in tuberculosis drug textbooks were hand-searched.

RESULTS

Clofazimine

Clofazimine is a riminophenazine initially synthesized in 1954 for the treatment of tuberculosis [4]. Inconsistent results in animal models hindered its development for tuberculosis, but it was licensed for treatment of leprosy in 1969 [5]. Its mechanism of action remains unclear, but existing evidence favors production of reactive oxygen species of *M. tuberculosis,* a mechanism which may lead to synergy with isoniazid, and inhibition of adenosine triphosphate synthesis [6]. It is currently used at a dose of 50–100 mg daily for treatment of MDR-tuberculosis or XDR-tuberculosis when few treatment options are available. The MIC of clofazimine against *M. tuberculosis* ranges from 0.06 to 2.0 μg/mL, and 1 μg/mL is the suggested susceptibility breakpoint [7]. In one study, the minimum bactericidal concentration against *M. tuberculosis* ranged from 0.12 to 0.48 μg/mL, compared with 8–125 μg/mL for *Mycobacterium avium* complex (MAC) infection [8], providing evidence that the limited efficacy of clofazimine against MAC infection should not be extrapolated to tuberculosis. Similarly, potent activity against hypoxic, nonreplicating *M. tuberculosis* [9] suggests clofazimine may have potential as a sterilizing drug. Mechanisms for resistance have not been reported.

Preclinical Studies

Clofazimine has substantial anti-tuberculosis activity in mouse models, but results are less impressive in guinea pigs and monkeys. In mice, a 20 mg/kg daily dose yields mean plasma concentrations of 0.55 μg/mL at steady state, but concentrations in tissues such as liver and lung are much higher [7, 10]. At this dose, clofazimine monotherapy is bactericidal [5, 7]. The onset of the bactericidal effect, however, is slow and may not prevent death in heavily infected animals. Thus, short-term evaluations of clofazimine activity for acute infection may underestimate the drug's expected activity.

A challenge in the measurement of clofazimine activity in animals is the "carryover" effect. The pronounced drug accumulation in tissues with repeated dosing results in clofazimine concentrations high enough to inhibit the growth of viable bacilli when organ homogenates are transferred onto culture media, leading to overestimation of clofazimine activity [11]. Nonetheless, recent studies of multidrug combinations using relapse as an outcome and activated charcoal in the culture media to reduce carryover effects identified a strong combined effect of clofazimine with pyrazinamide and the new diarylquinoline, bedaquiline [12].

Early results comparing clofazimine with isoniazid and streptomycin in guinea pigs were not as promising as those in mice, perhaps because of poor drug absorption or choice of early mortality and pathology scores (rather than cure without relapse) as endpoints [5, 13]. More damning at the time were results in rhesus macaques in which clofazimine was effective as prophylaxis but did not have sustained efficacy against established tuberculosis, despite adequate plasma concentrations [14]. However, clofazimine resistance emerged in the majority of treatment failures and may have explained the poor outcomes in this monotherapy study.

Clinical Studies

Clofazimine is a component of multidrug therapy for lepromatous leprosy but is no longer recommended for treatment

of AIDS-associated MAC infection because of its association with excess mortality in this patient population [15]. Among patients with leprosy taking the same dose (100 mg daily) that was studied for AIDS-associated MAC infection, however, the drug has little serious toxicity. Encouraging results from 2 recent studies sparked new interest in using clofazimine for DR-tuberculosis. One demonstrated cure of MDR-tuberculosis in nearly 90% of patients receiving a 9-month regimen including high-dose gatifloxacin, high-dose isoniazid, and clofazimine in addition to standard second-line drugs [16]. In another study, treatment of XDR-tuberculosis was successful in >60% of patients, and most received clofazimine [17].

PK studies demonstrate a prolonged lag time for absorption, high variability in bioavailability and clearance, and a terminal half-life of 70 days [18]. Mean steady state serum concentrations of approximately 0.24 μg/mL are achieved only after 1 month of 50 mg/day. Clofazimine is highly concentrated in fat, organs, skin, and bone with prolonged use. Despite such marked accumulation, clofazimine is relatively well-tolerated. Slowly reversible red-black skin discoloration occurs in virtually all patients treated for more than a few months. Intriguing reports suggest that co-administration with isoniazid may reduce tissue accumulation while increasing clofazimine concentrations in serum and urine [10].

Areas of Research Interest

Clofazimine is a poorly understood drug. It has promising antituberculosis activity in vitro and in mice, including strong combined effects with new agents, but its activity in larger animal models was discouraging. Given renewed interest in clofazimine for DR-tuberculosis and ongoing efforts to develop more water-soluble analogs with reduced potential for skin deposition, clofazimine warrants a more thorough evaluation in animal models and clinically as part of new combinations for DR-tuberculosis. Efficacy studies should be performed in animal models which exhibit more humanlike pathology than do mice, with careful attention to drug PK, outcome measures of sterilizing activity, and methods to reduce drug carryover effects. The clinical evaluation of clofazimine also presents challenges. Although EBA studies, studies that quantitatively assess the reduction in sputum colony counts over time (generally 2–14 days), can assist with dose-finding and evaluation of PK-pharmacodynamic relationships for tuberculosis drugs, the long time to steady state and slow onset of effect of clofazimine mean that 2-week EBA studies are unlikely to demonstrate the true potential of this drug. The lag in absorption, low serum concentrations even in the setting of adequate tissue concentrations, and long terminal half-life must be considered in design of longer-term clinical trials.

Linezolid

Linezolid is an oxazolidinone antibiotic developed to treat resistant gram-positive bacterial infections. It is increasingly used in salvage regimens for DR-tuberculosis. Linezolid inhibits bacterial protein synthesis by binding to 23S ribosomal RNA (rRNA). It also inhibits protein synthesis in mammalian mitochondria, giving rise to dose- and duration-dependent myelopoietic and neuropathic toxicity. The standard dose for treatment of gram-positive infections is 600 mg twice daily, but daily doses of 300–600 mg once daily are commonly used for DR-tuberculosis in an effort to reduce or prevent toxicity associated with prolonged use [19]. The MIC for linezolid against *M. tuberculosis* is 0.5 μg/mL [20]. Mutations affecting the 23s rRNA gene confer high-level resistance (MIC, 16–32 μg/mL), whereas the mechanism for low-level resistance (MIC, 4–8 μg/mL) is unknown [21].

Preclinical Studies

The activity of linezolid against gram-positive bacteria is most closely linked to the area under the curve (AUC) and MIC. Evidence from a mouse model suggests the same is true for *M. tuberculosis* [22], although time-dependent killing is suggested by a whole blood model [23]. Linezolid is bacteriostatic in mice at 50 mg/kg (AUC equivalent to 300 mg daily in humans) and weakly bactericidal at doses corresponding to 600 mg once or twice daily in humans [22, 24].

Clinical Studies

In an EBA study, 600 mg of linezolid given once and twice daily reduced sputum CFU counts by 0.18 and 0.26 $\log_{10}$ CFUs/mL/day, respectively, over the first 2 days. The EBA over the next 3 days was minimal [25]. Multiple case series suggest that linezolid contributes to sputum culture conversion in patients with few treatment options, but there are no data from controlled clinical trials [19, 26]. Dose- and duration-limiting mitochondrial toxicities such as peripheral neuropathy and bone marrow suppression limit the clinical utility of linezolid. Although adverse events, especially hematological toxicity, are less common with once-daily dosing of 300–600 mg, the impact of dose reduction on efficacy and the risk of neuropathy, which is often irreversible [27], remains unclear. It is possible that alternative dosing schemes may provide sufficient exposures to inhibit *M. tuberculosis* while minimizing inhibition of mitochondrial protein synthesis [28, 29]. An ongoing study of XDR-tuberculosis in Korea is evaluating 600 mg daily followed by either continuation of 600 mg daily or de-escalation to 300 mg daily together with an optimized background regimen. Preliminary results reveal culture negativity in liquid medium in 24% of patients at 2 months, 57% of patients at 4 months, and 76% of patients at 6 months coupled with radiographic improvement [30]. Newer oxazolidinones in clinical development may be associated with reduced toxicity and greater efficacy [28].

Areas of Research Interest

Although experimental studies confirm the anti-tuberculosis activity of linezolid and promising clinical results have been

reported, its use is limited by toxicity. Better understanding of the relationship of drug exposure with efficacy and toxicity could help optimize dosing strategies. An ongoing clinical trial should shed light on whether or not a lower daily dose of 300 mg will improve the risk-to-benefit ratio. However, because newer oxazolidinones in clinical development for tuberculosis are more potent than linezolid against *M. tuberculosis* in preclinical models and may prove to be less toxic, more investment in linezolid for tuberculosis may not be prudent.

Beta-Lactams

Beta-lactams were initially developed to treat gram-positive infections in the 1940s. Beta-lactams inhibit cell wall synthesis by binding the transpeptidases which catalyze peptidoglycan cross-linking. A handful of beta-lactams, including amoxicillin (a penicillin) and the carbapenems (imipenem, ertapenem, and meropenem), have been considered for tuberculosis treatment. Although the broad-spectrum beta-lactamase of *M. tuberculosis,* BlaC, limits the anti-tuberculosis potential of beta-lactams [31], it may be irreversibly inhibited by clavulanate, but not tazobactam or sulbactam, to enhance beta-lactam activity [32]. Co-administration of clavulanate reduces the amoxicillin MIC against *M. tuberculosis* from ≥16 μg/mL to 2–8 μg/mL [33, 34]. Carbapenems also are hydrolyzed by BlaC but at a slower rate than for amoxicillin [34]. Still, the addition of clavulanate to imipenem and meropenem reduces their MICs against *M. tuberculosis* by several dilutions. The ertapenem-clavulanate combination is less potent [35].

Recent work shows that an alternative form of peptidoglycan cross-linking (L,D-transpeptidation) predominates in *M. tuberculosis* and may confer tolerance to penicillins, especially in the stationary phase of growth [36]. Carbapenems, however, may still inhibit the L,D-transpeptidases involved in this process. This may confer sterilizing activity on carbapenems, which have been shown to kill hypoxic, nonreplicating, persistent *M. tuberculosis* in vitro. Beta-lactams are added only occasionally to DR-tuberculosis regimens in individual cases in which fewer than 4 drugs thought to be active against the organism are available. WHO guidelines suggest an amoxicillin-clavulanate dose of 500 and 125 mg to 1000 and 250 mg orally 3 times per day and an imipenem dose of 500–1000 mg intravenously every 6 hours, but these recommendations are not supported by dose-finding data from clinical trials. Of note, clavulanate is not commercially available in combination with carbapenems.

Preclinical Studies

The pharmacodynamic parameter that correlates best with the bactericidal activity of beta-lactams against other pathogens is time above MIC ($T_{>MIC}$). Only 2 studies have examined the efficacy of beta-lactams against tuberculosis in mice. An imipenem dose of 100 mg/kg twice daily reduced spleen and lung CFU counts by approximately 1.5 and 0.75 $\log_{10}$ CFUs/g, respectively, and reduced mortality despite achieving only 12% $T_{>MIC}$ [37]. In contrast, administration of imipenem, meropenem, or ertapenem at 100 mg/kg with clavulanate once daily prevented mortality but permitted bacterial growth [35].

Clinical Studies

The importance of achieving adequate $T_{>MIC}$ in humans may be evident in the results of 2 EBA studies. In a study using divided dosing of amoxicillin-clavulanate (1000 and 250 mg thrice daily), EBA_{0-2} was 0.34 $\log_{10}$ CFUs/mL/day [38]. In a study using 3000 and 750 mg once daily, however, EBA_{0-2} was similar to that for no drug [39]. New formulations of amoxicillin-clavulanate (2000 and 125 mg) may be safely administered twice or thrice daily, achieving the 50% $T_{>MIC}$ target against isolates for which amoxicillin-clavulanate MICs are 4 or 8 μg/mL, respectively. Anecdotal evidence suggests that 1 g of imipenem given twice daily contributes to sputum culture conversion among patients with MDR-tuberculosis [37]. Twice- or thrice-daily dosing of imipenem or meropenem (but not ertapenem) with clavulanate also may be expected to achieve the $T_{>MIC}$ values needed to treat patients infected with isolates for which the MIC is as high as 4 μg/mL and the majority of those in which the MIC is 8 μg/mL, but this remains to be proven. Overall, beta-lactams are well-tolerated, but anaphylactic reactions can occur in a minority (0.01%) of patients.

Areas of Research Interest

With greater appreciation of the time-dependent activity of beta-lactams and the potentiating effects of clavulanate, in vitro and animal model studies should be performed to identify optimized dosing strategies for amoxicillin-clavulanate as a relatively well-tolerated and safe oral option for DR-tuberculosis. Because carbapenems inhibit newly discovered transpeptidases that may be important for mycobacterial persistence, meropenem, which is less susceptible to hydrolysis by BlaC, may have sterilizing activity. Although meropenem must be given by intravenous infusion, which is highly impractical in most settings, investigational carbapenems with oral prodrug formulations could be evaluated. Dose-fractionation studies in hollow fiber models and/or mice can determine the target $T_{>MIC}$ associated with optimal killing and evaluate sterilizing activity, enabling prediction of the dose and schedule most likely to provide clinical benefit. However, divided dosing is almost certainly necessary.

Thiacetazone

Thiacetazone is a thiosemicarbazone initially evaluated in the 1940s for tuberculosis [40]. Its mechanism of action is unclear, but recent work suggests that thiacetazone inhibits cyclopropanation during mycolic acid biosynthesis [41]. It is used in rare cases for the treatment of DR-tuberculosis when no other options are available.

Table 2. World Health Organization Group 5 Drugs: Research Priorities for Use in Treatment Regimens for Drug-Resistant Tuberculosis

Drug (Formulation)	Major Toxicities Limiting Use for tuberculosis	Analogs in Development for Use in tuberculosis	Suggested Research Recommendations
Clofazimine (oral)	Red-black skin discoloration	Initial promising results of more water-soluble compound in mouse PK and efficacy studies	Evaluate in longer-term multidrug treatment regimens for MDR-tuberculosis or XDR-tuberculosis; follow closely as analogs move into clinical development
Linezolid (oral)	Myelotoxicity, peripheral neuropathy	In early clinical development; may be less potent inhibitors of mitochondrial protein synthesis; phase 2 EBA study recently completed	Consider testing efficacy of once-daily dosing in prospective clinical trials of multidrug therapy for DR-tuberculosis; further evaluations may be unwarranted if analogs are proven safe and effective
Amoxicillin-clavulanate (oral)	None	None	Determine PK-PD targets in preclinical studies; evaluate optimized twice- or thrice-daily dosing in clinical trial
Meropenem-clavulanate (intravenous meropenem; no commercially available coformulation)	None	Oral carbapenems under development but not for tuberculosis	Determine PK-PD targets in preclinical studies; consider clinical evaluation if clavulanate becomes available and optimized dosing likely to be twice-daily or less
Thiacetazone (oral)	Severe cutaneous hypersensitivity	None	Severe toxicity among certain patient populations (Asians and patients with HIV co-infection) makes it a poor candidate for further clinical evaluations
Clarithromycin (oral)	None	None	Intrinsic, inducible resistance precludes further evaluation of this drug for tuberculosis

Cost, availability, and patent information that impact the use of these drugs for drug-resistant tuberculosis are summarized elsewhere and are not included in this table [3].

Abbreviations: DR-tuberculosis, drug-resistant tuberculosis; EBA, early bactericidal activity; HIV, human immunodeficiency virus; MDR-tuberculosis, multidrug-resistant tuberculosis; PD, pharmacodynamic; PK, pharmacokinetic; XDR-tuberculosis, extensively drug-resistant tuberculosis.

Preclinical Studies

In mice and guinea pigs, thiacetazone has activity comparable with that of streptomycin and superior to that of para-aminosalicylic acid [40]. Even at high concentrations, thiacetazone is bacteriostatic and has poor sterilizing activity [42]. In a study to determine the utility of second-line drugs against MDR-tuberculosis, addition of thiacetazone did not enhance activity of low-dose moxifloxacin.

Clinical Studies

In the late 1940s, trials of thiacetazone efficacy were performed, but the results were limited by lack of controls and short periods of observation. In East Africa, thiacetazone was as effective as para-aminosalicylic acid when given together with isoniazid in effecting sputum conversion and preventing isoniazid resistance [43]. In EBA studies, however, thiacetazone had poor activity [44]. Thiacetazone was mostly used to prevent resistance to co-administered agents, although its capacity to do so was relatively poor. Its primary benefit was that it was extraordinarily cheap for resource-limited settings. When thiacetazone was first developed, severe toxicity, notably rash, related to thiacetazone was common in Asia and uncommon in Africa [45], hence its disproportionate use in Africa. However, among patients with human immunodeficiency virus (HIV) infection, thiacetazone can cause severe cutaneous hypersensitivity reactions that can be life-threatening, so its usefulness in settings of high HIV infection prevalence is diminished [46].

Areas of Research Interest

Given its low potency and toxicity profile, especially among patients with HIV co-infection, further study of thiacetazone is not warranted at this time.

Clarithromycin

Macrolides are important antibiotics for treatment of respiratory-tract infections. Macrolides inhibit protein synthesis by binding to the 50S ribosomal subunit. Although macrolides such as azithromycin and clarithromycin have been used successfully to treat nontuberculous mycobacterial infections, *M. tuberculosis* displays intrinsic, rapidly inducible resistance due to methylation of 23S rRNA by the *erm37* gene product, which prevents macrolide binding to the ribosome [47]. Values of MIC_{90} for clarithromycin against *M. tuberculosis* strains are typically

≥16 μg/mL and may be ≥128 μg/mL [48], especially after preincubation with clarithromycin [47]. In vitro synergy has been reported between clarithromycin and other drugs, including isoniazid, rifampin, pyrazinamide, and ethambutol, but the clinical significance of these findings is unknown [49]. Macrolides also are known to have anti-inflammatory properties which could contribute to clinical benefit despite microbial resistance.

Preclinical Studies

Monotherapy with clarithromycin in mice has weak inhibitory effects on bacterial growth but may reduce mortality associated with overwhelming infection [48, 50]. Addition of clarithromycin did not improve upon isoniazid or streptomycin monotherapy in mice. No other macrolide-containing regimens have been evaluated in animals.

Clinical Studies

No clinical studies evaluating the efficacy of clarithromycin for the treatment of tuberculosis have been reported to date.

Areas of Research Interest

M. tuberculosis is intrinsically resistant to currently available macrolides. Unless analogs are developed that do not induce or are not affected by the inducible *erm37* resistance mechanism, macrolides do not appear to have significant potential for the treatment of DR-tuberculosis.

CONCLUSIONS

Group 5 drugs are group 5 drugs for a reason, but that reason differs by drug and may be related to either toxicity or the lack of reliable information about clinical efficacy. Thiacetazone and linezolid have potentially serious adverse effects; *M. tuberculosis* is intrinsically resistant to clarithromycin; and clofazimine and beta-lactams have uncertain activity. However, although our review found that thiacetazone and macrolides are unlikely to be helpful except in deep salvage, linezolid is potentially useful in DR-tuberculosis treatment, if not limited by cost or toxicity (Table 2). Clofazimine and beta-lactams may have greater unrealized potential in the treatment of DR-tuberculosis. Clofazimine has intriguing activity in mice and may contribute to short-course treatment of DR-tuberculosis. Beta-lactams are well-tolerated and safe agents which may only require optimization of dose and schedule and co-administration with clavulanate to be effective agents for DR-tuberculosis. Better understanding of the potential contribution of the oxazolidinones, riminophenazines, and beta-lactams to DR-tuberculosis therapy also may inform the development of newer agents in these classes and spur new discovery efforts.

Notes

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Potential conflicts of interest. E. L. N. received a research grant from Pfizer within the past 3 years and is an inventor on a patent for combination therapy of tuberculosis including PNU-100480. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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