Streptomycin-Starved \textit{Mycobacterium tuberculosis} 18b, a Drug Discovery Tool for Latent Tuberculosis

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\textit{Mycobacterium tuberculosis} 18b, a streptomycin (STR)-dependent mutant that enters a viable but nonreplicating state in the absence of STR, has been developed as a simple model for drug testing against dormant bacilli. Here, we further evaluated the STR-starved 18b (SS18b) model both \textit{in vitro} and \textit{in vivo} by comparing the behavior of 22 approved and experimental tuberculosis drugs. Using the resazurin reduction microplate assay (REMA), rifampin (RIF), rifapentine (RPT), TMC207, clofazimine (CFM), and linezolid (LIN) were found to be active against SS18b \textit{in vitro}, and their bactericidal activity was confirmed by determining the number of CFU. A latent 18b infection was established in mice, and some of the above-mentioned drugs were used for treatment, either alone or in combination with RIF, RIF, RPT, TMC207, CFM, and pyrazinamide (PZA) were all active \textit{in vivo}, while cell wall inhibitors were not. A comparative kinetic study of rifamycin efficacy was then undertaken, and the results indicated that RPT clears latent 18b infection in mice faster than RIF. Intrigued by the opposing responses of live and dormant 18b cells to cell wall inhibitors, we conducted a systematic analysis of 14 such inhibitors using REMA. This uncovered an SS18b signature (CWPRED) that accurately predicted the activities of cell wall inhibitors and performed well in a blind study. CWPRED will be useful for establishing the mode of action of compounds with unknown targets, while the SS18b system should facilitate the discovery of drugs for treating latent tuberculosis.

Tuberculosis (TB) represents a major threat to human health in both developing and industrialized countries. WHO reported that one-third of the world’s population is latently infected with \textit{Mycobacterium tuberculosis}, the etiologic agent of the disease, and millions of lives are lost every year worldwide (16). The pathogen can enter a dormant or latent state characterized by limited growth and metabolism and, most importantly, by phenotypic resistance to the commonly used medications (8, 10), thereby allowing indefinite persistence in the human body. The current directly observed therapy short course (DOTS) regimen requires a four-drug combination of rifampin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB) for 2 months, followed by RIF and INH for 4 months (7). Poor adherence to such a lengthy treatment has resulted in the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains (18) that have worsened the global problem and forced the research community to explore new avenues to fight \textit{M. tuberculosis}. This scenario is exacerbated in TB patients with advanced HIV infection or diabetes (13, 15). Shortening the duration of therapy and finding drugs that could effectively kill dormant TB bacilli is therefore imperative (4).

\textit{M. tuberculosis} strain 18b, a member of the Beijing family (our unpublished data), is a streptomycin (STR)-dependent mutant that enters a viable but nonreplicating state in the absence of STR (19). In an attempt to develop a simple and robust model for testing drugs against nonreplicating bacilli, STR-starved 18b (SS18b) was the subject of our previous study, which established the \textit{in vitro} and \textit{in vivo} experimental conditions for evaluating drug efficacy (34). Compared to other \textit{in vitro} dormancy models, such as hypoxia (44, 45) or nutrient starvation (6), SS18b requires no special equipment, thus simplifying subsequent high-throughput screening (HTS). Most importantly, 18b mimics latent TB in animals, which makes it an appealing tool for \textit{in vivo} studies. Compared to other animal models used to reproduce latency, including the establishment of latent \textit{M. tuberculosis} infection 12 weeks after \textit{Mycobacterium bovis} BCG vaccination (50) or the Cornell model, which induces persistence by a 3-month treatment with high doses of INH and PZA (25, 26), the 18b model has the advantages of being considerably faster and simpler. Currently, there are several TB drug candidates in the early stages of development or in clinical trials that have been found to target various essential processes, including cell wall, DNA, RNA, and protein synthesis. While they are all effective against actively multiplying cells, their efficacy against dormant bacilli is sometimes unknown or unclear. In order to further assess its robustness and utility, we have systematically used the SS18b model to examine the \textit{in vitro} and \texti
SS18b cultures were maintained at an optical density at 600 nm (OD600) between 0.2 and 0.5 for 2 weeks (with the addition of fresh medium if necessary), by which time they had stopped replicating.

Antimicrobials. STR, INH, RIF, PZA, clofazimine (CFM), EMB, ethionamide (ETH), amoxicillin (AMOX), clavulante (CLAV), and cycloserine (CYS) were purchased from Sigma-Aldrich. Meropenem (MER) powder was the pharmaceutical standard. Other drugs were kindly provided by Sanofi-Aventis (rifapentine [RPT]), AstraZeneca (thiaclavomycin [TLM] and linezolid [LIN]), GlaxoSmithKline (GSK compounds), Tibetoc (TMC207), V. Makarov (the second-generation benzothiazone PBTZ169), H. Boshoff, C. E. Barry III (SQ109 and KRT2029), and M. Jackson (isoxyl), while pyridomycin (PYR) was purified as described previously (18a). Drugs for in vivo experiments were either dissolved or suspended before gavage as follows: RIF, RPT, and PZA in water (49); PBTZ in 0.3-fold serial dilutions of each test compound were prepared in 96-well plates containing tubercle bacilli in a total volume of 100 μl and then incubated for 7 days at 37°C before addition of 10 μl of 0.25% resazurin. After overnight incubation, the fluorescence of the resazurin metabolite resorufin was determined (excitation at 560 nm and emission at 590 nm; gain, 80) by using a Tecan Infinite M200 microplate reader.

Drug susceptibility testing. Drug treatment was performed both in the presence of STR (actively growing cells) and in the absence of STR (dormant cells) at 37°C with shaking. Mid-logarithmic-phase 18b or SS18b cultures were diluted to an OD of 0.05 and split into 10 ml samples, and drugs were added at the concentrations indicated (see Fig. 2), with an untreated sample serving as the control. Serial dilutions of the cultures were plated on 7H10 supplemented with glycerol, OADC, and STR at day 7 after the addition of drugs. The STR-dependent phenotype was checked by plating the same dilutions on 7H10 without STR. CFU were counted after 4 to 5 weeks of incubation at 37°C.

Mice and infection. Female BALB/c mice, aged 5 to 6 weeks, were obtained from Charles River Laboratories. For infections, logarithmic-phase 18b or SS18b cultures were diluted to an OD of 0.01 and an H37Rv culture (OD600 = 0.0002) were used in resazurin reduction microplate assays (REMA). Twofold (or 3-fold) serial dilutions of each test compound were prepared in 96-well plates containing tubercle bacilli in a total volume of 100 μl and then incubated for 7 days at 37°C before addition of 10 μl of 0.25% resazurin. After overnight incubation, the fluorescence of the resazurin metabolite resorufin was determined (excitation at 560 nm and emission at 590 nm; gain, 80) by using a Tecan Infinite M200 microplate reader.

Study 1. PBTZ, PZA, RPT, and CFM, as well as the combinations RIF-PZA and RIF-CFM, were tested. All drugs were administered by gavage 5 times a week (5/7) for 8 weeks. Treatment efficacy was assessed on the basis of lung CFU counts at the end of treatment. Control and treated mice were sacrificed, the lungs were homogenized, and dilutions were plated on 7H10 agar enriched with 10% OADC and supplemented with STR (50 μg/ml), cycloheximide (10 μg/ml), and ampicillin (50 μg/ml). The plates were incubated for 28 days at 37°C before CFU were enumerated.

Study 2. TMC207 was used alone and in combination with RIF. Both drugs were administered by gavage 5/7 for 8 weeks. Treatment efficacy was assessed on the basis of lung CFU counts after 4 weeks and at the end of treatment. Lung CFU counts of control and treated mice were obtained as described for study 1.

Study 3. A kinetic study of RPT with different drug exposures against latent 18b in vivo was performed. RPT was given at 10 mg/kg/5/7, 15 mg/kg twice weekly (2/7), or 20 mg/kg once weekly (1/7) for 12 weeks. Treatment efficacy was assessed on the basis of lung CFU counts after 2, 4, and 8 weeks and at the end of treatment. Lung CFU counts of control and treated mice were obtained as described for study 1.

Statistical analysis. CFU counts were log_{10} transformed before analysis, expressed as mean log_{10} CFU ± standard deviation (SD), and compared using unpaired Student’s t tests in Prism version 5.0 (GraphPad).

RESULTS

Study rationale. In our initial work (34), we established the experimental conditions for using SS18b to assess the efficacy of TB drugs against latent TB in vitro and in a murine model. Highly encouraging results were obtained under both conditions with RIF and the candidate drugs PA-824 and moxifloxacin (MOXI), suggesting that SS18b could be a valuable tool for finding new drugs active during latency. In contrast, the cell wall inhibitors INH, BTZ043, and MER were effective only against actively growing 18b. To evaluate the system more extensively, we tested a much larger set of compounds, including drug candidates that are currently being evaluated in clinical trials against MDR TB. In addition, we tested as many cell wall inhibitors as possible in order to determine whether their apparent lack of effect against SS18b is a general feature or restricted to the three tested previously (34).

In vitro drug susceptibility testing by REMA. The activities of a few front-line and several experimental TB drugs were tested against SS18b using REMA. RIF and TMC207 were selected as positive controls, since their activities on dormant cells have been well demonstrated, while INH was used as a negative control (34). After incubating SS18b for 7 days with the various compounds (Fig. 1), INH showed no activity against dormant cells, whereas a concentration-dependent decrease in fluorescence was seen with RIF (activity starting at 12 ng/ml) and TMC207 (156 ng/ml). Furthermore, another cell wall inhibitor, the second-generation benzothiazone PBTZ169 (24), caused no appreciable decrease in fluorescence. These data agree with the findings of our previous report (34).

Among the drugs tested against SS18b for the first time (Fig. 1), RPT had activity similar to that of RIF against dormant 18b (activity starting at 12 ng/ml), while CFM (156 ng/ml) and the oxazolidinone LIN (390 ng/ml) also caused a decrease in fluorescence. However, PZA did not affect resazurin reduction even at the high-concentration starting at 12 ng/ml), while CFM (156 ng/ml) and the oxa-
crease in CFU, whereas CFM performed less well, with only a 0.3-log-unit decrease in viability recorded ($P = 0.0364$). These data indicated that all drugs were active against multiplying 18b cells.

In parallel, the same drugs were tested against SS18b cells (Fig. 2B). No growth was seen in the absence of STR, confirming that the 18b cells were arrested. Contrary to what was observed when STR was present, addition of INH ($P = 0.1591$) or SQ109 ($P = 0.1133$) did not cause a significant decrease in CFU levels, although PBTZ169 had modest activity against nongrowing 18b cells, causing a 0.6-log-unit reduction in CFU ($P = 0.0451$). Both RIF ($P = 0.0199$) and RPT ($P = 0.0024$) were highly active, but RPT performed better and caused an additional 0.7-log-unit decrease in CFU. Similar results were obtained with CFM ($P = 0.0052$), which, interestingly, displayed greater killing activity against nonreplicating than growing cells. Taken together, these data confirmed the results obtained by REMA.

**In vivo activities of drugs against latent 18b.** To evaluate the efficacies of the drugs against SS18b *in vivo*, we performed three mouse studies with a chronic 18b infection model. BALB/c mice were infected, and after administration of STR for 3 weeks, a 1-log-unit increase in lung CFU counts was observed. Treatment began 10 days after STR withdrawal. In the first experiment, we compared the activity of RIF to those of RPT, PBTZ169, PZA, and CFM, as well as RIF plus PZA and RIF plus CFM (Fig. 3). After 8 weeks of treatment, RIF caused a 3.3-log-unit reduction in lung

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**FIG 1** Resazurin microplate assay with SS18b. Serial 2- or 3-fold dilutions of each drug were prepared in a 96-well plate, and their activities against strains H37Rv and SS18b were evaluated by REMA. The graphs show the correlation between drug concentrations and fluorescence levels.
CFU (P < 0.0001) compared to the lungs of untreated mice, whereas PBTZ treatment had a negligible bactericidal effect (P = 0.28), in agreement with the in vitro REMA data and previous findings for the first-generation benzothiazinone BTZ043 (34). PZA (P = 0.0009) and CFM (P < 0.0001) both displayed good killing, reducing the bacterial load by 2.1 and 1.5 log units, respectively. The number of bacteria in the lungs of mice treated with RPT was below the detection limit (<1.5 log CFU), confirming the higher efficacy of RPT against latent TB than was obtained with RIF. In the animals treated with RIF in combination with PZA or CFM, no CFU could be detected in the lungs either, suggesting that there was no antagonism between RIF and PZA or CFM. Together, these results mirror the data obtained in vitro by REMA, except for PZA, which had no activity in vitro, probably due to the neutral pH of the assay medium used.

**In vivo potency of TMC207.** TMC207, characterized by significant activity against SS18b in vitro (34), was tested in the second mouse study. After only 4 weeks of TMC207 monotherapy, no CFU were detected in the lungs (Fig. 4A). However, the combination of RIF and TMC207 did not clear the bacilli, as more than 1 log CFU remained, suggesting a possible antagonism between the two drugs, which was also reported when RIF was added to a TMC-PZA combination regimen (1, 21). At the end of treatment (i.e., after 2 months), TMC207, either alone or in combination with RIF, reduced the CFU levels below the limit of detection in the lungs, whereas RIF-treated mice still had almost 3 log CFU. Although the risk of some carryover may exist due to the accumulation of TMC207 in tissue, these data nonetheless confirm the potency of TMC207 against nonreplicating 18b in vivo and demonstrate that this activity is even higher than that of RIF.

**Kinetic study of rifapentine against latent 18b in vivo.** The potency of RPT against latent 18b was demonstrated in the first mouse study (Fig. 3). To investigate the kill kinetics of RPT and to gain insight into the effectiveness of different RPT regimens, we performed another mouse experiment whose results are reported in Fig. 5. After infection with 18b, groups of mice received three different RPT regimens: RPT10, 10 mg/kg 5/7; RPT15, 15 mg/kg 2/7; or RPT20, 20 mg/kg 1/7, for 12 weeks. Treatment efficacy was assessed on the basis of lung CFU counts after 2, 4, and 8 weeks and at the end of treatment. Chronic 18b infection was successfully established with an initial count of 7 log CFU. This value decreased to 4.7 log CFU after 3 months. During the course of treatment, RPT20 (1/7) and RIF (5/7) showed very similar activities, thus confirming that RPT is the more powerful rifamycin against latent 18b. More interestingly, the potency of RPT15 (2/7) was very close to that of RPT10 (5/7), and both regimens reduced the bacterial load in the lungs at the end of treatment below the levels of detection. Taken together, the data indicate that higher rifamycin exposure resulted in greater activity against latent 18b in mice.

**Cell wall inhibitor signature.** Interestingly, three cell wall inhibitors with different mechanisms, INH, PBTZ169, and SQ109, caused an increase in fluorescence in REMA, starting at concentrations near their MICs (Fig. 1). This increase was not accompanied by cidality. With growing H37Rv, an indication of an increase in fluorescence levels was also seen with these compounds at sub-MIC levels but was followed by a sharp loss in viability (Fig. 1 and 6). These observations suggested that such differential behavior.
and GSK7). Interestingly, the target of two of them (GSK5 and GSK6; see Fig. S1 in the supplemental material, GSK3 and AMOX-CLAV combinations (Fig. 6). Although the weak ETH, TLM, SQ109, EMB, and KRT2029 and with the MER-CLAV concentration-dependent increase in fluorescence was seen with drugs isoxyl and CYS (see Fig. S1 in the supplemental material) did not cause an increase in fluorescence, no decrease was observed, either, confirming their lack of activity against SS18b.

The existence of a signature for cell wall inhibitors, termed CWPRED (Table 1). The series included five inhibitors of mycolic acid synthesis or translation, namely, INH, ETH, TLM, SQ109, and isoxyl (3, 24, 29, 38, 41, 42); three compounds targeting the arabinogalactan layer, PBTZ169, KRT2029, and EMB (5, 24); and three inhibitors of peptidoglycan synthesis, MER, AMOX (with and without CLAV), and CYS (9, 17, 20, 51). Consistent with our hypothesis, a marked concentration-dependent increase in fluorescence was seen with ETH, TLM, SQ109, EMB, and KRT2029 and with the MER-CLAV and AMOX-CLAV combinations (Fig. 6). Although the weak drugs isoxyl and CYS (see Fig. S1 in the supplemental material) did not cause an increase in fluorescence, no decrease was observed, either, confirming their lack of activity against SS18b.

**Blind evaluation of the CWPRED signature.** The existence of a signature for cell wall inhibitors, termed CWPRED, could be valuable for predicting the modes of action (MOA) of compounds with an unknown target. Consequently, four compounds with whole-cell activity from GlaxoSmithKline were tested in an unbiased manner by REMA, without prior knowledge of their respective targets. All of them clearly displayed the signature (Fig. 6, GSK5 and GSK6; see Fig. S1 in the supplemental material, GSK3 and GSK7). Interestingly, the target of two of them (GSK5 and GSK6) had been previously identified as InhA (unpublished results), thus supporting the notion of the cell wall signature. Given the above results, the remaining compounds, GSK3 and GSK7, may also target cell wall synthesis. PYR, a natural product with antitycobacterial activity (23), also showed such a signature (Fig. 6), suggesting that it too targets cell wall synthesis, and this MOA has since been confirmed (Hartkoorn et al., submitted). Collectively, these results substantiate the existence of an SS18b REMA signature for cell wall inhibitors and demonstrate that CWPRED can reliably predict the MOA for novel compounds.

**DISCUSSION**

The need for drugs acting against latent TB and persisting tubercle bacilli has been eloquently articulated, but finding such compounds represents a major challenge (4). In this work, we have further developed and validated SS18b as a dormancy model for drug susceptibility testing by assessing the activities of a series of 22 currently approved and experimental TB drugs. They include inhibitors of cell wall synthesis, transcription, translation, and ATPase activity, as well as drugs with unknown targets or MOA. The activities of some known drugs in vitro were different against nonreplicating 18b cells compared to growing M. tuberculosis. INH displayed no effect against SS18b; in contrast, RIF, RPT, TMC207, and CFM were very efficacious, whereas PZA had essentially no activity. Some of these drugs have been tested in other dormancy models (6, 37, 44, 47), and our results are again in general agreement with previous findings. The efficacy of some experimental TB drugs was also demonstrated here, with particular attention to bactericidal compounds that kill only growing M. tuberculosis. Among these, the cell wall inhibitors PBTZ169, SQ109, KRT2029, and PYR and the four GSK inhibitors showed no activity on dormant cells. Most importantly, the SS18b model can be easily adapted to in vivo drug testing. In the mouse model, bactericidal activities were detected for RIF, RPT, PZA, TMC207, and CFM, with the data obtained in vivo reflecting the in vitro results, thereby reinforcing the usefulness of SS18b as a model for discovering and testing compounds that target dormant M. tuberculosis.

Our findings identify the cellular processes, transcription, translation, and ATP synthesis as being vital for the survival of M. tuberculosis in the nonreplicating state, both in vitro and in murine models of TB. The results obtained with two different rifamycins imply either that transcription is required for SS18b to survive in
this state or, alternatively, that there is insufficient functional RNA polymerase remaining when cells exit latency to allow transcription and regrowth. In either case, inhibition of RNA synthesis kills latent \textit{M. tuberculosis}.

With respect to ribosome inhibitors, we previously found that fusidic acid and tetracycline derivatives had no effect against SS18b (34), whereas in the present study, PZA and LIN showed activity. Pyrazinoic acid (POA), the active form of PZA, was recently shown to target RpsA, the ribosomal protein S1, and thus to inhibit trans-translation, a means of freeing stalled ribosomes that allows survival of nonreplicating bacilli (36). Our results suggest that the residual trans-translation, present in SS18b cells, is strongly inhibited by POA, thereby explaining the ability of PZA to eradicate nonreplicating organisms. Another protein synthesis inhibitor, the oxazolidinone LIN, also killed nonreplicating 18b in vitro. LIN is showing promise for the treatment of MDR TB, and two other oxazolidinones, AZD5847 and PNU-100480, are in phase I and phase II clinical trials, respectively (12, 43, 46). Since LIN showed activity against SS18b, it is desirable to compare all three oxazolidinones in the murine model of latent TB to assess their potential efficacy against this form of the disease.

ATP synthesis and hydrolysis are presumably highly important.
TABLE 1 Drugs tested for evaluation of the cell wall inhibitor signature

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Cell wall layer</th>
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<tbody>
<tr>
<td>Isoniazid</td>
<td>Enoyl-ACP reductionase (InhA)</td>
<td>Mycolic acid</td>
</tr>
<tr>
<td>ETHIONAMIDE</td>
<td>Enoyl-ACP reductase (InhA)</td>
<td></td>
</tr>
<tr>
<td>GSK5, GSK6</td>
<td>Enoyl-ACP reductase (InhA)</td>
<td></td>
</tr>
<tr>
<td>Thiolactomycin</td>
<td>Beta-ketoacyl-synthase III</td>
<td></td>
</tr>
<tr>
<td>Isoxyl</td>
<td>Fatty acid desaturase (DesA3)</td>
<td></td>
</tr>
<tr>
<td>SQ109</td>
<td>Transporter of trehalose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>monomycolate (MmpL3)</td>
<td></td>
</tr>
<tr>
<td>PBTZ169/BTZ2043</td>
<td>DprE1 epimerase</td>
<td>Arabinogalactan</td>
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<tr>
<td>Ethambutol</td>
<td>Arabinosyl transferases</td>
<td></td>
</tr>
<tr>
<td>KRT2029</td>
<td>Unknown possibly UbiA</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>DD-Transpeptidase</td>
<td>Peptidoglycan</td>
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<tr>
<td>Amoxicillin</td>
<td>DD-Transpeptidase</td>
<td></td>
</tr>
<tr>
<td>Cycloserine</td>
<td>Alanine racemase, δ-alanine ligase</td>
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*ACP, acyl carrier protein.

during all stages of TB. The extraordinary sterilizing activity of TMC207 in mouse lungs confirmed that ATP depletion is also lethal for nongrowing cells, indicating that ATP synthase is a highly vulnerable target (1, 30). CFM was first launched as an antiileprosy agent (11) and decades later was recommended as a second-line drug in combination therapy for the treatment of MDR TB (27), although its use has been hampered by side effects, such as skin discoloration and an exceptionally long half-life. The bactericidal activity of CFM (31) was confirmed in the 18b murine model. Although the exact MOA of CFM remains unclear, it has been suggested that the primary site of action is the cell envelope, whereas other putative targets include redox cycling and K + transport (14, 28, 35, 39, 48). However, since the signature characteristic of cell wall inhibitors was not seen with CFM in our SS18b REMA, we consider that it most likely impacts electron transfer through redox cycling.

RIF is the most important sterilizing drug in modern short-course chemotherapy. Replacement of RIF with RPT, a long-lived rifamycin, in the standard daily regimen shortens the duration of treatment from 6 months to 3 months in mice (32, 33). For this reason, and also because of its potential for the chemoprophylaxis of latent TB (40), we evaluated the effect of RPT in the 18b latent-infection model; the results confirmed that RPT has greater sterilizing activity than RIF. Rosenthal et al. (33) suggested that the superior activity of RPT could be explained by greater rifamycin exposure, as measured by the percentage of time above MIC per week for free drug (RIF 10 5/7, 60%; RPT 10 5/7, 100%; RPT 15 2/7, 90%). Our data are consistent with this concept and demonstrate that the activity of RPT 15 2/7 is very close to that of RPT 10 5/7 during the whole treatment course, as both regimes cleared mouse lungs after 3 months. The recent clinical trial findings of Sterling et al. (40) also suggest that RPT is effective against latent TB infection in humans. In the same vein, based on its bactericidal activity against 18b in mice, adequate RPT exposure (e.g., RPT 15 2/7) may also be important for preventing reactivation and reinfection in cases of latent TB in HIV-positive patients and persons in high-incidence countries.

REMA and SS18b allowed the identification of a cell wall inhibitor signature that should be helpful to predict the MOA and identify the targets of new drugs. REMA exploits the ability of viable cells to reduce resazurin to resorufin and dihydrorresorufin (2). Curiously, although the latent cells are not killed, treatment with cell wall inhibitors results in an increase in fluorescence, possibly due to a more permeable cell wall and thus greater production of resorufin. Currently, drug target identification is a long process and relies increasingly on selection of resistant mutants, whole-genome sequencing, single-nucleotide polymorphism (SNP) analysis, and genetic confirmation. Implementing the SS18b REMA, CWPreD, gives a rapid indication that a compound may function as a cell wall inhibitor, thereby allowing earlier investigation of its impact on biochemical activities. The signature was obtained with 12 out of 14 cell wall-active compounds, with only the two weakest drugs, isoxyl and cycloserine, showing anomalous results. Furthermore, as demonstrated by the blind study of the GSK compounds, CWPreD accurately identified two new InhA inhibitors, GSK5 and GSK6, and predicted that GSK3 and GSK7, whole-cell hits with unknown function, may also inhibit cell wall biogenesis. This prediction has since been confirmed by characterizing resistant mutants (A. Mendoza-Losana, unpublished results). The CWPreD signature was not seen with drugs that target nucleic acid metabolism (quinolones and rifamycins), translation (aminoglycosides, oxazolidinones, and PZA), or energy production (TMC207 and CFM) or with nitroimidazole compounds (34). We consider that the SS18b model represents a powerful tool for finding potential drugs for the treatment of latent TB and that CWPreD will facilitate drug discovery by rapidly identifying cell wall inhibitors.

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REFERENCES


