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Extremely Low Hit Rate in a Diverse Chemical Drug Screen Targeting *Mycobacterium abscessus*

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Mycobacterium abscessus, a rapidly growing nontuberculous mycobacterium (NTM), is increasingly recognized as an important pathogen causing soft tissue and lung infections as well as severe disseminated infections in immunocompromised patients (1, 2). Due to the intrinsic resistance to most of the classic antituberculous drugs and many other antibiotics, infections with *M. abscessus* are extremely difficult to manage (3, 4). The spread of strains which acquired additional resistance-causing mutations and the requirement for extended treatment periods, often leading to severe adverse events, stress the need for identification of new compounds active against this pathogen (2). Recent drug screening efforts have focused on testing of preselected compounds and compound libraries containing substances with known antimycobacterial or antibacterial activity which led to identification of some compounds inhibiting growth of *M. abscessus* (5–9). Given the high attrition rate of hit and lead compounds during preclinical and clinical development, additional efforts may be needed to identify promising clinical candidates against *M. abscessus*. Phenotypic whole-cell screening of large, diverse synthetic small-molecule libraries led to the identification of potent and selective antituberculous drugs, some of which have been tested successfully in clinical trials (8, 10, 11). While the expected hit rate for these classic *M. tuberculosis* whole-cell screens is between 0.3 and 1%, this important information is missing for *M. abscessus* strains (12).

Here, we report on a head-to-head comparison of *M. abscessus* and *Mycobacterium tuberculosis* in a whole-cell phenotypic screen using a diverse chemical library of 10,000 synthetic small molecules (World Diversity Set III, SPECS, Netherlands) (13). We established a resazurin microtiter assay (REMA) for *M. abscessus* in analogy to the *M. tuberculosis* REMA which was previously described (14, 15). Log phase *M. abscessus* ATCC 19977 with an optical density at 600 nm (OD_{600}) of 0.0001 was incubated in 96-well plates at 37°C in Middlebrook 7H9 broth supplemented with 10% ADC (albumin, dextrose, catalase), 0.5% glycerol, and 0.05% Tween 80. After 72 hours, resazurin was added (0.025% wt/vol), and fluorescence was measured after 2 hours of incubation (excitation at 560 nm and emission at 590 nm). When using dimethyl sulfoxide (DMSO) and clarithromycin (20 μM) as controls, the assay had a Z-factor of 0.78 in a 96-well microplate format. Molecules were tested in parallel REMA against *M. abscessus* or *M. tuberculosis* Erdman in duplicates at concentrations of 20 μM and 10 μM, respectively.

In the *M. tuberculosis* primary screen, we observed the expected hit rate of 0.7% (72 hits). In contrast to that, the *M. abscessus* screen revealed only 7 substances causing growth inhibition of 50% or higher (hit rate of 0.07%) (Table 1). *M. abscessus* hits were retested, followed by MIC determination in *M. abscessus* and *M. tuberculosis* using 2-fold serial dilutions and a starting concentration of 100 μM. To estimate the cellular toxicity

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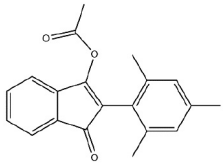
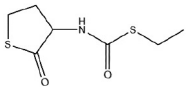
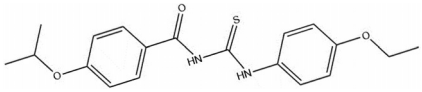
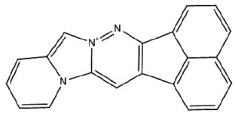
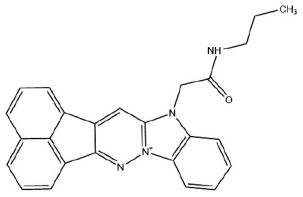
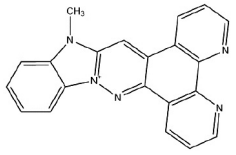
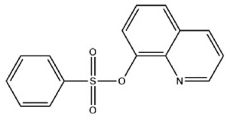
TABLE 1 Properties of *M. abscessus* REMA hit compounds[a]

Substance	Molecular structure	MIC (μM) *M. abscessus*	MIC (μM) *M. tuberculosis*	IC_{50} (μM)[b]	SI (*M. abscessus*)[c]
1		6.1	6.3	54.2	8.9
2		22	>100	>100	>4.5
3		23.7	25.4	>100	>4.2
4		>100	3.5	2.1	<0.02
5		40.8	6.9	39.7	0.97
6		>100	45.4	17.2	<0.17
7		>100	>100	>100	ND
Positive control	Clarithromycin	0.7	ND	ND	ND
Positive control	Isoniazid	ND	1.3	ND	ND

[a]ND, not determined.
[b]IC^{50}, half maximal inhibitory concentration, measured in human hepatoma HepG2 cells.
[c]SI, selectivity index (MIC against *M. abscessus*/IC_{50}).

(50% inhibitory concentration [IC_{50}]) of the seven hit compounds, we used standard human hepatoma HepG2 cell cytotoxicity assays and calculated the corresponding selectivity indices (SI) (IC_{50}/MIC). Growth inhibition of *M. abscessus* was confirmed for all 7 hit compounds. However, only compound 1 had a MIC below 20 μM (6.1 μM). The selectivity index for this substance was 8.9 (Table 1). Hit compound 2, a carbamothioate, and hit compound 3, a thiourea, had MICs of 22 μM and 23.7 μM, respectively. IC_{50} cytotoxicity values were >100 μM. A database search revealed that both substances display known antituberculous activity (Collaborative Drug Discovery Vault) (16). This also holds true for the imidazo-pyridazine compounds 4 to 6, all which displayed an SI of <1 due to pronounced cytotoxicity. Hit compound 7, a quinoline, did not reach a MIC of $\leq$100 μM.

In conclusion, we show that high-throughput screening of randomly selected molecules yields extremely low hit rates for compounds targeting *M. abscessus*. Despite using a two-times-higher compound concentration in the *M. abscessus* assay, the hit rate was 10 times lower than results obtained for *M. tuberculosis*. From a total of seven hits, only one showed a MIC below 10 μM and moderate cytotoxicity. Five of the seven *M. abscessus* hits were either positive in our *M. tuberculosis* REMA or had been described previously as hits in other *M. tuberculosis* screening campaigns (16). Upon testing of *M. abscessus* hits against *M. tuberculosis*, we identified only one substance (number 2) which seems to possess selective activity against *M. abscessus* (Table 1). The disappointing results observed in this *M. abscessus* small-molecule screening study can be explained by the high intrinsic drug resistance of NTM that might reflect an evolutional adaptation to hostile environments (soil and water) (4, 17). NTM have a highly flexible system of gene regulation altering growth rate, metabolism, and inducible expression of genes directly facilitating drug resistance (e.g., genes encoding efflux pumps). One example is the transcriptional regulator WhiB7, which is involved in various mechanisms of inducible drug resistance in mycobacteria (18–22). Recently, it was shown that WhiB7 confers species-specific patterns of gene induction that might explain differences in drug susceptibilities among different mycobacterial species. For *M. abscessus*, it was shown that resistance to amikacin is induced by the WhiB7-regulated gene *eis2*, which seems to be unique for this species (23).

Based on our findings, we suggest retesting and repurposing substances that have already been tested positive in screens targeting *M. tuberculosis* and Gram-positive bacteria, rather than performing large-scale phenotypic screening against *M. abscessus* (5–7, 24, 25). In addition, known antimycobacterial drugs may exhibit synergistic effects as shown for combinations of various drug classes acting against *M. abscessus* (26–28). Changing assay conditions and using different media may also increase hit rates, as recently shown for experiments performed using Mueller-Hinton broth (6). Whether this will translate into compounds with good *in vivo* activity against *M. abscessus* requires further investigation.

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