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REVIEWS

Spotlight

Resistance Reversed in KatG Mutants of *Mycobacterium tuberculosis*

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A peptidomimetic containing a thiazolo ring-fused 2-pyridone (C10) has now been reported to inhibit hypoxia-induced tolerance to isoniazid (INH) in *Mycobacterium tuberculosis* (Flentie *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, 2019). The C10 compound could also potentiate the bactericidal activity in aerobically grown bacilli, prevented selection of drug-resistant strains, and reversed INH resistance in *katG* (catalase-peroxidase) mutants.

Tuberculosis (TB) caused 1.6 million deaths worldwide in 2017, and multidrug-resistant TB remains a public health crisis and health security threat [1]. Extracellular *Mycobacterium tuberculosis* in human TB lesions forms biofilms (pellicles) in which the high cell density, cell–cell contacts, and different nutrient and oxygen gradients within their interiors result in unique phenotypes, including drug tolerance [2]. A library of 91 compounds sharing a thiazolo ring-fused 2-pyridone bicyclic chemical scaffold was screened for inhibitors of pellicle formation in *M. tuberculosis*, the main causative agent of TB [3]. These authors employed a modified version of the culture-based hypoxia model in which *M. tuberculosis* in liquid media was incubated for 3 weeks in airtight containers (a phase in which drug-tolerant bacilli evolved), followed by 2 weeks of aerated cultures, and formation of a pellicle biofilm at the air–liquid interface was observed. The most potent chemical compound (C10, Figure 1) inhibited a process involved in pellicle formation.

As pellicle formation and drug tolerance are developed by hypoxia, the authors [3] evaluated physiological processes linked to stress and drug tolerance. The C10 compound blocked drug tolerance due to oxidative stress induced by hydrogen peroxide and hypoxia-induced phenotypic tolerance to isoniazid (INH), a first-line chemotherapeutic agent used to treat TB. Interestingly, C10 appears to be specific for INH as no effect was observed for hypoxia-induced tolerance to rifampicin, streptomycin, and ethambutol. C10 in combination with INH increased the killing of bacilli in aerobic liquid cultures and blocked the selection of INH-resistant strains on solid agar plates. INH in combination with C10 inhibited the growth of INH-resistant strains harboring *katG* gene mutations [3], which encodes the catalase-peroxidase (KatG) enzyme. KatG activates INH to form a hypothetical isonicotinoyl anion or radical that reacts with NAD^+ to yield an INH–NAD adduct which, in turn, inhibits the NADH-dependent *trans* Δ2-enoyl reductase (InhA) enzyme activity [4]. InhA plays a role in fatty acid elongation of the meromycolate branch of mycolic acids and its inhibition by an INH–NAD adduct results in cell death [4]. Gene expression results implicated C10 in up-regulation of components of the electron transport chain (ETC) in both normoxid and hypoxic conditions [3]. In short, C10 blocked oxygen consumption and depleted ATP levels. C10 did not increase reactive oxygen species (ROS) production as the authors reasoned that the latter could have led to increased susceptibility to INH killing.

Another important environmental factor is the pH value, which varies for different subpopulations of *M. tuberculosis* during infection, ranging from 6.2 within the phagosome of immature macrophage to 4.5 in the phagolysosome following gamma interferon activation, and 7.4 for extracellular bacilli in the necrotic core of a granuloma [5]. Respiration plays a role in intracellular pH homeostasis [5] and C10 affected components of the ETC. Accordingly, the authors [3] evaluated whether or not C10 could inhibit respiration and impair the ability of bacilli to survive exposure to acidic pH in aerobic conditions. C10 decreased *M. tuberculosis* viability at pH 5.5, suggesting that this chemical compound increases the susceptibility of bacilli to low pH values that is consistent with alterations in transcription of ETC components.

The interaction between drugs targeting energy metabolism and classical first- and second-line anti-TB agents should be exploited to try to maximize treatment efficiency [6]. Q203 is a clinical-stage drug candidate targeting the terminal respiratory oxidase cytochrome bc1:aa3 [6]. Q203 is bacteriostatic because of the presence of the alternate cytochrome bd oxidase [6]. The C10 compound in combination with a Q203 clinical candidate resulted in a significant decrease in *M. tuberculosis* viability after 15 days of treatment in liquid cultures [3]. Although C10 increased expression of *cydABDC* and potentiated killing by Q203, it showed a similar inhibitory effect on respiration of the wild-type as compared with the

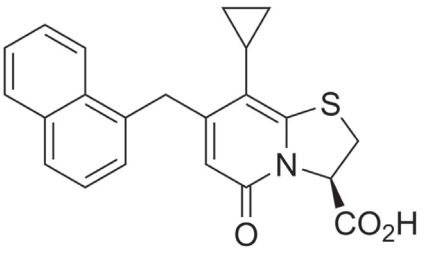

Figure 1. Chemical Structure of C10. (*R*)-8-cyclopropyl-7-(naphthalen-1-ylmethyl)-5-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyridine-3-carboxylic acid (C10).

Δ*cydA M. tuberculosis* strain [3]. These results suggest that C10 does not target cytochrome bd. As inactivation of cytochrome bd increases susceptibility to cytochrome bc1 inhibitors (e.g., Q203), C10 does not target cytochrome bc1 either, as inhibitors of bc1 show increased activity in *cydAB* mutants due to no compensatory pathway availability.

Although mutations in various DNA sequences have been found in INH-resistant clinical isolates of *M. tuberculosis*, only mutations in *katG* and *inhA* have been correlated with INH resistance [7]. The proportion of mutations in *katG* and *inhA* varies geographically, but it is estimated that at least 80% of all INH-resistant clinical isolates have either the mutated Ser315 codon of *katG* or the C-15 T nucleotide substitution in the *inhA* promoter [7]. Accordingly, the C10 would be effective only to treat patients infected with *M. tuberculosis* INH-resistant strains harboring *katG* mutations. Notwithstanding, the *in vivo* mechanism of INH resistance appears not to be reflected by *in vitro* experiments [8]. Neither of the two most common *in vivo* mutations were found in seven *in vitro*-selected INH-resistant strains as insertions and deletions (frameshifts) as well as missense mutations (W328L, A172T, and A144E) in the *katG* gene were identified [3]. To reach a specific drug target in *M. tuberculosis*, the chemical agent must be transported from the blood compartment to a nonvascularized pulmonary lesion, diffuse into necrotic foci and the caseum, permeate the lipid-rich cell envelope of bacilli, bind to its intended target at adequate concentrations and act upon it for a required time frame [9]. Several physiological barriers need thus to be overcome when drugs are orally administered, including first-pass metabolism, adequate permeability in the lungs, and uptake into *M. tuberculosis* to reach the intracellular target(s) [9]. Furthermore, chemical stability under different physiological conditions of the multicellular structures that are characteristic of TB pathology, such as necrotizing or caseum granulomas, must be considered [10]. Accordingly, further efforts should be pursued to translate the C10 compound into a chemotherapeutic agent to treat TB infection in human hosts infected with INH-resistant strains of *M. tuberculosis* harboring *katG* mutations. Elucidation of the mode of action of C10 may unveil novel targets that can be valuable for the development of new chemotherapeutic agents to treat TB.

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Spotlight

Structures Unveil the Invasion Mechanism of Chikungunya Virus

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Structures of the multiple arthritogenic alphavirus receptor MXRA8 as well as MXRA8 in complex with chikungunya virus (Song *et al.*, *Cell*, 2019; Basore *et al.*, *Cell*, 2019) have revealed the mechanism underlying viral invasion and could facilitate the development of novel vaccines and entry inhibitors.

Alphaviruses are enveloped, single-stranded RNA viruses transmitted primarily by mosquitoes; they include Sindbis virus (SINV), Semliki Forest virus (SFV), Ross River virus (RRV), chikungunya virus (CHIKV), Venezuelan equine encephalitis virus (VEEV), Barmah Forest virus (BFV), o'nyong-nyong virus (ONNV), and Mayaro virus (MAYV). These viruses cause endemic diseases and widespread epidemics. However, no specific therapeutic approaches have been developed to treat infections caused by alphaviruses [1]. The arthritogenic alphavirus CHIKV is the causal agent of an emerging widespread outbreak of a debilitating human disease with symptoms varying from fever or rash to severe arthritis. Similar to other alphaviruses, the membrane fusion-related envelope glycoproteins of CHIKV,