

The implication of *Mycobacterium tuberculosis*-mediated metabolism of targeted xenobiotics

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Abstract

Drug metabolism is generally associated with liver enzymes. However, in the case of *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB), *Mtb*-mediated drug metabolism plays a significant role in treatment outcomes. *Mtb* is equipped with enzymes that catalyse biotransformation reactions on xenobiotics with consequences either in its favour or as a hindrance by deactivating or activating chemical entities, respectively. Considering the range of chemical reactions involved in the biosynthetic pathways of *Mtb*, information related to the biotransformation of antitubercular compounds would provide opportunities for the development of new chemical tools to study successful TB infections while also highlighting potential areas for drug discovery, host-directed therapy, dose optimization and elucidation of mechanisms of action. In this Review, we discuss *Mtb*-mediated biotransformations and propose a holistic approach to address drug metabolism in TB drug discovery and related areas.

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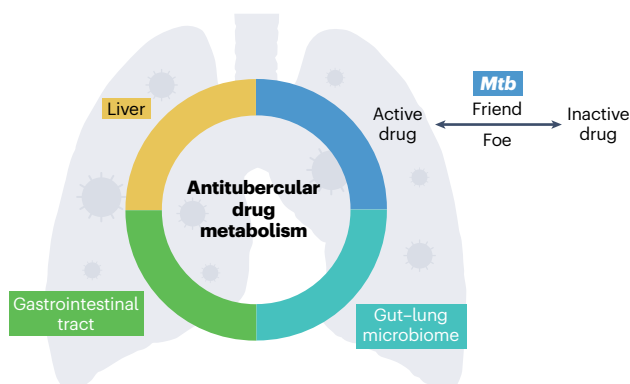
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Introduction

Tuberculosis (TB), a highly infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*), primarily affects the lungs, causing pulmonary TB, and may affect other parts of the human body, to cause extrapulmonary TB^{1,2}. After the severe acute respiratory syndrome coronavirus 2-related-COVID-19, TB is the second highest leading cause of death by a single infectious agent, claiming an estimated 1.6 million lives in 2021, a slight increase from 2020 (ref. ¹) (here it is to be noted that the real numbers could be higher as there are no clear measures to differentiate the cause of COVID-19-related deaths in patients with TB). Drug-susceptible TB is curable; however, the treatment involves a first-line four-drug standard regimen for 6 months: an intensive phase comprising a 2-month regimen of isoniazid, rifampicin, pyrazinamide and ethambutol, followed by a continuous phase involving a 4-month regimen of isoniazid and rifampicin (Box 1). The major obstacle in eradicating TB is the increasing incidence of drug-resistant TB. Several second-line drugs are available for the treatment of drug-resistant TB, such as fluoroquinolones, injectables (capreomycin, kanamycin and amikacin), ethionamide, cycloserine, linezolid, bedaquiline and delamanid. However, the challenge of a successful TB treatment remains daunting owing to the emergence of multidrug-resistant (MDR) TB – that is, resistance to at least both isoniazid and rifampicin – and extensively drug-resistant (XDR) TB, in which MDR strains develop resistance to any fluoroquinolone and at least one of the three second-line injectable drugs. TB drug resistance is further complicated by reports of resistance to bedaquiline^{3–5}, the first drug to be approved for MDR-TB treatment in over 40 years. These examples of resistance emphasize the urgent need to discover and develop novel molecules with unique mechanisms of action (MoAs) for the treatment of MDR-TB and XDR-TB. Therefore, there is a critical need to expand our understanding of the interaction between the microorganism and xenobiotics and various mechanisms of resistance presented by *Mtb*, identify new anti-TB chemical matter, expand our knowledge of microorganism and host interaction, and establish molecular targets for host-directed therapy.

TB-causing mycobacteria are collectively known as the *Mtb* complex, which includes: *Mtb*, the aetiologic agent of TB in humans; *M. africanum*, which causes TB in humans in certain regions of Africa; *M. bovis*, *M. caprae*, *M. canetti*, *M. mungi* and *M. pinnipedii*, responsible for TB in wild and domesticated mammals; and *M. microti*, the causative agent of TB in voles. Co-evolution of these strains with enhanced virulence, transmissibility and selectivity for specific hosts can be attributed to the introduction of agriculture, civilization and clustered human population in urban areas⁶. Classically, *Mtb* infection in humans can result in one of the two clinically defined states: latent TB infection or active TB disease. *Mtb* is carried in airborne particles, called droplet nuclei (1–5 µm in diameter), which are generated when patients with pulmonary TB cough or sneeze. For a successful infection, the aerosolized droplet containing infectious *Mtb* must be inhaled, travel to the lower lung and be ingested by a certain type of white blood cell (alveolar macrophage – a type of tissue-resident macrophage, monocyte-derived macrophages, dendritic cells and neutrophils)⁷.

Macrophages can induce a pro-inflammatory phenotype (for the containment of *Mtb*) and granuloma (a pathological hallmark of human TB) formation during this initial stage of infection. However, the phenotypic response of the macrophage is subsequently altered to allow *Mtb* growth⁸: a phenomenon that is less understood but believed to involve metabolic reprogramming, which has a role in controlling the response of immune cells⁹. Unlike other intracellular pathogens that depend on macrophages for nutrients (auxotrophs), *Mtb*, as a prototroph, has

metabolic flexibility of catabolic and anabolic reactions to convert organic precursors into products¹⁰. This metabolic flexibility allows *Mtb* to survive under various stresses encountered during its life. Moreover, several human microbiome research studies conducted in a wide variety of diseases, including TB¹¹, indicated that the microbiota exert a major influence on TB disease progression by affecting host-innate and adaptive immune responses in which metabolism has a critical role.

The biotransformation of xenobiotics in humans involves an array of metabolic reactions and is generally associated with liver enzymes, as in hepatic metabolism¹² (Fig. 1). In general, these metabolic reactions can be classified as either phase I – functionalization reactions that can transform a substrate into an active or inactive species – or phase II – reactions in which small endogenous polar molecules conjugate with other molecules via functional groups either formed in phase I reactions or already present (Fig. 1). Phase II reactions increase the hydrophilicity of molecules to enhance excretion. At this juncture, it is noteworthy that biotransformation reactions may convert: 1) an active compound to a less active compound (inactivation or detoxification); 2) an active compound to a more active metabolite (bioactivation); 3) an inactive compound (prodrug) to an active metabolite (activation); 4) an active compound to an equally active metabolite; and 5) an active compound to an active metabolite with an entirely different pharmacological activity profile (Fig. 2a). In relation to the effect on TB, *Mtb*-mediated biotransformation reactions can be categorized into two groups: either ‘foe’ (having a negative impact on *Mtb*) or ‘friend’ (having a positive impact on *Mtb*) (Fig. 2a). Foe refers to the scenario in which the *Mtb* metabolic machinery converts an antitubercular prodrug into an active molecule, for example, activation of isoniazid (29) by a catalase peroxidase encoded by *katG*, pyrazinamide (31) by nicotinamidase (PncA) and ethionamide (33) by monooxygenases (Fig. 2b). Two nitroimidazole drugs delamanid and pretomanid (36) are also bioactivated by *Mtb* nitroreductases (Fig. 2b). In the context of friend, *Mtb* can aid TB by metabolically deactivating ‘potent’ antituberculars, such as second-line aminoglycosides in amidation, esterification or phosphorylation reactions catalysed by acetyltransferases and phosphotransferases¹³ (Fig. 2c). These examples highlight the need for a deeper understanding of *Mtb*-mediated biotransformation reactions in the context of drug discovery, host-directed therapy and MoA studies.

In this Review, we discuss the current knowledge of capability of *Mtb* to remodel its metabolism for biotransformation reactions on small molecule xenobiotics in the context of beneficial and detrimental outcomes for the pathogen. Additionally, we briefly discuss the role of the host microbiome in influencing the survival of *Mtb*. We propose using approaches to learn the metabolic liabilities of the active molecules beyond hepatic metabolism and MoA studies on generated metabolites in identifying molecular targets for TB drug discovery and host-directed therapy.

Biotransformation in mycobacteria

Owing to the complex pathophysiology of TB disease, it remains puzzling to determine the accurate metabolic status of *Mtb* at the disease site. Most metabolic studies have been performed using genetic tools and metabolomics to confirm various enzymatic or regulatory functions, thereby validating the metabolic role and essentiality^{14,15}. Some of these findings and *Mtb*-mediated drug metabolism (pre-2017) have been reviewed previously¹⁶. In recent years, mode of action or resistance and metabolomics studies have contributed immensely towards understanding the metabolic arsenal accessible to *Mtb* in eluding TB treatment. In this regard, examples of biotransformation reactions on

Box 1

Current WHO recommendation for TB

Drug-susceptible TB treatment

Six-month regimen

New patients with pulmonary tuberculosis (TB) should receive a regimen of: 2 months with isoniazid, rifampicin, ethambutol and pyrazinamide followed by 4 months of isoniazid and rifampicin. Daily dosing is preferred.

Four-month regimen

Patients aged >12 years with drug-susceptible TB may receive a 4-month regimen of: 2 months of isoniazid, rifapentine, moxifloxacin and pyrazinamide followed by 2 months of isoniazid, rifapentine and moxifloxacin.

Patients aged 3 months to 16 years with non-severe TB may receive a 4-month treatment regimen of: 2 months with isoniazid, rifampicin and pyrazinamide (ethambutol) followed by 2 months of isoniazid and rifampicin.

Drug-resistant TB treatment

Regimen for rifampicin-susceptible and isoniazid-resistant TB

Treatment with rifampicin, ethambutol, pyrazinamide and levofloxacin for 6 months.

Shorter all-oral regimen for multidrug-resistant or rifampicin-resistant TB

Bedaquiline, in combination with levofloxacin or moxifloxacin, ethionamide, ethambutol, isoniazid, pyrazinamide and clofazimine for 4 months (6 months, if the patient remains smear positive at the end

of 4 months); followed by 5 months of levofloxacin or moxifloxacin, clofazimine, ethambutol and pyrazinamide.

Longer regimens for multidrug-resistant or rifampicin-resistant TB

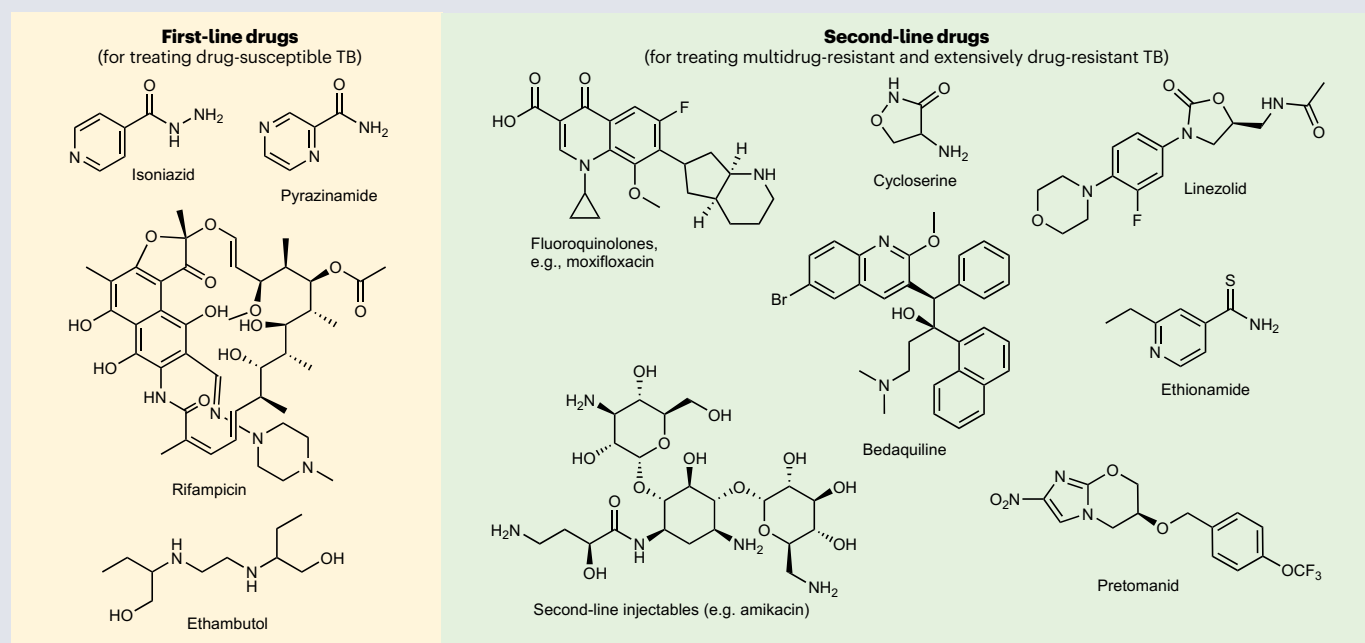
All three group A agents and at least one group B agent (see the table). If only one or two group A agents are used, both group B agents are to be included. If the regimen cannot be composed with agents from groups A and B alone, group C agents should be added.

Drug groupings for multidrug-resistant or rifampicin-resistant TB

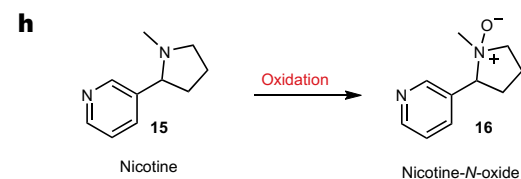
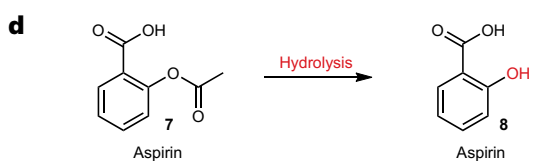
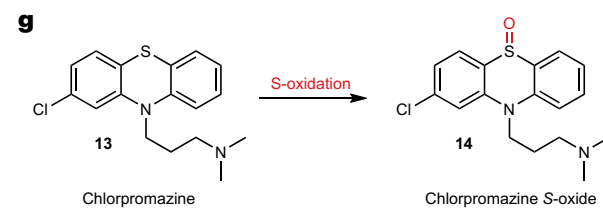
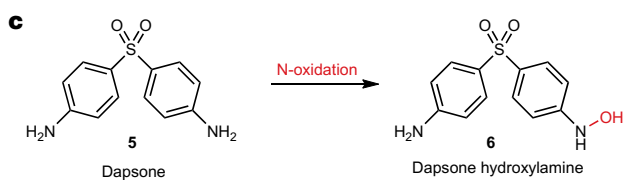
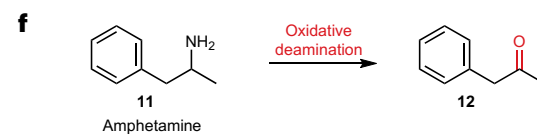
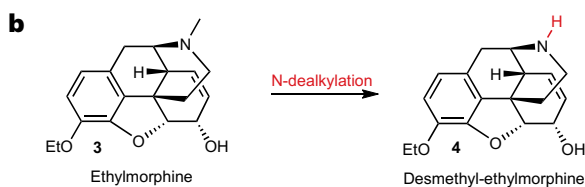
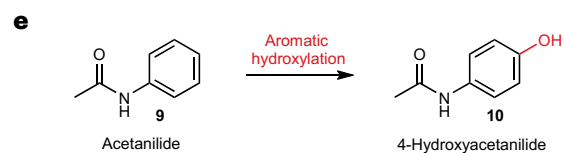
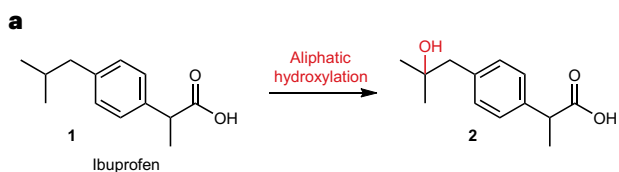
Groups (and steps)	Drugs
Group A: include all three medicines	Levofloxacin or moxifloxacin; bedaquiline; linezolid
Group B: add one or both medicines	Clofazimine; cycloserine or terizidone
Group C: add to complete the regimen and when medicines from groups A and B cannot be used	Ethambutol; delamanid; pyrazinamide; imipenem–cilastatin or meropenem; amikacin (or streptomycin); ethionamide or prothionamide; <i>P</i> -aminosalicylic acid

Regimen for multidrug-resistant TB with additional fluoroquinolone resistance

Bedaquiline, pretomanid and linezolid for 6–9 months. As per the recent WHO update, the use of a 6-month treatment regimen composed of bedaquiline, pretomanid, linezolid and moxifloxacin is recommended for multidrug-resistant or rifampicin-resistant TB⁹¹.



A Phase I



B Phase II

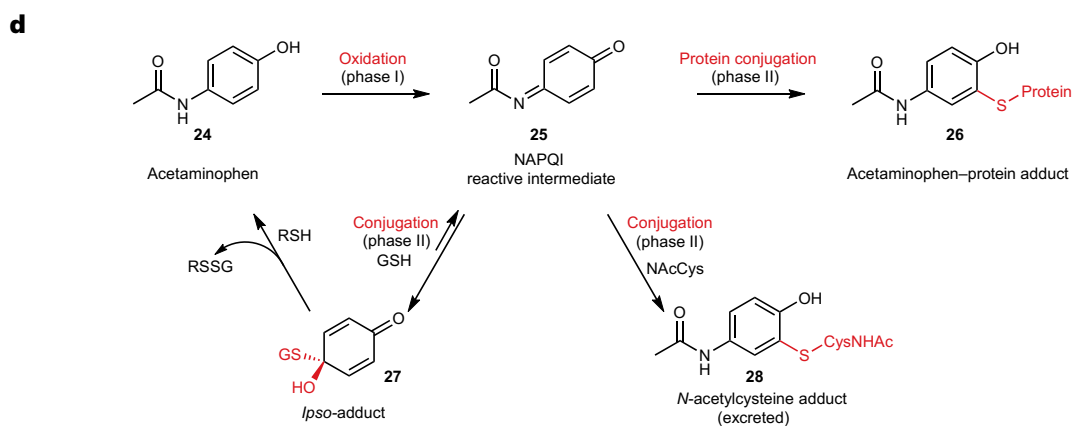
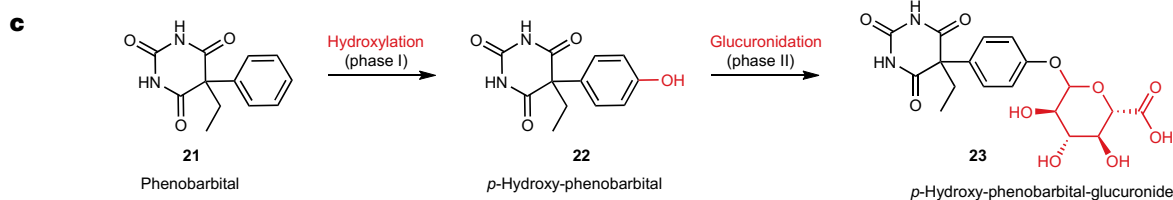
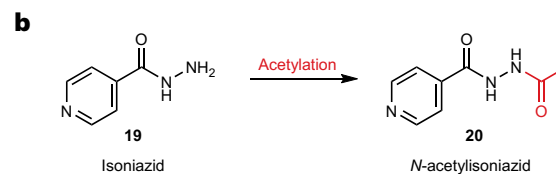
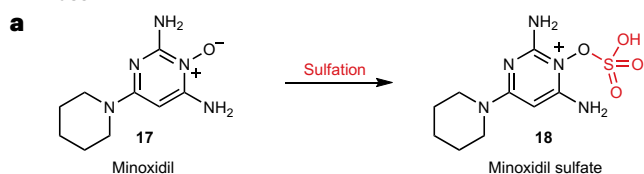


Fig. 1 | Biotransformation reactions. **A**, Phase I: involved in exposing or adding a functional group; following phase I, the drug may be activated or unchanged or inactivated. **B**, Phase II: synthetic or conjugation reactions; following phase II,

generally, the drug is inactive. GSH, glutathione; NAcCys, *N*-acetylcysteine; NAPQI, *N*-acetyl-*p*-benzoquinoneimine; RSH, thiol-containing protein; RSSG, protein-bound glutathione.

some newly identified anti-*Mtb* agents are briefly discussed subsequently. The discussion focuses on: 1) biotransformation mechanisms, including enzymes and chemical transformations involved, and 2) the MoA of active metabolites or mechanisms of resistance or inactivity of metabolites.

Foe biotransformation examples

Biotransformation reactions can have a negative impact on *Mtb*, denoted here as foe.

Triaza-coumarin congeners

A new class of 1,3,5-triazaspiro[5.5]undeca-2,4-dienes has been identified as a selective inhibitor series of dihydrofolate reductases (DHFRs), an essential enzyme involved in folate metabolism, with potent whole-*Mtb* cell activity¹⁷. Congeners of triaza-coumarin (**39**) (Fig. 3a), which bear a coumarin moiety, showed low nanomolar minimum inhibitory concentration (MIC) values of 10–200 nM against *M. bovis* bacillus Calmette–Guérin (BCG). Against *Mtb*, **39** possessed an MIC value of 25 nM but a moderate *Mtb* DHFRIC₅₀ value of 820 nM, which was 18-fold higher than human DHFR. Further studies to elucidate the underlying mechanisms responsible for the whole-cell-to-enzyme potency disconnect identified two resistance mechanisms. The first mechanism was associated with low-frequency mutations in thymidylate synthase (*thyA*), confirming that **39** is involved in the folate pathway. The second mechanism is based on the loss of enzymes (FbiC and FgdI) required for the biosynthesis of redox cofactor F₄₂₀, which implicated metabolism by *Mtb* F₄₂₀-dependent oxidoreductases (FDORs)¹⁷. The latter was corroborated by the generation of mutant strains harbouring loss-of-function mutations in *fbiC* and *fgdI*, which are involved in the biosynthesis of the redox cofactor F₄₂₀H₂, a bioactivator of pretomanid. At a molecular level, the α,β-unsaturated carbonyl group in the coumarin motif of **39** is reduced to deliver **40**, which is spontaneously hydrolysed to **41** – a potent DHFR inhibitor – thereby affecting the folate pathway¹⁷ (Fig. 3a). Chemically synthesized **41** showed an IC₅₀ value of ~10 nM against recombinant *Mtb* DHFR, about 100-fold more potent than the parent **39** and improved selectivity over the human DHFR. However, **41** showed lower whole-cell activity compared with **39** (*M. bovis*, MIC = 0.8 and 0.02 μM, respectively), which may be due to its limited permeability into *Mtb* cells. Compound **39** with a methyl substituent at the β-position to block reduction of the unsaturated bond showed loss of whole-cell potency (MIC = 3.2 μM), but moderate DHFR inhibitory activity with an IC₅₀ value of 0.63 μM. Although FDORs bioactivate **39**, the specific enzymes involved are yet to be determined: biochemical analyses using recombinant *Mtb* FDORs confirmed that **39** is a substrate of both deazaflavin-dependent nitroreductase (Ddn) and its homologue Rv1558. From a drug discovery perspective, **39** exhibits properties of a good starting point. For example, it has low cytotoxicity (SI > 4,000; SI is the selectivity index; SI = IC₅₀:MIC₉₀, mammalian cell-line:*Mtb*) and hemolytic activity (LC₅₀ > 100 μM; LC₅₀ is the lethal concentration that kills 50% of the target population), as well as good aqueous solubility (>100 μM, pH 7.4) and encouraging metabolic stability in rat liver microsomes¹⁸. Considering its low resistant-mutation frequency and possible pleiotropic mode of action, characterizing the specific targets of **39** while addressing the reduction will be useful

to inform requisite structure–activity relationship (SAR) strategies to overcome the metabolism-induced resistance.

Nitrofuranyl calanolides

Nitrofuranyl calanolides are natural product-inspired molecules based on calanolide A, possessing potent selective bactericidal activity against replicating and non-replicating *Mtb* and an ability to eliminate detectable *Mtb* from primary human macrophages in vitro. The nitrofuranyl calanolides **42** and **43** (Fig. 3b) showed MIC values of 0.84 and 2.25 μM and 1.68 and 2.81 μM against replicating and non-replicating *Mtb*, respectively¹⁹. To understand their mode of action, *Mtb* or *M. bovis* BCG-resistant mutants of **42** or **43** were generated and relevant mutations were identified in the mycothiol-dependent nitroreductase Rv2466c (Mrx-2, mycoredoxin); by investigating these mutants, Mrx-2 was found to be involved in the activation of **42** by reduction of its nitro group, resulting in amine **44** as the major metabolite: confirmed by chemical synthesis²⁰. In addition to its function as a nitroreductase, Mrx-2 conferred partial protection to menadione stress. However, **44** showed low antimycobacterial activity when used exogenously: this low activity could be due to either poor cellular uptake or that a minor metabolite of **42**, which could not be identified, is responsible for the observed in vitro antimycobacterial activity. Despite the encouraging antimycobacterial activity, **42** was found to be genotoxic presumably on account of the presence of the nitrofuranyl moiety, precluding its further progression.

Thienopyrimidines

Compound **45** is a new thienopyrimidine analogue with antimycobacterial activity against both replicating and non-replicating *Mtb* at MIC values of 0.43 and 2.7 μM, respectively. Subjecting *Mtb* to **45** drug pressure conferred mutations associated with the Rv2466c gene, which was shown to bioactivate **45** by reduction of its nitro group²¹ (Fig. 3c). However, the chemically synthesized amino derivative of **45** lacked antimycobacterial activity (MIC > 390 μM). To further understand the MoA, an assessment has been performed in purified enzyme assays and mycobacteria cultures, which showed that the mycothiol-dependent nitroreductase Mrx-2 released nitric oxide (NO) from **45** in both²². To identify the active metabolite, high-density culture of a recombinant *Mtb* strain overexpressing Mrx-2 was exposed to high drug pressure (400× MIC). This resulted in the generation of metabolite **46**, formed by cleavage of the reduced thiophene ring to reveal a reactive thiol moiety, and is hypothesized to be responsible for the pleiotropic effects of **45** on *Mtb* cell growth by inhibiting lipid biosynthesis, transcription and protein synthesis.

Pyrano[3,2-b]indolones

Compound **47** (Fig. 3d) is a pyrano[3,2-b]indolone with potent MIC values of 1.26 and 1.68 μM against replicating and non-replicating (SS18b) strains of *Mtb*²³. *M. bovis* BCG-resistant mutants of **47** were cross-resistant to pretomanid, suggesting a similarity in MoA. Activation of pretomanid involves either Ddn or proteins responsible for the synthesis (FbiA, FbiB and FbiC) or reduction (FgdI) of a mature active form of cofactor F₄₂₀. However, owing to the absence of a nitro group in **47**, the involvement of Ddn was ruled out and focus turned to the MoA involving F₄₂₀.

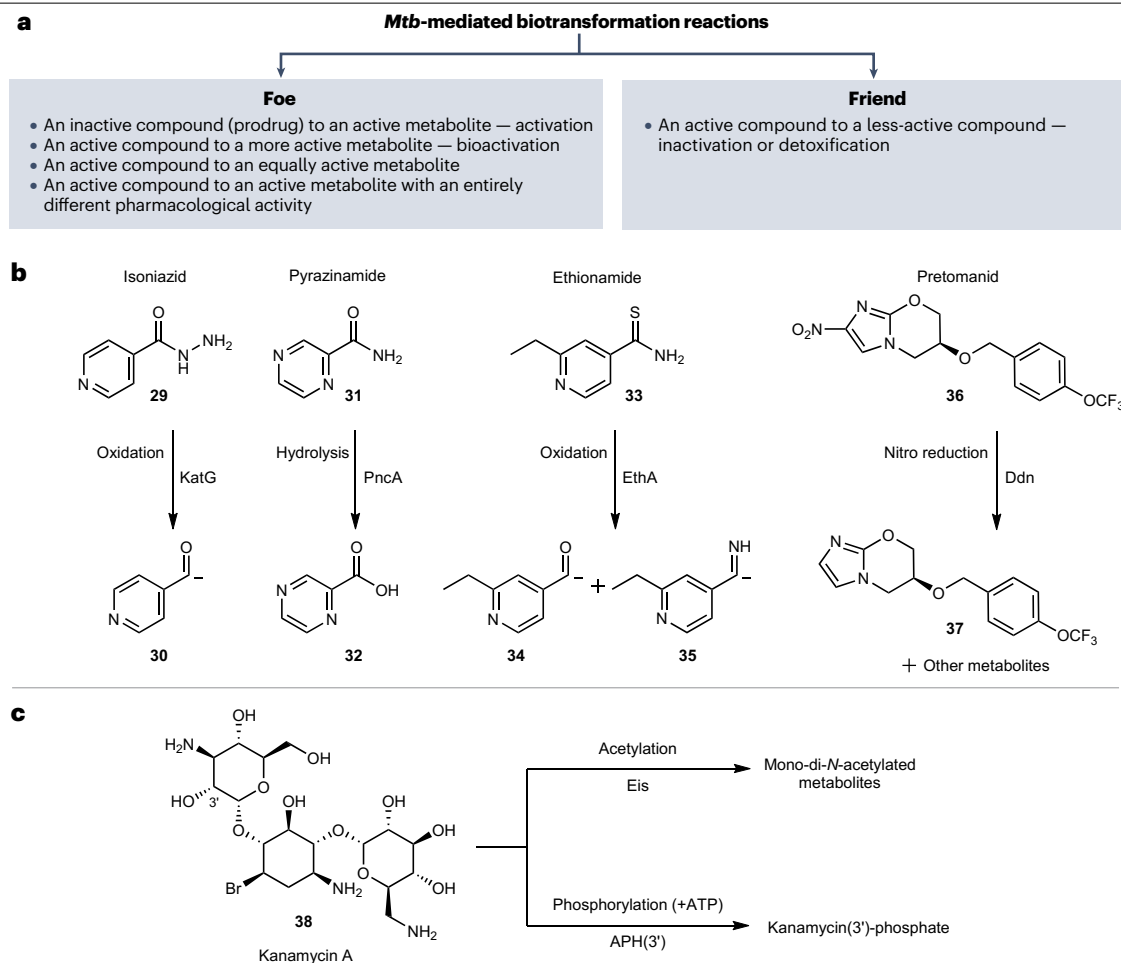


Fig. 2 | Biotransformation reactions mediated by *Mtb*. **a**, Common biotransformation reactions in *Mycobacterium tuberculosis* (*Mtb*) in the context of friend or foe. **b**, Activation of anti-tuberculosis prodrugs by *Mtb*. **c**, Kanamycin

A (38), an aminoglycoside antibiotic, is metabolized by *Mtb* through N-acetylation by enhanced intracellular survival protein (Eis) and O-phosphorylation by aminoglycoside-3'-phosphotransferase (APH) to afford inactive metabolite.

Sanger and whole-genome sequencing of 47-resistant mutants established a role of F₄₂₀-dependent enzymes (FbiC or Fgd1) in the activation of 47.

Thioalkylbenzoxazoles

Thioalkylbenzoxazole analogue (48; MIC = 10 μM) is bioactivated by *Mtb* flavin monooxygenase (MymA) through a sulfur oxidation metabolism²⁴ (Fig. 3e). Resistant mutants to 48 possessed an SNP (insertion of an adenine nucleotide at position 382 of the *mymA* (also known as *rv3083*) gene), translating into high MIC values against the *mymA* mutant strains but a similar MIC value of 10 μM against a wild-type *Mtb* strain. The role of MymA in *Mtb*-mediated biotransformations is crucial as it is also responsible for an alternative monooxygenase, which activates ethionamide²⁵. Additionally, the role of MymA as a Toll-like receptor agonist in stimulating the release of pro-inflammatory cytokines can be crucial in designing next-generation vaccines²⁶.

Indole-4-carboxamides

Host CD4 T cells (involved in the immune response) manage *Mtb* growth by starving it of tryptophan; however, *Mtb* can subvert this by synthesizing the amino acid under stress conditions. Examples of tryptophan

biosynthesis inhibitors (Fig. 3f) include BRD4592 (49), indole propionic acid (50) and 5-fluoroanthranilate (51), which target different enzymes in the pathway²⁷. Conversely, a new series of indole-4-carboxamides is metabolized by *Mtb* to 4-aminoindole which is incorporated into the tryptophan biosynthesis pathway affording 4-amino-L-Trp (53), a toxic metabolite resulting in *Mtb* cell death²⁸ (Fig. 3g). *Mtb* has developed three resistance mechanisms against indole-4-carboxamides (exemplified by 52) (Fig. 3g), shedding light on the potential MoA of these compounds. The first mechanism involved mutations in a non-essential putative amidase AmiC. Mutated AmiC lost the ability to hydrolyse the amide bond to generate the metabolite, which maintains activity against *Mtb* (MIC = 4.68 μM). The second resistance mechanism involved mutations in *trpE* (anthranilate synthase), which converts chorismite to anthranilate, the first step in the tryptophan biosynthesis pathway. The TrpE-H170R mutant was less sensitive than TrpE (250-fold) to allosteric inhibition by L-Trp. Additionally, this mutation enhances the flux of intermediates through the pathway irrespective of L-Trp concentrations that would otherwise shut down biosynthesis, allowing *Mtb* to overcome inhibition by the metabolite. Finally, 52 and its metabolite conferred mutations in TrpAB (tryptophan synthase)

in residues spread throughout the protein rather than in the α -subunits and β -subunits only, which are usually observed owing to TrpAB inhibitors. These mutations altered the enzymatic activity of TrpAB, which decreased the formation of toxic 4-amino-L-Trp (**53**) along with normal L-Trp needed for bacterial survival.

Benzoheterocyclic oxime carbamates

The anti-*Mtb* activity of a library of small polar molecules has been evaluated^{29,30} and yielded a new series of benzoheterocyclic oxime carbamate hits. Compounds **54** and **55** (Fig. 3h), two molecules explored, have good selectivity towards mycobacteria, including a panel of five

clinical isolates with MIC values in the range of 0.08–0.30 μM (refs. ^{29,30}). These compounds were hypothesized to act as prodrugs by undergoing *Mtb*-mediated hydrolysis of the carbamate moiety to release the more stable oximes, which are responsible for the observed activity. The oximes can bind to their protein target in *Mtb*; however, they have poor *Mtb* permeability. By exploring another hit, **56**, liquid chromatography coupled with mass spectrometry in *Mtb* live cultures confirmed rapid metabolism to **57** (Fig. 3h), relative to culture media and heat-killed cultures, in support of the prodrug hypothesis. In addition, the chemically synthesized oxime congeners of the carbamates were inactive against *Mtb* (MIC > 160 μM). It has been further hypothesized

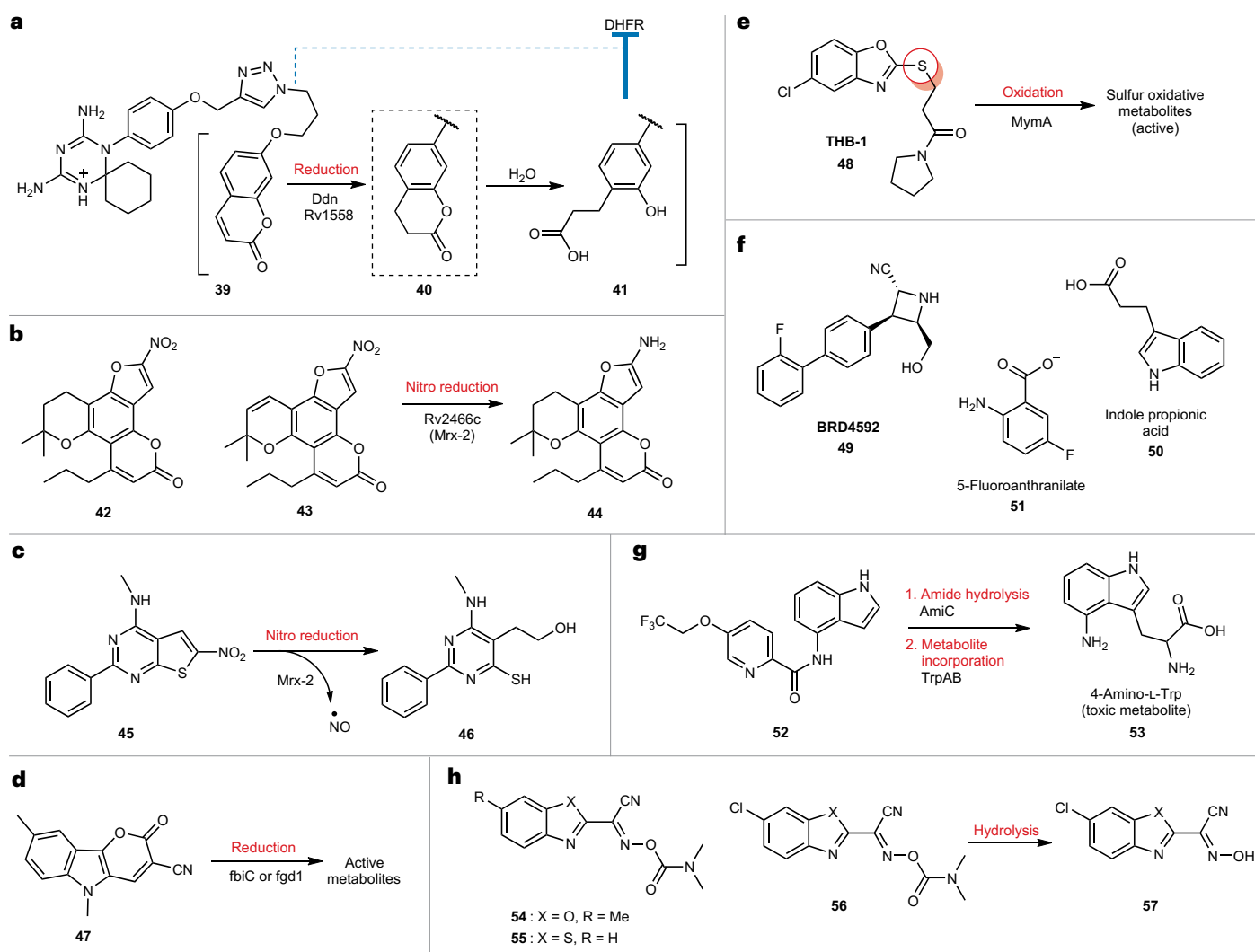


Fig. 3 | Examples of *Mtb*-mediated biotransformation reactions on small molecule antimycobacterial agents in the context of foe. **a**, The triaza-coumarin (**39**) is reduced in its coumarin ring inside *Mycobacterium tuberculosis* (*Mtb*) cells to form the unstable intermediate **40**, which undergoes spontaneous ring opening to form the more active **41**. **b**, The nitrofuranyl calanolides **42** and **43** are reduced to the amino metabolite **44**. **c**, The thienopyrimidine analogue **45** prodrug is metabolized through nitro group reduction to the reactive **46**. **d**, The pyrano[3,2-b]indolone compound **47** releases active metabolites after reduction by *Mtb*. **e**, The thioalkylbenzoxazole analogue (THB-1; **48**) underwent

sulfur oxidation to produce active metabolites. **f**, Chemical structures of **49**, 5-fluoroanthranilate **50** and indole propionic acid **51**. **g**, Indole-4-carboxamide **52** is hydrolysed to 4-aminoindole, which is incorporated in the tryptophan biosynthetic pathway to produce toxic tryptophan metabolite (4-amino-L-Trp; **53**). **h**, The benzoheterocyclic oxime carbamate hit compounds **54** and **55**, *Mtb*-mediated hydrolysis of **56** and related compounds release the oxime metabolite **57**. AmiC, putative amidase; Ddn, deazaflavin-dependent nitroreductase; DHFR, dihydrofolate reductase; FbiC, Fgd1, probable F₄₂₀ biosynthesis protein; Mrx-2, mycoredoxin; MymA, flavin monooxygenase; TrpAB, tryptophan synthase.

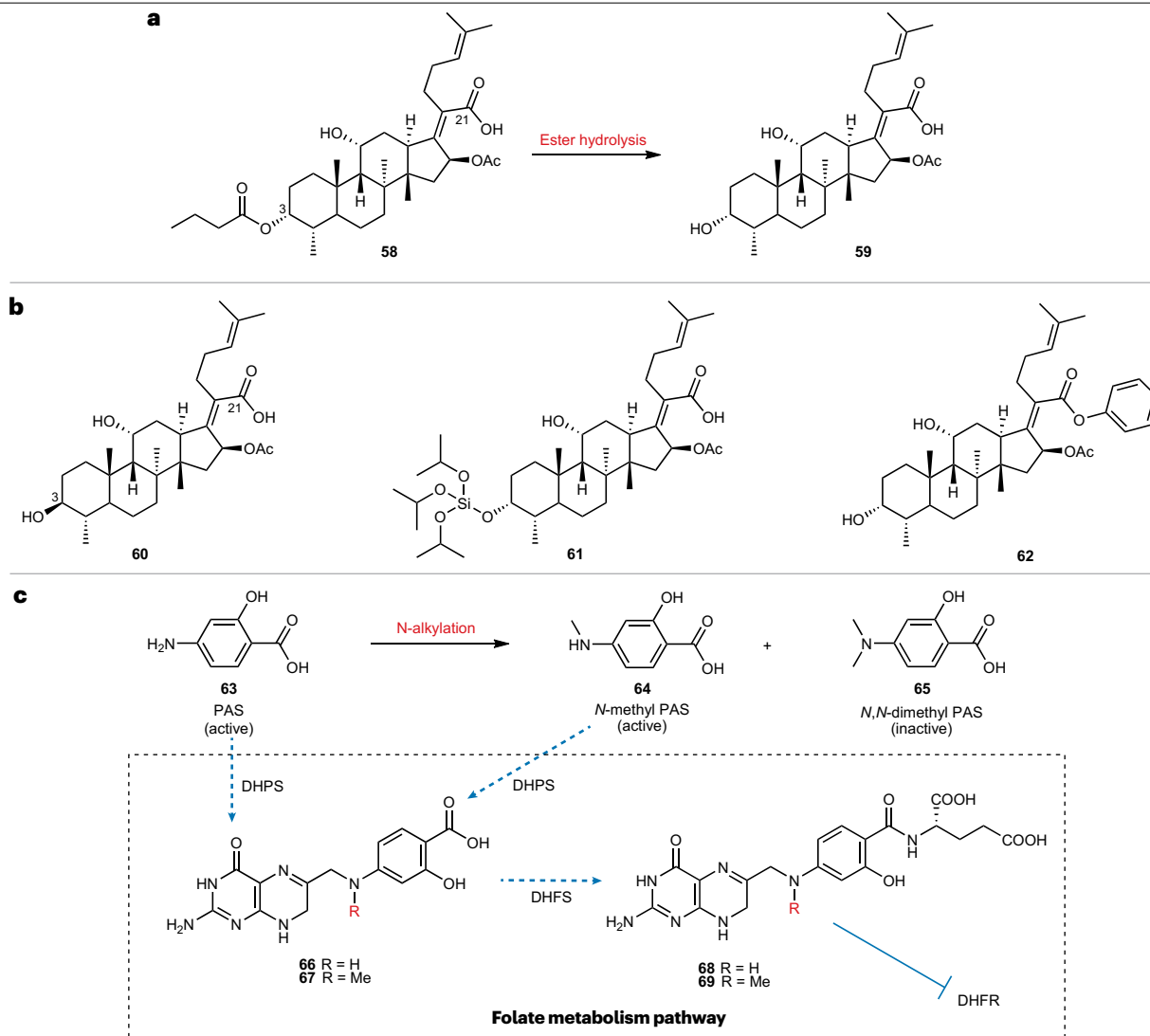


Fig. 4 | *Mtb*-mediated biotransformation on fusidic acid and cases of foe.

a, The fusidic acid alkyl ester (**58**) as a prodrug is metabolized by *Mycobacterium tuberculosis* (*Mtb*) to the parent drug fusidic acid (**59**). **b**, Chemical structures of 3-epifusidic acid (**60**), fusidic acid 3-silicate ester (**61**) and fusidic acid C-21 aryl ester (**62**). **c**, *p*-Aminosalicylic acid (PAS; **63**) undergoes mono-amino and

di-amino alkylation in *Mtb* to produce **64** and **65**. Compounds **63** and **64** are incorporated in the folate metabolism pathway; mimicking the natural substrate *p*-aminobenzoic acid, they form conjugates (**66**–**69**) that are able to inhibit *Mtb* dihydrofolate reductase (DHFR). DHFS, dihydrofolate synthase; DHPS, dihydropteroate synthase.

that the carbamate group is essential to facilitate *Mtb* cell membrane permeability as free oximes of the same series were completely inactive.

Fusidic acid-3-esters

The natural product antibiotic fusidic acid (**59**) (Fig. 4a) showed potent antimycobacterial activity with an in vitro MIC value of <0.15 μM against *Mtb*. However, **59** was inactive in a mouse TB infection model presumably owing to its rapid metabolism to the substantially weakly whole-cell-active 3-epifusidic acid (**60**) (Fig. 4b), which was confirmed by metabolite identification studies following incubation with mouse liver microsomes^{31,32}. Towards repositioning **59** for TB, its active alkyl esters (Fig. 4a) were used as classic prodrugs: when **58** is incubated with *Mtb* H37Rv cultures, **59** is slowly released as an even more active metabolite.

Moreover, as expected, the levels of the biotransformation reaction product of **58** were 500-fold more when incubated with live *Mtb* H37Rv cultures, in comparison to metabolically inactive heat-killed *Mtb* cells. At this juncture, it is noteworthy that in the same experiments, the C-3 silicate ester **61** (MIC = 1.25 μM) (Fig. 4b) was not hydrolysed to **59** or any other metabolite, maintaining comparable amounts in both cell cultures. In addition, the C-21 aryl ester **62** was not metabolized to the free acid; **59** is in clinical use against Gram-positive infections, including methicillin-resistant *Staphylococcus aureus*³³. In *Mtb*, **59** targets an essential elongation factor, *G*, which is crucial for protein translation³⁴. Despite **58** showing improved absorption and tissue distribution in pharmacokinetic and organ distribution experiments³², preclinical development of fusidic acid as an anti-TB drug has been hampered as a

Glossary

Auxotrophs

Organisms that are unable to synthesize one or more essential growth components.

Bioavailability

Measure of the rate and fraction of the drug dosage that reaches either the site of action or the bodily fluid from which the intended targets of the drug have unimpeded access.

Bioisosteres

Substituents or groups that have chemical or physical similarities and related molecular shapes and may result in similar biological activities.

Dysbiosis

Negative imbalance in the microbial communities of the human body (for example, gut bacteria) that are linked to health problems and diseases.

Functional foods

Foods that offer health benefits beyond their nutritional value, for example, whole, fortified, enriched or enhanced foods.

Granuloma

A distinctive pattern of chronic inflammation characterized by cellular aggregates that assume an epithelioid appearance; although capable of limiting *Mycobacterium tuberculosis* growth, it also provides a survival niche from which *M. tuberculosis* may disseminate.

Latent TB infection

A state of persistent anti-*Mycobacterium tuberculosis* immune response without evidence of clinically manifested active disease.

pharmacology proof-of-concept study in a mouse TB infection model showed rodent-mediated metabolism to yield inactive **60**. Considering this, it may be useful to progress and evaluate the pharmacokinetics of **59** in non-rodent models of TB, such as the marmoset, in which metabolism, and thus drug exposure, may be like that in humans.

p-Aminosalicylic acid

The anti-TB drug *p*-aminosalicylic acid (PAS; **63**) inhibits the folate pathway (crucial for the synthesis of cell-function precursors) of *Mtb*. It was assumed that **63** inhibits dihydropteroate synthase (which is involved in folic acid synthesis) by mimicking the substrate *p*-aminobenzoate.

Minimum inhibitory concentration

(MIC). The lowest concentration of a compound that inhibits $\geq 90\%$ in vitro growth of *Mycobacterium tuberculosis*.

Nutraceuticals

Products that are food or part of food providing physiological benefit or protection against chronic disease.

Persister cells

A subpopulation of *Mycobacterium tuberculosis* that is genetically identical to drug-susceptible *M. tuberculosis*, but can survive antibiotic treatment.

Pleiotropic

Responsible for or affecting more than one phenotypic characteristic.

Prototroph

Organism that can synthesize its required nutrients from inorganic substances.

Structure–activity relationship

(SAR). An approach designed to investigate relationships between chemical structure and biological activity of studied compounds, allowing the selection and optimization of ideal drug candidates.

Xenobiotics

Chemicals found within an organism that are not naturally produced or expected to be present within the organism and are extrinsic to the normal metabolism of that organism.

PAS is metabolized by *Mtb* via N-alkylation to the active *N*-methyl PAS (**64**) and inactive *N,N*-dimethyl PAS (**65**) and progresses through the folate pathway forming conjugates that inhibit enzymes in the pathway³⁵ (Fig. **4c**). Conjugates **66–69** were identified after exposure of *Mtb* to **63** through metabolomics analyses. Conjugate **68**, which is responsible for the antitubercular activity of **63**, was chemically synthesized and its biochemical activity was confirmed against *Mtb* DHFR (inhibitor constant $K_i = 174$ nM)³⁶.

Friend biotransformation examples

The biotransformations described earlier detail instances which are detrimental to *Mtb*, but beneficial to its host. It is also possible for biotransformations to act as a friend to *Mtb* and aid its survival.

Bio-AMS

Bio-AMS (**70**) is a 5'-[*N*-(*D*-biotinoyl)sulfamoyl]amino-5'-deoxyadenosine bisubstrate inhibitor of mycobacterial biotin protein ligase (involved in lipid metabolism) with an IC₅₀ value of 135 nM (Fig. **5a**). It is potent against *Mtb* (MIC = 0.31–0.78 μ M) with MIC values in the range of 0.16–0.625 μ M against MDR–XDR strains of *Mtb*^{37–39}. *Mtb*-resistant mutants to **70** showed overexpression of the type II sulfatase Rv3406. Rv3406 is involved in the oxidation of the 5'-methylene carbon of **70** to the hemiaminal (**71**), resulting in molecular cleavage to produce the inactive substrates, biotinoyl sulfamide (**72**) and adenosine 5'-aldehyde (**73**)³⁷. By blocking the labile 5'-position of **70** through the introduction of a methyl substituent, a modified 5'-C-methyl derivative was no longer susceptible to enzyme inactivation⁴⁰.

Benzofurazan scaffold

The antitubercular activity of the benzofurazan scaffold is well documented⁴¹; for example, the benzofurazan analogue JSF-2164 (**74**) is an inhibitor of purified 2-*trans*-enoyl-acyl carrier protein reductase (InhA, which catalyses a crucial step in type II fatty acid synthesis in *Mtb*; IC₅₀ = 51 μ M) with an in vitro MIC value of 10 μ M against *Mtb*⁴². Unlike the known InhA inhibitor isoniazid, which exhibits bactericidal activity, **74** was bacteriostatic, which, along with the observed disconnect between InhA inhibition and whole-cell activity, suggests that InhA inhibition is secondary to another possible mode of action. This was supported by the observation when resistant mutants to **74** exhibited mutations in the genes *fbtC* and *fgdI*. The essentiality of F₄₂₀H₂ to **74** activity was confirmed through resistance conferred by the *fgdI::tn* strain, bearing a functionally inactivated *fgdI* gene. Similar to pretomanid, **74** undergoes intrabacterial F₄₂₀H₂-dependent NO production for the inhibition of *Mtb* growth (Fig. **5b**). Apart from releasing NO, *Mtb* biotransforms **74** into two metabolites with concomitant reduction of the nitro group to an amino group. The metabolites (**75**; JSF-3616 and **76**; JSF-3617) that were confirmed by chemical synthesis, relative to **74**, showed less potent MIC values of 32 and 250 μ M, respectively, against the wild-type *Mtb* strain, but 46% and 77% InhA inhibition at 50 μ M compared with 53% for **74** at the same concentration. Metabolites **75** and **76** were produced in the *fgdI::tn* strain but at a slower rate compared with the wild-type *Mtb* strain, suggesting the involvement of other genes besides F₄₂₀H₂ in the metabolism of **74**. Furthermore, given that the desnitro metabolite from the loss of NO in **74** was not detected, the formation of **75** and **76** supports the involvement of other nitroreductases in the intrabacterial metabolism of **74**. Although **74** has a high in vitro MIC value, it may be useful as a starting point for medicinal chemistry SAR exploration as *N*-oxide benzofurazans with potent in vitro and in vivo antitubercular activities have been reported⁴¹.

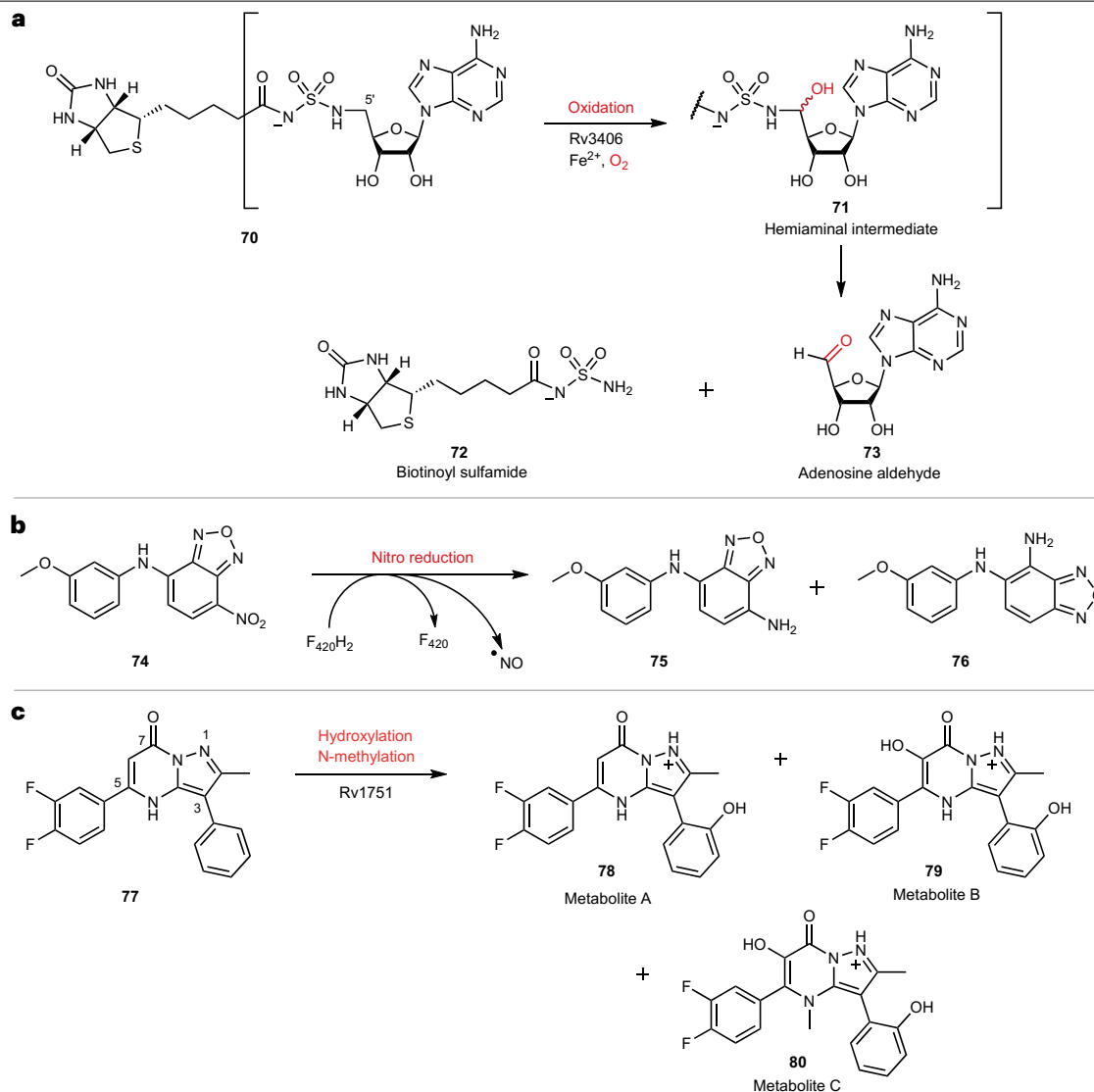


Fig. 5 | Examples of *Mtb*-mediated biotransformation reactions on small molecule antimycobacterial agents in the context of friend. a, The biotinoyl adenosine analogue Bio-AMS (**70**) is oxidized (**71**) and subsequently cleaved to produce inactive metabolites (**72** and **73**). Rv3406 is a sulfotase. **b,** The benzofurazan analogue **74** releases nitric oxide (NO) inside *Mycobacterium*

tuberculosis (*Mtb*) via nitro reduction to produce two amino metabolites (**75** and **76**). **c,** Pyrazolo[1,5-*a*]pyrimidin-7(4H)-one analogue **77** is metabolized by *Mtb* through hydroxylation and N-methylation to produce several metabolites (**78**–**80**). Rv1751 is an oxidoreductase.

Pyrazolo[1,5-*a*]pyrimidin-7(4H)-one

Antitubercular pyrazolo[1,5-*a*]pyrimidin-7(4H)-ones that act by targeting cell wall biosynthesis, isoprene biosynthesis and iron chelation have been reported^{43–45}. Structurally, these compounds contain a substituted phenyl group at the C-3 position of the pyrazolo[1,5-*a*]pyrimidin-7(4H)-one core scaffold (Fig. 5c). A promising pyrazolo[1,5-*a*]pyrimidin-7(4H)-one hit compound (**77**) was identified from a high-throughput whole-cell screen⁴⁶. SAR exploration revealed the importance of the C7-oxo group for antimycobacterial activity. A methyl group at the C2 position and a phenyl group at C5 were also required for potency. MoA studies by spontaneous-resistant mutant generation towards identifying the molecular target of these compounds revealed the role of a flavin

adenine dinucleotide-dependent hydroxylase (Rv1751) in compound metabolism via hydroxylation leading to hydroxylated metabolites (**78**–**80**) (Fig. 5c). This finding highlights an additional MoA of the 3-phenylpyrazolo[1,5-*a*]pyrimidin-7(4H)-onescaffold in *Mtb*. On the basis of this, it is suggested that the pyrazolo[1,5-*a*]pyrimidin-7(4H)-one scaffold has the potential to target several proteins in *Mtb*, which may make compounds based on this scaffold less susceptible to resistance.

Other examples

Contrary to PAS, the well-known sulfonamide inhibitors of the folate pathway dihydropteroate synthase are inactive (sulfanilamide and dapsone) or weakly active (sulfamethoxazole) against *Mtb*.

These undergo *Mtb*-mediated metabolism by N-alkylation, N-acetylation and/or acetyl reduction to produce inactive metabolites^{16,35} (Supplementary Fig. 1a–c). Similarly, the methotrexate diethyl ester **87** (Supplementary Fig. 1d) and its dimethyl congener show MIC values of 0.6 and 0.2–0.4 μM against *Mtb*, with potent *Mtb* DHFRIC₅₀ values of 30 and 50 nM, respectively. However, methotrexate is poorly active against *Mtb* (MIC > 100 μM), but highly active against purified DHFR with an IC₅₀ value of 6.8 nM. Compound **87** undergoes intrabacterial hydrolysis to afford weakly whole-cell active mono-ethyl metabolites (MIC = 33 μM), which maintained DHFR inhibitory potency (IC₅₀ = 24 and 172 nM for **88** and **99**, respectively)⁴⁷. A study⁴⁸ uncovered N-methylation as a potential biotransformation reaction mechanism inside *Mtb* in the metabolism of a bactericidal pyrido-benzimidazole compound (**90**) (Supplementary Fig. 1e), thereby developing resistance. The S-adenosyl-L-methionine-dependent methyltransferase (Rv0560c) was involved in transferring a methyl group from S-adenosyl-L-methionine to the N-5 position of **90** to form **91** (ref. ⁴⁸).

Host microbiome versus tuberculosis

The human host, born sterile, starts developing a microbiota along with the related immune system within minutes of birth. The microbiota can be defined as a collection of microbial communities living on or in a defined environment: the human body carries trillions of microbes. The related term microbiome refers to a micro-ecosystem (microbiota and their genomes). Evolution of the microbiome is a continuous process throughout the life cycle of an individual and its composition is majorly affected by, for example, diet, hormones, stress, genetics and impacting the body in healthy and adverse states. In recent times, there has been a strong interest in microbiome research resulting in a handful of applications, such as the use of probiotics, functional foods and nutraceuticals^{49–51}. The microbiome–immune interactions, reviewed elsewhere⁵², make a strong case for host-microbiota-directed therapies as a potential adjunctive strategy to improve TB treatment for its effectiveness, treatment shortening and relapse prevention.

Additionally, as drugs can also impact the composition of microbial communities (dysbiosis)⁵³, microbial interaction with drugs or

chemicals inside the host paved the way to the new field of ‘pharmacomicrobiomics’, which studies the interactions of microbes and pharmaceuticals⁵⁴. The majority of these interactions occur within the gastrointestinal tract where distinct microbial communities reside and influence or carry metabolic processes across the different regions of the gastrointestinal tract that are varying in cell type and physiology⁵⁵. Generally, the bioavailability of orally administered drugs depends on the extent of first-pass metabolism by intestinal and hepatic enzymes⁵⁶. However, oral drugs may encounter the gut microbiota before reaching host tissues that can affect the bioavailability of drugs by: 1) biotransformation of drugs by common reduction and hydrolysis reactions; 2) affecting drug absorption during treatment; and/or 3) bioaccumulation (Fig. 6). There is an emerging interest in understanding the effect of TB chemotherapy on gut microorganisms. An occurrence of dysbiosis was reported in which *Bacteroides* species were increased, whereas *Clostridiales* species were reduced during TB chemotherapy⁵⁷. In another study, it was suggested that TB chemotherapy has a long-lasting effect on microbiome composition as the microbiome of TB-cured individuals was unique⁵⁸. These observations were complemented by animal *Mtb* infection studies in a TB mouse infection model⁵⁹. Although these studies highlight the crosstalk between gut microbiome and TB treatment, as TB is a pulmonary disease, there is a need to understand the pulmonary microbiome role in various stages of TB disease. Moreover, there are knowledge gaps in the role of microbiome composition versus latent TB infection, dysbiosis versus efficacy of TB treatment and/or role of dysbiosis in re-infection or relapse after successful treatment. Nonetheless, the fact mentioned earlier signifies the importance of understanding the ‘pharmacomicrobiomics’ of TB drugs and the impact of *Mtb*-mediated drug metabolism on the pharmacokinetics of antitubercular and/or coadministered drugs.

Addressing biotransformation peril

To avoid the *Mtb*-friendly biotransformation setbacks during various phases of the drug discovery and development process as highlighted earlier, cognisant of recommendations by Awasthi and Freundlich¹⁶, we propose a small molecule hit compound progression cascade that

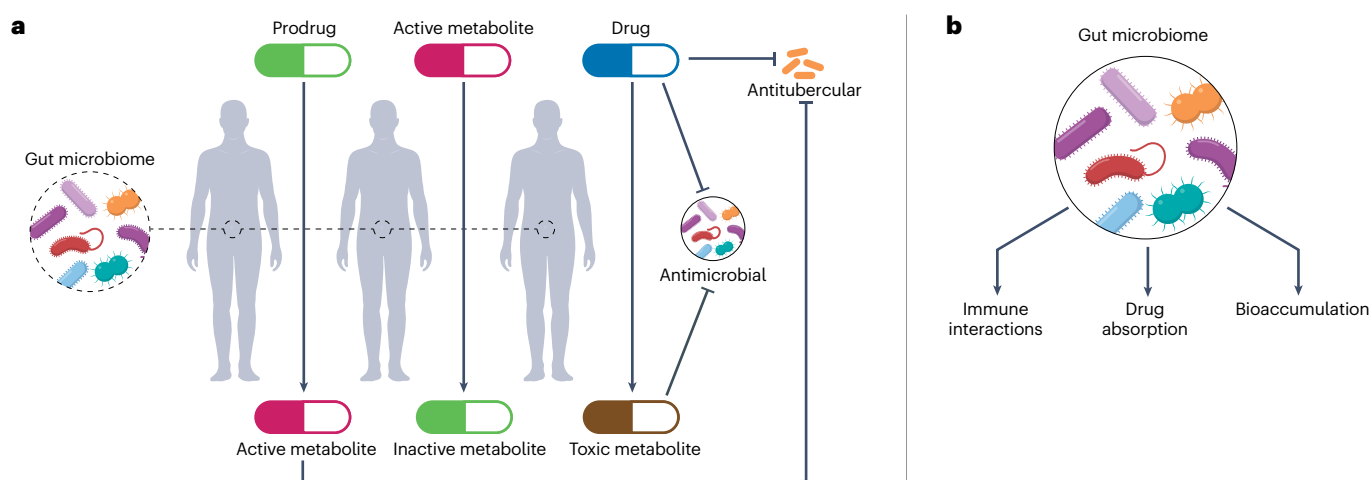


Fig. 6 | Role of gut microbiome in antitubercular drug metabolism. a, The gut microbiome can directly influence anti-tuberculosis drugs by metabolizing them into active, inactive or toxic metabolites. **b**, The gut microbiome can also affect *Mycobacterium tuberculosis* infection by modulating host

metabolites or pathways resulting in immune interactions: before entering systemic circulation, the bioavailability of anti-tuberculosis drugs may be affected by drug absorption and/or bioaccumulation of the drugs by the gut microbiome.

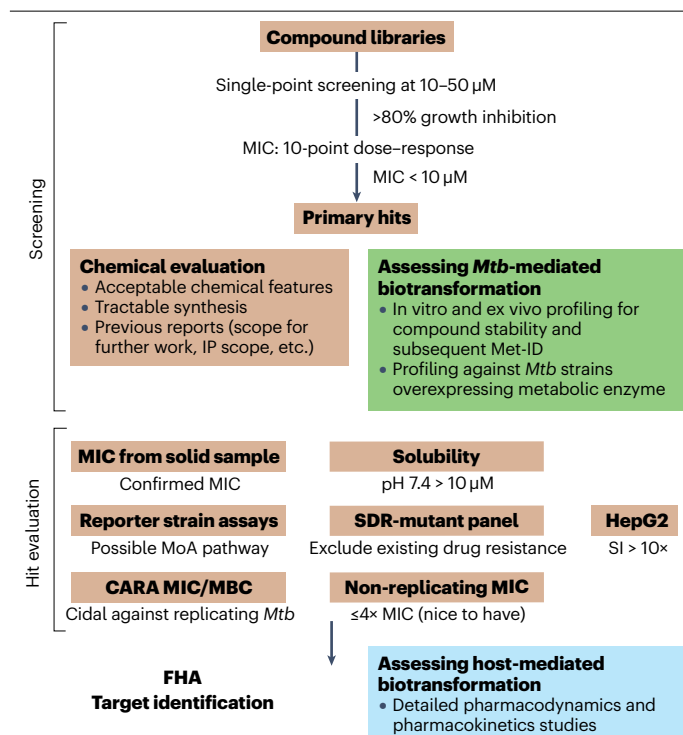


Fig. 7 | Addressing *Mtb*-mediated drug metabolism during early drug discovery phase. An early tuberculosis drug discovery cascade is proposed, in which the potential hits can be triaged for associated *Mycobacterium tuberculosis* (*Mtb*)-mediated biotransformation liability immediately after screening. This can be achieved by incubating the compound in the relevant growth medium and/or with *Mtb* (in vitro), with THP.1 human cell-line-derived macrophages and intracellular *Mtb* (ex vivo), followed by metabolite identification. Additionally, the compounds can be titrated against recombinant *Mtb* overexpressor strains, expressing validated recombinant enzymes involved in biotransformation. CARA, charcoal agar resazurin assay; FHA, formal hit assessment; HepG2, human liver cancer cell line; MBC, minimum bactericidal concentration; Met-ID, metabolite identification; MIC, minimum inhibitory concentration (7–14 days); MoA, mechanism of action; SDR, single drug resistant; SI, selectivity index.

includes frontloading *Mtb*-mediated metabolism studies (Fig. 7). Here, we propose to evaluate the stability of hit compounds in 1) assay media, 2) the presence of *Mtb*, 3) the host cell line and 4) intracellular *Mtb*. Additionally, our current efforts are focused on developing and using in vitro medium-throughput assays involving recombinant *Mtb* overexpressor strains, expressing common compound-metabolizing recombinant enzymes. This will be complemented with metabolite identification to determine likely *Mtb*-mediated metabolic outcomes of newly identified small molecule starting points, which will facilitate hit triaging and identification of an active metabolite (of a prodrug) and may guide MoA deconvolution. Subsequently, to predict in vivo stability of lead molecules in humans, studies in model assays (for example, solubility, hepatocyte or microsomal fractions) offer an opportunity to further probe compound metabolism.

The outcomes will then guide the optimization of lead compounds for optimal in vivo pharmacokinetic and pharmacodynamic properties with minimum safety liabilities and more accurate human dose prediction. There are useful approaches to identify metabolic ‘soft

spots’ (that is, labile or susceptible groups) of drugs⁶⁰. For example, it is widely accepted that the allylic methyl, benzylic C–H bond and the *O*-methyl, *N*-methyl and *S*-methyl groups are preferred metabolic soft spots of P450-mediated metabolism, in the absence of steric hindrance⁶¹. These soft spots can be addressed by structure–metabolism relationship studies in which bioisosteres are used to replace the identified soft spots; for example, replacing a benzylic methyl group with a fluorine or a chlorine atom, or a trifluoromethyl group. Furthermore, these metabolic studies may inform the selection of suitable candidates with the optimum dosing route for the required oral formulation of TB drugs: a long treatment is required for this slow-growing *Mtb* pathogen.

Application of metabolic wisdom

Historically, in TB drug discovery, when compared with target-based screening, phenotypic whole-cell screening has been relatively more successful to deliver desired outcomes, from hit to preclinical and clinical candidate identification⁶². The current TB drug discovery and clinical pipeline comprise a diverse set of drug-like molecules acting on various metabolic targets. However, the attrition rate in drug development is high and demands continued discovery efforts to replenish the pipeline. The most advanced drug candidates target energy metabolism, for example, a pyrazolo[1,5-*a*]pyridine-3-carboxamide hybrid (**92**) (Supplementary Fig. 2) is active against both drug-susceptible and drug-resistant clinical *Mtb* isolates and targets the QcrB subunit of the respiratory cytochrome *bc1* complex⁶³. Moreover, a novel class of benzoxaboroles (**93**) (Supplementary Fig. 2) inhibits leucyl-tRNA synthetase, an essential enzyme for protein synthesis in *Mtb*, and displays excellent in vivo efficacy in TB animal infection models⁶⁴. However, as stated earlier, owing to the complexity of the disease pathophysiology, metabolic target: inhibitor pairs do not always translate into successful outcomes and caution is needed. Generally, validation of target essentiality or vulnerability is governed by genetic depletion of the target, which is indicative of the consequences of target depletion. Whereas the less-validated chemical inhibition of the target would result in modulating the related metabolome, which is not always predictable. This is further complicated by the rescue of target depletion or chemical toxicity by available metabolites. For example, the essential inosine monophosphate dehydrogenase was validated as genetically essential but the chemical inhibitor could not translate the genetic vulnerability into chemical vulnerability in the mouse TB infection model, possibly owing to the availability of target metabolite (metabolites) at the disease site^{65,66}. Similarly, the presence of an alternative pathway can rescue bacteria from the adverse effect of an inhibitor, as in the case of Q203 and related derivatives in which the inhibitor activity is alleviated by the presence of glycerol supplementation via upregulation of the cytochrome *bd* oxidase, which compensates for the inhibition of target cytochrome *bc1:aa3* terminal oxidase⁶⁷. Therefore, it is necessary to ascertain the genetic and chemical vulnerability of a target along with confirmation of on-target activity in in vitro and in vivo environments. Nonetheless, information on drug metabolism remains crucial. For example, a new metabolic target mycobacterial membrane protein Large 3 (MmpL3) – an inner membrane protein transporting trehalose-monomycolates, precursors for trehalose-dimycolates and mycolic acids – has been reported to be a target of a diverse range of compounds⁶⁸. One of these is the clinical candidate SQ109 (**94**) (Supplementary Fig. 2), a 1,2-ethylenediamine that interacts synergistically with other anti-TB drugs such as rifampicin, isoniazid and bedaquiline^{69,70}. Compound **94** is currently in phase II clinical trials for the treatment of MDR-TB. Since the discovery

of **94**, it has been suggested that **94** inhibits multiple targets in *Mtb*. Owing to the various possible soft spots in **94** that can be oxygenated by cytochromes (CYPs), **94** is metabolized by human, dog and murine liver microsomes⁷¹. As *Mtb* also carries multiple cytochromes⁷², a role for *Mtb* CYP124 (Rv2266) in hydroxylating **94** to a monohydroxylated metabolite has recently been established⁷³. It was proposed that this newly formed **95** (Supplementary Fig. 2) metabolite affects binding to the relevant pocket in mycobacterial membrane protein Large 3. This study supported the hypothesis that **94** is a prodrug activated by *Mtb* CYPs, in which CYP124 exemplifies an *Mtb* CYP that functions similarly to drug-metabolizing human CYPs.

The adaptability of *Mtb* can also be measured in developing drug-tolerant persister cells, which may emerge due to the metabolic alteration, induced drug resistance or genetic mutation to survive drug toxicity or host environmental stress⁷⁴. For example, conditional tolerance to isoniazid, ofloxacin and rifampicin was associated with a mutation in transcription factor, *prpR*, that affects propionyl-CoA metabolism⁷⁵. Similarly, the significance of a detailed understanding of metabolic reactions or pathways in reversing TB drug resistance cannot be overlooked. For example, the prodrug ethionamide is activated by a monooxygenase (EthA) generating an active metabolite that inhibits mycolic acid biosynthesis by targeting InhA^{76,77} (mycolic acids, α -branched, β -hydroxy long-chain fatty acids, are the hallmark of the cell envelope of *Mtb*). Overexpression of EthA is directly under the control of EthR, a TetR-type transcriptional repressor⁷⁸. This implies that EthR can be targeted to stimulate EthA expression, thereby boosting the potency of ethionamide⁷⁹. Recently, **96** (Supplementary Fig. 2) was identified from the spiroisoxazoline class of small molecules aborting resistance (SMART): it stimulated ethionamide activity and reversed EthA-mediated resistance owing to its unique action in the *ethR2-ethA2* region⁸⁰. This work was further extended and resulted in the identification of **97** (Supplementary Fig. 2) from an *N*-acylated 4-phenylpiperidine compound series that interacts with VirS, another transcriptional regulator for regulating the *mymA* operon that encodes an alternative monooxygenase responsible for activating ethionamide²⁵.

Additionally, caution needs to be exercised in evaluating covalent inhibitors that may behave unexpectedly under different conditions, for example, 'irreversible' under chemically simple conditions but 'reversible' in the complex in vivo environment⁸¹. This concept is illustrated by β -lactam antibiotics, which target peptidoglycan biosynthesis, an essential component of bacterial cell envelope, commonly by irreversible, covalent inhibition of penicillin-binding proteins. Meropenem (**98**) (Supplementary Fig. 2) is a broad-spectrum carbapenem β -lactam antibiotic that inhibits the L,D-transpeptidase biosynthetic enzymes in *Mtb*. Recently, it has been observed that meropenem unexpectedly engages with its target in the form of reversible and nonhydrolytic offloading reactions⁸². This reversibility could lead to degradation of meropenem to inactive meropenem β -lactone and hydrolysed forms, resulting in ineffective treatment. A similar observation has been noted with D-cycloserine (DCS, **99**) (Supplementary Fig. 2), a key antitubercular used for the treatment of MDR-TB and XDR-TB, which targets two essential enzymes, alanine racemase and D-Ala:D-Ala ligase, in the peptidoglycan biosynthesis pathway. It is commonly believed that the alanine racemase inhibition mechanism involves an irreversible route. In *Mtb*, alanine racemase activity was detected after exposure to clinically relevant DCS concentrations. A detailed mechanistic investigation revealed a slow, reversible covalent mechanism of DCS inhibiting the alanine racemase in *Mtb*⁸¹. The knowledge gained from these two unconventional mechanisms

strengthens the approach of having mechanistic insight in guiding the design and synthesis of improved chemical matter.

Conclusions

The mechanisms underpinning intrabacterial biotransformation are useful in discovering next-generation antituberculars well suited to treat TB. Although the search for new antitubercular agents remains an active research endeavour, with a plethora of very promising agents under development, understanding the innate metabolic capabilities of *Mtb* will require combined learnings from the biotransformation of specific antitubercular agents. Such knowledge will inform chemists of potential metabolic soft spots in compounds that lend themselves to *Mtb*-mediated metabolism early in the drug discovery process and how to potentially address this liability. Additionally, using machine learning tools, predicting the biotransformation of xenobiotics in the human gut by metabolic enzymes of gut microbes⁸³ combined with predictions of phase I and phase II drug metabolism using various software such as MetaSite⁸⁴, Metaprint2D⁸⁵, ADMET predictor (simulations plus, USA), RS-WebPredictor⁸⁶ and FAME⁸⁷ could be a powerful strategy towards identifying metabolic liabilities of novel small molecules. Novel putative drug targets have been identified through analyses of metabolic pathways and the proteins associated with bacterial drug resistance⁸⁸. Furthermore, *Mtb* displays a co-evolution of virulence and antibiotic resistance in which enhanced intracellular survival protein not only protects *Mtb* from host immunity and helps in the survival of the pathogen in macrophages but also acetylates various aminoglycosides and cyclic peptide antibiotics rendering them inactive^{89,90}. Therefore, understanding metabolic pathways involved in making *Mtb* refractory to anti-TB drugs and targeting these metabolic mechanisms can lead to the development of effective strategies for the management of anti-TB drugs as well as potentially faster cures and thus treatment-shortening. In parallel, metabolism has a crucial role in successful TB infection as host cells undergo profound metabolic changes to influence the function of immune cells. This metabolic reprogramming in immune cells has developed a new field of study known as immunometabolism. Here, a clear understanding of metabolic interactions between the host and *Mtb* can potentially lead to the identification of molecular targets for host-directed therapy.

Published online: 20 March 2023

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Acknowledgements

The Bill and Melinda Gates Foundation (Global Health Grant Number OPP1066878), South African Medical Research Council (SAMRC), Strategic Health Innovation Partnerships unit of the SAMRC, Technology Innovation Agency and South African Department of Science and Innovation/National Research Foundation Research are gratefully acknowledged for support. K.C. is the Neville Isdell Chair in African-centric Drug Discovery and Development and thanks N. Isdell for generously funding the Chair.

Author contributions

V.S. and K.C. designed the content and flow of the paper. V.S. and G.A.D. collected the necessary information and data. V.S., G.A.D. and K.C. wrote the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41570-023-00472-3>.

Peer review information *Nature Reviews Chemistry* thanks Jianping Xie and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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