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Novel targets for tuberculosis drug discovery

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Since the determination of the *Mycobacterium tuberculosis* genome sequence, various groups have used the genomic information to identify and validate targets as the basis for the development of new anti-tuberculosis agents. Validation might include many components: demonstration of the biochemical activity of the enzyme, determination of its crystal structure in complex with an inhibitor or a substrate, confirmation of essentiality, and the identification of potent growth inhibitors either *in vitro* or in an infection model. If novel target validation and subsequent inhibition are matched by an improved understanding of disease biology, then new antibiotics could have the potential to shorten the duration of therapy, prevent resistance development and eliminate latent disease.

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Introduction

Tuberculosis (TB) remains one of the deadliest infectious diseases for humans [1]. Approximately eight million people develop active disease each year and between two and three million cases of active disease result in death [2,3]. This review (and Table 1) surveys several newly identified targets as well as those that have been revisited in the past few years, and the levels to which they have been validated to demonstrate their potential role in improving chemotherapy against TB.

Targeting cell wall biosynthesis

The mycobacterial cell wall is comprised of three covalently linked macromolecules: peptidoglycan, arabinogalactan and mycolic acids. Cell wall biosynthesis is a particularly good source of molecular targets because the biosynthetic enzymes do not have homologues in the mammalian system. Currently used anti-TB drugs include inhibitors of mycolic acids (isoniazid and ethionamide), arabinogalactan (ethambutol) and peptidoglycan (cycloserine). One of the major issues, however, is whether inhibition of cell wall synthesis can reduce the duration of therapy, as the known cell wall inhibitors do not appear to shorten the duration of treatment for active disease.

Peptidoglycan biosynthesis

Both alanine racemase (Alr) and D-Ala-D-Ala ligase are targets of D-cycloserine, a second-line anti-TB drug. These two enzymes catalyze the first and second committed steps in bacterial peptidoglycan biosynthesis. Alr is a pyridoxal 5′-phosphate-containing enzyme that catalyzes the racemization of L-alanine into D-alanine, a major component in the biosynthesis of peptidoglycan. A recent report has described the crystal structure of Alr from *Mycobacterium tuberculosis*, revealing conserved residues near the active site that could be incorporated into the rational design of more specific inhibitors [4].

Arabinogalactan biosynthesis

Studies into the mechanism of action of ethambutol in *M. avium* identified a gene cluster that conferred resistance to this antibiotic when overexpressed [5]. Further studies showed that the products of this gene cluster — EmbA, EmbB and EmbC — are involved in the formation of the terminal hexaarabinofuranoside portion of arabinogalactan, where mycolic acids are attached [6]. A recent study has identified AftA, a novel arabinofuranosyl transferase that catalyzes the addition of the first key arabinofuranosyl residue to the galactan core, priming it for subsequent modifications by the Emb proteins [7•]. This enzyme is not sensitive to inhibition by ethambutol but has been shown to be essential for viability [8].

The first committed step in the synthesis of decaprenylphosphoryl-D-arabinose, the lipid donor of mycobacterial D-arabinofuranosyl residues during arabinogalactan biosynthesis, is the transfer of a 5-phosphoribosyl residue from phosphoribose diphosphate to decaprenyl phosphate to form decaprenylphosphoryl–5-phosphoribose. This step is catalyzed by a ribosyltransferase that has recently been characterized and shown to be essential for growth [9].

Other enzymes essential for arabinogalactan biosynthesis have been identified, including UDP-galactopyranose mutase (encoded by the *M. tuberculosis glf* gene) [10], galactofuranosyl transferase [11] and dTDP-6-deoxy-L-lyxo-4-hexulose reductase, the enzyme that catalyzes the final step in the formation of dTDP-rhamnose. dTDP-rhamnose is a product of four enzymes, RmlA–D, and a recent report has demonstrated that both RmlB and RmlC are essential for mycobacterial growth [12].

Table 1

Validated drug targets in *Mycobacterium tuberculosis*

Metabolic pathway	Gene product	Validation					References
		Knockout *in vitro* growth	Human homologue	Knockout *in vivo* growth	Complexed with crystal structure	Small-molecule inhibitor	
Cell wall biosynthesis							
Peptidoglycan biosynthesis	Alanine racemase	Not viable	None		D-cycloserine	D-cycloserine	[4]
	D-Ala-D-Ala ligase	Not viable	None			D-cycloserine	
Arabinogalactan biosynthesis	EmbA–C	EmbA–B not viable	None		Cofactor (PLP)	Ethambutol	[5,6]
	AftA	Not viable	None				[7•,8]
	Phospho-ribosyltransferase	Not viable	None				[9]
	Galactofuraosyl transferase	Not viable	None				[10]
	dTDP-deoxy-hexulose reductase	Not viable	None				[11]
	RmlA–D		None				[12]
Mycolic acid biosynthesis	ENR (InhA)	Not viable	None			Isoniazid	[14,18,22]
	AcpM	Not viable	None				[15]
	FabD	Not viable	None				[15]
	FabH	Not viable	None		Lauroyl-CoA		[16,20]
	MabA	Not viable	None				[17,21]
	KasA	Not viable	None			Thiolactomycin	[19]
	KasB	Not viable	None			Thiolactomycin	[19]
	MmaA4		None	Attenuated			[23]
	Pks13	Not viable	None				[25]
	Acyl-AMP ligase	Not viable	None				[26••]
	FadD32	Not viable	None				[26••]
	AccD4	Not viable	None				[26••]
	AccA3	Not viable	None				[27•]
	AccD5	Not viable	None				[27•]
	AccE5	Not viable	None				[27•]
Amino acid biosynthesis	LysA		None	Attenuated	Lysine/coenzyme PLP		[28,31]
	LeuD		None	Attenuated			[29]
	TrpD		None	Attenuated			[29]
	ProC		None	Attenuated			[29]
	LeuA		None	Attenuated	Substrate/product/ cofactor	Leucine	[30]
	Didydropicolinate reductase		None	Attenuated			[32]
Shikimic acid pathway	AroA, B, C, E, G, K, Q	Not viable	None				[33–39]
Arginine biosynthesis	ArgF	Not viable	None			Substrate	[40]
	ArgA		None	Attenuated			[41]
Branched-chain amino acid biosynthesis	Acetolactate synthase		None				[42,43]
	Branched-chain amino acid aminotransferase	Not viable	None				[44]
Cofactor biosynthesis							
Folic acid biosynthesis	Dihydropteroate synthase	Not viable	None			Trimethoprim	[45]
	Dihydrofolate reductase	Not viable	Yes		NADP/methotrexate/ trimethoprim	Trimethoprim	[45–47]
Pantothenic acid biosynthesis	PanB–PanE		None	PanCD attenuated			[48]
CoA biosynthesis	CoA (PanK)	Not viable	None				[49]
Riboflavin biosynthesis	LS, riboflavin synthase	Not viable	None		Purinetrione inhibitors		[51••]

Table 1 (*Continued*)

Metabolic pathway	Gene product	Validation					References
		Knockout *in vitro* growth	Human homologue	Knockout *in vivo* growth	Complexed with crystal structure	Small-molecule inhibitor	
Reductive sulfur assimilation	APS reductase	Not viable	None				[52–54]
Mycothiol biosynthesis	MshA–MshD	Not viable?	None		Octylglucoside for MshB/CoA and acetyl CoA for MshD		[55–57]
Terpenoid biosynthesis	IspC–H	Not viable	None		Fosmidomycin for IspC	Fosmidomycin	[55,58]
DNA synthesis	Ribonucleotide reductase	Not viable	Yes				[59,60,64]
	Thymidine monophosphate kinase	Not viable	Yes		3-azidodeoxythymidine monophosphate	3-azidodeoxythymidine monophosphate	[65]
	LigA	Not viable	None			Glycosyl ureides glycofuranosylated diamines	[67,68••, 69••]
	DNA gyrase	Not viable	Yes			Fluoroquinolones	[70,71]
Glyoxylate shunt	Icl 1/2		None	Attenuated	Nitropropionate	Nitropropionate	[72,73••,74]
	Malate synthase		None	Attenuated?			[72]
Regulatory proteins	GlnE	Not viable	None				[75]
	MtrA	Not viable	None				[76]
	IdeR	Not viable	None				[77]
	DosR	Conditionally impaired	None				[78]
Menaquinone biosynthesis	MenA-E and MenH	Not viable?	None				[79]
Stringent response	RelMTB		None	Attenuated			[80–83]
ATP synthesis	ATP synthase		Yes			R207910	[84••]

'?' indicates that a result is not yet confirmed.

Fatty acid biosynthesis

M. tuberculosis contains both type I (FAS I) and type II (FAS II) fatty acid biosynthetic pathways, which is unique to this genus [13]. FAS I is responsible for the *de novo* synthesis of C16-C26 fatty acids. The FAS II system extends these fatty acids up to C56 to make precursors of mycolic acids, which are essential for growth. Whereas FAS II consists of several distinct enzymes, all of the FAS I enzyme activities reside on a single multifunctional enzyme. The mammalian system is similar to the FAS I configuration, making the FAS II system distinct to microbes.

The enoyl acyl carrier protein reductase (ENR) catalyzes the final enzymatic step in the elongation cycle of the FAS II pathway. The *M. tuberculosis* ENR is InhA, the target of the first-line drug isoniazid (INH) [14]. Other essential FAS II enzymes in *M. tuberculosis* have been well characterized [15–19], and crystal structures have been determined for FabH [20], MabA [21] and InhA [22].

A gene cluster that encodes four closely related methyltransferases that introduce modifications in the meromycolate chain of the pathogenic *M. tuberculosis* complex has been characterized. A mutant of one of these methyltranferases, *mmaA4*, was shown to be attenuated in a mouse model of infection [23]. All four enzymes are closely related and share a common cofactor, S-adenosyl methionine. Analogues of S-adenosyl methionine have been successfully synthesized and are effective inhibitors of bacterial and fungal methyltransferases [24].

Pks13, the enzyme that catalyzes the final condensation step in mycolic acid biosynthesis, has been described and shown to be essential for mycobacterial growth [25]. Recently, three enzymes — the acyl-AMP ligase, FadD32, and the AccD4-containing acyl-coenzyme A (CoA) carboxylase — which catalyze the final steps in producing the substrates for the ligase have been described and also shown to be essential for mycobacterial growth [26••].

Mycocerosic acids are found uniquely in the cell wall of pathogenic mycobacteria, and their biosynthesis is essential for growth and pathogenesis. The biosynthesis of the unique precursor methylmalonyl-CoA is an attractive potential drug target, as an essential acyl-CoA carboxylase from *M. tuberculosis* involved in the synthesis of methylmalonyl-CoA has been identified [27•].

Targeting amino acid biosynthesis

M. tuberculosis auxotrophs have been described that are attenuated in mouse infection models, including mutants in the biosynthesis of lysine (*lysA*) [28], proline (*proC*), tryptophan (*trpD*) and leucine (*leuD*) [29]. The structures of both LeuA [30] and LysA in complex with the coenzyme pyridoxal 5′-phosphate (PLP) and the product lysine, as well as in complex with lysine only [31], have been solved. Amino acid biosynthesis should yield important antibiotic targets if the organism is unable to scavenge for nutrients from the human host. Another attractive target in lysine biosynthesis is the enzyme dihydrodipicolinate reductase, for which potent inhibitors have been identified [32]. This is a particularly important pathway because, although lysine is required for protein synthesis, its precursor, meso-diaminopimelic acid, is incorporated into peptidoglycan.

The shikimate pathway is an attractive target for antibiotic development because it is absent from mammals despite being essential in algae, higher plants, fungi and bacteria. The pathway consists of seven enzymatic reactions designated AroG, AroB, AroQ, AroE, AroK, AroA and AroC, and the product of the pathway — chorismate — is the main intermediate in the biosynthesis of aromatic amino acids and other metabolites, including folic acid and quinones. The whole pathway is essential in *M. tuberculosis* [33], and the *aroK* gene could only be disrupted in the presence of a functional copy elsewhere on the chromosome. AroE [34] and AroA [35] have been characterized in detail; furthermore, the structures of AroQ [36], AroC [37] and AroK [38] in complex with shikimic acid, revealing the amino acid residues involved in binding this substrate [39], have been determined.

Arginine biosynthesis was shown to be essential in *M. tuberculosis*, as an *ArgF* mutant requiring exogenous L-arginine for growth *in vitro* had reduced virulence in immunodeficient SCID mice and was highly attenuated in immunocompetent mice, suggesting that L-arginine availability is restricted *in vivo* [40]. A recent study has identified ArgA, an essential enzyme that catalyzes the conversion of L-glutamate to α-*N*-acetyl-L-glutamate, the initial step in L-arginine biosynthesis [41].

Plants and bacteria synthesize branched-chain amino acids such as leucine, isoleucine and valine that are essential for growth. Acetolactate synthase (ALS) catalyzes the first step in the biosynthesis of branched-chain amino acids [42]. ALS is essential for growth, and inhibitors of plant ALS are used as herbicides [43].

The unique pathway that recycles methionine from methylthioadenosine, a byproduct of polyamine biosynthesis, is also important for growth. The final step of the methionine regeneration process is the transamination of ketomethiobutyrate to methionine; this step can be catalyzed by an aspartate, tyrosine or branched-chain amino acid aminotransferase. The *M. tuberculosis* enzyme has been cloned, expressed and demonstrated to be a branched-chain amino acid aminotranferase that is inhibited by aminooxy compounds which also inhibit bacterial growth *in vitro* [44].

Targeting cofactor biosynthesis

Folate derivatives are cofactors utilized in the biosynthesis of essential molecules including purines, pyrimidines and amino acids. Whereas bacteria synthesize folate *de novo*, mammals must assimilate preformed folate derivatives through an active transport system. Two enzymes of the folate biosynthesis pathway — dihydropteroate synthase and dihydrofolate reductase — are the validated targets of the widely used antibacterial sulfonamide drug trimethoprim [45]. An inhibitor of dihydrofolate reductase, WR99210, also possesses whole-cell activity against *M. tuberculosis* [46]. Three crystal structures of ternary complexes of *M. tuberculosis* dihydrofolate reductase with NADP and three different inhibitors (methotrexate, trimethoprim and WR99210) were determined, as well as the binary complex with NADP [47].

Pantothenate (vitamin B_5) is involed in CoA biosynthesis and is also attached to acyl carrier proteins for the purpose of coordinating acyl groups during fatty acid biosynthesis. The four enzymes in pantothenate biosynthesis (Pan B–E) are good targets for antibiotic discovery, but in organisms such as *Escherichia coli*, which are capable of assimilating pantothenate from the environment through a membrane-bound pantothenate permease (PanF), these enzymes are not essential for growth. However, there are no Pan F homologs in *M. tuberculosis*, and a *panCD* deletion mutant was attenuated in a mouse model of infection [48].

CoA is an essential cofactor in lipid biosynthesis, and enzymes involved in its biosynthesis are attractive targets. Pantothenate kinase (PanK) is essential for growth and catalyzes the first step of CoA biosynthesis. This enzyme from *M. tuberculosis* in complex with a derivative of the feedback inhibitor, CoA, has been crystallized and its structure solved [49].

Genomic analysis studies have suggested that the riboflavin (vitamin B_2) biosynthesis pathway is essential in *M. tuberculosis* [50]. Lumazine synthase (LS) catalyzes the penultimate step of riboflavin biosynthesis, and a recent study has performed structure determination for the *M. tuberculosis* LS, made a comparison with previously known structures of LSs from different species, and performed calorimetric binding studies of novel purinetrione inhibitors [51••], thus validating LS as a target for anti-TB drug discovery.

Reduced sulfur is required by algae, plants, fungi and bacteria for the biosynthesis of essential biomolecules

such as cysteine, CoA and methionine. Bacteria possess a reductive sulfur assimilation pathway that converts inorganic sulfate into sulfide, the oxidation state required for biomolecular synthesis; humans do not have a similar pathway. Adenosine-5′-phosphosulphate (APS) reductase catalyzes the first committed step of reductive sulfur assimilation [52], and was shown to be essential in *M. bovis* [53]. A recent study has described the reaction mechanism for APS reductase, paving the way to inhibition studies [54].

Targeting mycothiol biosynthesis

M. tuberculosis synthesizes mycothiol in a multistep process involving four enzymatic reactions designated MshA, MshB, MshC and MshD for protection against the damaging effects of reactive oxygen species. This pathway is absent in humans. Targeted disruption of MshC yielded no colonies unless a second copy of the gene was supplied, an indication of essentiality [55]. The structures for both MshB, a metal-dependent deacetylase that catalyses the second step in mycothiol synthesis [56], and MshD, the mycothiol synthase that catalyzes the last step [57], have been determined.

Targeting terpenoid biosynthesis

Whereas mammals exclusively use the mevalonate pathway for synthesis of terpenoids, many pathogenic bacteria, including *M. tuberculosis*, utilize the non-mevalonate pathway, making this pathway a good source of novel targets. The non-mevalonate pathway is catalyzed by a sequence of enzymes (IspC–H). Four of these enzymes, IspC–F, have been crystallized and their three-dimensional structures determined [58]. This pathway still awaits validation in *M. tuberculosis*.

Targeting DNA synthesis

Ribonucleotide reductases (RNRs) catalyze the first committed step in DNA synthesis, reducing ribonucleotides to deoxynucleotides, and are essential for bacterial growth. There are two classes of RNR in *M. tuberculosis*: class Ib and class II. Class Ib RNRs consists of two subunits, the large subunit R1 and the small subunit R2; both subunits are required for catalytic activity. Extensive genetic, biochemical and biophysical characterization of *M. tuberculosis* RNR [59] has revealed several major differences between the *M. tuberculosis* and the mammalian enzymes [60], including the observations that R1 lacks an allosteric regulatory site for dATP [61]; R2 manifests a weak magnetic interaction between the radical and the iron center compared with its homologues; and the *M. tuberculosis* R2 tyrosyl radical is not hydrogen bonded, but instead is stably held in a hydrophobic pocket where it is difficult to scavenge [62,63]. The crystal structure for the *M. tuberculosis* small subunit of RNR has been solved [64].

Thymidine monophosphate kinase (TMPK) is an essential enzyme that catalyses the conversion of dTMP to dTDP. This step is common to both *de novo* biosynthesis and the salvage pathway. The structure of the *M. tuberculosis* enzyme has been determined [65], and it shows important differences from the mammalian enzyme. As opposed to the human and other TMPKs, catalysis by the *M. tuberculosis* TMPK necessitates the transient binding of a magnesium ion coordinating the phosphate acceptor [66]. Also, 3′-azidodeoxythymidine monophosphate is a competitive inhibitor of *M. tuberculosis* TMPK, whereas it is a substrate for human and other TMPKs [66].

DNA ligases are important for DNA replication and repair, whereby they catalyze the formation of phosphodiester linkages between adjacent double-stranded DNA termini. DNA ligases are classified as either NAD^+- or ATP-dependent, depending upon their specific cofactor. Whereas ATP-dependent ligases are widely distributed in all species, NAD^+-dependent ligases (LigA) are only found in some viruses and in eubacteria. *M. tuberculosis* LigA is essential for growth [67]. A recent study reported the crystal structure of *M. tuberculosis* LigA with bound AMP, and this structure was used to guide computational approaches that identified glycosly ureides as novel inhibitors of LigA that distinguished between NAD^+- and ATP-dependent ligases [68••]. Glycofuranosylated diamine-based inhibitors were identified that also distinguished between the two types of ligases, and had some anti-TB activity [69••].

Genome analysis has revealed that in *M. tuberculosis* DNA gyrase is the only type II topoisomerase [70]. DNA gyrase has been validated as a target for anti-TB drug discovery, as its inhibition by fluoroquinolones results in highly mycobactericidal activity; this compound class has been used to treat multiple-drug-resistant TB. The *M. tuberculosis* enzyme has recently been overexpressed and purified, and is currently being used in *in vitro* inhibition assays to direct structure–activity relationships in the development of novel and more potent inhibitors [71].

Targeting the glyoxylate shunt

Isocitrate lyase (ICL) catalyzes the first step in the glyoxylate shunt, a carbon assimilatory pathway that allows the net synthesis of C4 dicarboxylic acids from C2 compounds such as acetate. In *M. tuberculosis*, the glyoxylate cycle is comprised of a single gene encoding malate synthase, but two genes encoding ICL [72]. The smaller gene encodes Icl1, an enzyme closely related to ICLs in other eubacteria; the larger gene encodes Icl2, a protein more homologous to eukaryotic isocitrate lyases. A recent study found that deletion of both *icl1* and *icl2* resulted in complete impairment of mycobacterial intracellular replication and rapid elimination from the lungs [73••]. The structure of ICL from *M. tuberculosis* has been solved in complex with the inhibitors 3-nitropropionate and 3-bromopyruvate [74].

Targeting regulatory proteins

Three regulatory proteins have been shown to be essential for growth of *M. tuberculosis*, including GlnE [75], MtrA [76] and IdeR [77], which encode the regulator of glutamine synthetase, a response regulator of a two-component regulator pair, and an iron-responsive regulator, respectively. Another two-component regulator, DosR, is essential for growth under conditions of low oxygen [78]. Inhibiting a regulatory protein would have the added downstream effect of disrupting a whole network of proteins under the influence of the regulator, giving one inhibitor a pleotropic effect.

Inhibiting menaquinone biosynthesis

If menaquinone is indeed the only quinone in *M. tuberculosis*, as current evidence suggests, its biosynthesis is essential for growth. Menaquinone biosynthesis has been studied in detail in *E. coli* [79], where the pathway is catalyzed by a series of enzymes including MenF, MenD, MenC, MenE, MenB, MenA and MenG. This pathway is not present in humans, who cannot synthesize menaquinone and must obtain it from diet or from gut-dwelling bacteria. The *M. tuberculosis* homologs of MenA–E and MenH have been described and their respective genes are clustered in one region of the genome.

Targeting the stringent response enzyme

The bacterial stringent response is characterized by a decrease in ribosomal RNA, transfer RNA and protein synthesis, modified RNA polymerase activities, diminished activity of some transport systems and decreased carbohydrate, amino acid and phospholipid metabolism. The stringent response is mediated by the rapid accumulation of hyper phosphorylated guanosine [(p)ppGpp], which interacts with RNA polymerase [80] and alters its interaction with mRNA through mechanisms that are still poorly understood. In *M. tuberculosis*, synthesis and hydrolysis of (p)ppGpp is carried out by a single enzyme, Rel_{Mtb}, [81]. The contribution of the stringent response and (p)ppGpp in *M. tuberculosis* cultures and in an animal infection model has been reported [82,83] suggesting that it is important during infection. The stringent response is presumably critical for adaptation to *in vivo* conditions.

Targeting ATP biosynthesis

The recent discovery of an ATP synthase inhibitor (R207910) validates ATP biosynthesis as a target for antibiotic discovery [84••].

Conclusions

The resilience of *M. tuberculosis* is manifest in the requirement for half a year of treatment with multiple drugs for active TB. This extended treatment leads to poor compliance, which promotes resistance development. The various antibiotics that constitute first- and second-line drugs for TB therapy target only a small number of essential functions in the organism; identification of further pathways that are required for bacterial growth should provide more (novel) targets for the rational design of effective antibiotics that shorten therapy and eliminate drug-resistant strains. The preceding discussion and Table 1 extend the list of target pathways with several enzymes that have been validated to varying degrees, from the demonstration of essentiality for growth to the identification of compounds that inhibit bacterial growth *in vitro* and/or during infection. As more targets are validated, a pattern will hopefully emerge that correlates the inhibited pathway with the shortening of therapy, and targets will be prioritized accordingly.

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The authors have accomplished heterologous protein expression and purification of the individual subunits and achieved the successful reconstitution of an essential acyl-CoA carboxylase from *M. tuberculosis*, whose main role appears to be the synthesis of methylmalonyl-CoA. The enzyme complex was reconstituted from the α-biotinylated subunit AccA3, the carboxyltransferase β subunit AccD5, and the ε subunit AccE5 (Rv3281). The kinetic properties of this enzyme showed a clear substrate preference for propionyl-CoA compared with acetyl-CoA (specificity constant fivefold higher), indicating that the main physiological role of this enzyme complex is to generate methylmalonyl-CoA for the biosynthesis of branched-chain fatty acids.

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DNA ligases that utilize NAD^+ are attractive drug targets because of the unique cofactor requirement for ligase activity. The authors report the crystal structure of the adenylation domain of the *M. tuberculosis* NAD^+-dependent ligase with bound AMP. They have identified a novel class of inhibitors for the enzyme using *in silico* docking calculations. The glycosyl ureide-based inhibitors were able to distinguish between NAD^+- and ATP-dependent ligases as evidenced by *in vitro* assays using T4 ligase and human DNA ligase I. The results can be used as the basis for rational design of novel antibacterial agents.

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The authors have identified a diarylquinoline, R207910, that inhibits growth of both drug-sensitive and drug-resistant *M. tuberculosis in vitro*. In mice, R207910 exceeded the bactericidal activities of isoniazid and rifampin by at least 1 log unit. Plasma levels associated with efficacy in mice were well tolerated in healthy human volunteers. Mutants selected *in vitro* suggest that the drug targets the proton pump of ATP synthase.