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"DRUGS IN TREATMENT OF TUBERCULOSIS: A REVIEW"

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KEYWORDS:

Drug Resistance, Mycobacteria, Genes, Ribosome, Clinical Trials, Minimum Inhibitory Concentration (MIC), Scaffolds.

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ABSTRACT

Recent years have shown a rise interest in the application of modern drug-discovery techniques to the field of TB, leading to an unprecedented number of new TB drug candidates in clinical trials. On downside, recent years also saw the application of many prejudices of modern drug development to anti-infective and antitubercular drugs. By some estimates there are some new agents approaching clinical trials for treating MDR and XDR disease. The current R&D pipeline for new TB drugs is inadequate to address emerging drug-resistant strains. Accordingly, filling the early drug development pipeline with novel therapies likely to slow additional drug resistance is urgent. Combination chemotherapy has been a standard of care for TB since 1950s, when it was shown that combining drugs slowed development of drug resistance, particularly for bactericidal compounds. Currently, anti-infective therapeutics are discovered and developed by either *de novo strategies*, or through extension of available chemical compounds that target protein families with the same or similar structures and functions. *De novo drug discovery* involves use of high throughput screening techniques to identify new compounds, both synthetic and natural, as novel drugs. Unfortunately, this approach has yielded very few successes in field of anti-infective drug discovery. Indeed, progression from early-stage biochemical *hits* to robust lead compounds is commonly an unfruitful process. This review is including various conventional First/Second-line antiTB agents along with some new leads under clinical trials which will be ready for use. The main objective of review is to introduce a part of antiTB agents to healthcare professionals.

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Abbreviations:

Tuberculosis (TB);

Mycobacterium Tuberculosis *(M.tb.);*

Multidrug Resistant Tuberculosis (MDR-TB);

Extensive Drug Resistant Tuberculosis (XDR-TB);

World Health Organization (WHO);

Non-Tuberculous Mycobacteria (NTM);

Pyrazinoic Acid (POA);

Adenosine Triphosphate (ATP)

Arabinogalactan (AG);

Lipoarabinomannan (LAM),

Deoxyribo Nucleic Acid (DNA),

Ribonucleic Acid (RNA),

Reactive Oxygen Species (ROS),

Early Bactericidal Activity (EBA);

Area Under Curve (AUC),

Fatty Acid Synthase (FAS);

Inhibitory Concentration (IC),

Colony Forming Unit (CFU);

Diversity Oriented Synthesis (DOS)

Strategy, Sulfuryl Transferase (ST); etc.

1. INTRODUCTION:

Tuberculosis, which is caused by the bacterial pathogen Mycobacterium tuberculosis (*M.tb.*), is a leading cause of mortality among infectious diseases. It has been estimated by the World Health Organization (WHO) that almost one-third of the world's population, around 2 billion people, is infected with the disease. Every year, more than 8 million people develop an active form of the disease, which subsequently claims the lives of nearly 2 million. This translates to over 4,900 deaths per day, and more than 95% of these are in developing countries. In 2002, the WHO estimated that if the worldwide spread of tuberculosis was left unchecked, then the disease would be responsible for approximately 36 million more deaths by the year 2020. Despite the current global situation, anti-tubercular drugs have remained largely unchanged over the last four decades. The widespread use of these agents, and the time needed to remove infection, has promoted the emergence of resistant *M.tb.* strains. Multi-drug resistant tuberculosis (MDR-TB) is defined as resistance to the first-line drugs Isoniazid and Rifampin. The effective treatment of MDR-TB necessitates the long-term use of second-line drug combinations, an unfortunate consequence of which is the emergence of extensively drug resistant tuberculosis (XDR-TB) – M.tuberculosis strains that are resistant to Isoniazid plus Rifampin, as well as key second-line drugs, such as ciprofloxacin and moxifloxacin. XDR-TB is extremely difficult to treat because the only remaining drug classes exhibit very low potency and high toxicity. The rise of XDR-TB around the world, including in industrialized nations, imposes a great threat on human health, therefore emphasizing the need to identify new anti-tubercular agents as an urgent priority.[1]

The development of new chemotherapeutic regimens capable of shortening the duration of tuberculosis (TB) treatment and/or improving the treatment of MDR-TB is a major research objective. Therapy for TB is arduous due to its long duration and the need to use multidrug regimens. The current standard regimen of isoniazid (*INH*), rifampin (*RIF*), and pyrazinamide (*PZA*) requires 6 to 8 months of daily treatment. In part due to noncompliance with treatment, therapy is now further complicated by the emergence of drug-resistant strains, with the global prevalence of drug resistance being from 1 to 3%. A further, equally important issue with tuberculosis therapy is the treatment of patients in which the infection may be in a latent state.

Supposedly, 1:3 people throughout the world harbor latent bacilli, which have the potential to reactivate and cause active disease. Current anti-TB drugs are mainly effective against replicating and metabolically active bacteria, and therefore, there is an urgent need for novel drugs that are also effective against persisting or latent bacterial infections, as well as those that can overcome the increasing problem of drug resistance.[2]

2. Conventional AntiTB First Line Drugs:

2.1 Isoniazid (*INH*): Isoniazid is considered primarily an antituberculosis agent, but it does have activity against several NTM species, including *M. kansasii* and *M. xenopi*. Isoniazid, or isonicotinic acid hydrazide (INH), is a prodrug that target organism must modify to its active form. The active derivatives of isoniazid are not fully characterized, but the main form is believed to be an isoniazidisoniazid- NAD adduct. Isoniazid-NAD forms from the interaction of INH and NAD+ in the presence of Mn^{2+} and O_2, which is catalyzed by the catalase-peroxidase KatG. Indeed, the lack of a suitable catalase-peroxidase to catalyze this process is the primary reason why most mycobacteria are not susceptible to isoniazid. The first insights into the mechanism of action of isoniazid were made over 3 decades ago with the demonstration that this agent inhibited mycolic acid synthesis. More recently, the primary target of activated isoniazid was identified as one of the fatty acid synthesis II (FAS II) enzymes, enoyl-acyl carrier protein (ACP) reductase, or InhA. The inhibition of InhA explains the primary effects of isoniazid on mycobacteria, and the crystal structure of the isoniazid-NAD adduct bound to InhA has been determined. There is evidence that activated isoniazid may target other proteins, including dihydrofolate reductase (DHFR) and the FAS II enzyme KasA. The role of these alternative targets in the antimycobacterial activity of activated isoniazid is still in question.[3]

2.2 Pyrazinamide (PZA): It is an important first-line tuberculosis (TB) drug used in combination with other TB drugs for treatment of both drug-susceptible TB. PZA is a peculiar persister drug that acts only on dormant non-growing persisters and has poor activity against growing *M.tb*. Its high activity against persister bacteria is responsible for PZA's unique sterilizing activity, which shortens the TB treatment period from 9–12 months to 6

months. Because of its indispensible sterilizing activity, all new TB regimens in clinical development include PZA. PZA is a prodrug that requires activation to its active form, pyrazinoic acid (POA), by an *M. tuberculosis* pyrazinamidase/nicotinamidase enzyme encoded by the pncA gene. Mutations in pncA are the major mechanism of PZA resistance in *M.tb*. However, some PZA-resistant strains do not have mutations in either the pncA or rpsA genes indicating the presence of a possible new resistance mechanism or target of PZA. A new gene, panD encoding aspartate decarboxylase and involved in β-alanine biosynthesis, mutations in which are associated with PZA resistance in *M.tb*. PanD is involved in synthesis of pantothenate (vitamin B5), which in turn is required for the synthesis of coenzyme A (CoA), a molecule that is at the center of all energy metabolism and allows carbohydrates, fats, and proteins to be burned as energy sources. However, how panD mutations cause PZA resistance and how PZA might interfere with pantothenate and CoA function are unclear. In an attempt to shed light on possible new targets of PZA, mutants of *M.tb*. resistant to POA, active form of PZA, and characterized mutations potentially involved in POA resistance. Whole-genome sequencing of select POA-resistant mutants without pncA or rpsA mutations together with targeted sequencing mapped all mutations in panD gene. Biochemical and genetic studies suggest that PanD is a new target involved in PZA action and resistance. Although RpsA (ribosomal protein S1, involved in trans-translation) has recently been shown to be a target of POA/PZA, whole-genome sequencing has identified mutations in panD gene encoding aspartate decarboxylase in PZA-resistant strains lacking pncA and rpsA mutations.[4]

2.3 Ethambutol (*ETH*): Although ethambutol is primarily an antituberculosis agent, it is a component of some regimens for infections with slowly growing mycobacteria. It is of limited use for rapidly growing mycobacteria and is considered inactive against other microorganisms. The direct effect of ethambutol on mycobacteria is a disruption of cell wall synthesis, in particular the inhibition of the synthesis of arabinogalactan (AG) and, to a lesser extent, lipoarabinomannan (LAM). A likely candidate for the actual molecular target of ethambutol is the arabinosyl transferase encoded by the *embB* gene; this has come largely

from analyses of mutations in the *emb* operon that confer resistance to ethambutol. Although the disruption of the synthesis of AG and LAM is believed to be the primary direct effect of ethambutol, evidence suggests that a secondary target of this agent may be spermine metabolism. Studies from more than 2 decades ago demonstrated that the growth inhibition caused by ethambutol could be reversed by the addition of spermidine, and the mycobacterial spermidine synthase enzyme is inhibited by ethambutol.[5]

2.4 Rifamycins: The rifamycins (e.g., rifampin and rifabutin) bind to the prokaryotic DNA-dependent RNA polymerases and are potent inhibitors of these enzymes. The RNA polymerase is comprised of five subunits, and the binding site of rifamycins is the β-subunit, which is the catalytic center of the enzyme. However, evidence suggests that rifampin (and presumably the other rifamycins) binds to the DNA/RNA channel rather than in the catalytic center of the β-subunit. Thus, rifamycins act by blocking the initiation of RNA synthesis; once the enzyme has loaded the DNA template and has begun RNA elongation, rifamycins are blocked from access to their binding site. In this respect, the mechanism of action for rifamycins has some similarity to that of macrolides. There is a significant variability in the susceptibilities of mycobacteria to the rifamycins, although much of this variability is believed to result from the impermeability of the mycobacterial cell wall, as the purified DNA-dependent RNA polymerases are sensitive to these agents.[6]

2.5 Aminoglycosides: Streptomycin was discovered in 1944 by *Waksman.* This was the result of a purposeful search among soil micro-organisms for antibiotic agents active against the Gram-negative bacteria and the (Gram +ve) mycobacteria, and suitable for clinical application. The antibiotic was originally found in one particular strain of *Streptomyces griseus* and indeed production has been noted in only a few of the other strains of this species since examined. The aminoglycosides inhibit protein synthesis by binding to the ribosome near the A site. This changes the state of the tRNA binding to theA site and also interferes with mRNA decoding, although some aminoglycosides can also inhibit the ribosomal translocation of tRNA. As a consequence of the interference of tRNA in the ribosome, aminoglycosides also disrupt the proofreading function of the A site, which can lead to

frameshift errors and the read through of stop codons, with an increase in the frequency of mistranslated proteins. Aminoglycosides target the ribosome by direct interaction with ribosomal RNA, and they affect protein synthesis by inducing codon misreading and by inhibiting translocation of the tRNA–mRNA complex. Their binding site is within a conserved loop of 16S rRNA helix 44, which is part of the small ribosomal subunit's aminoacyl-tRNA acceptor site (A-site). Mutagenesis studies have dissected the contribution of individual rRNA nucleotides to drug–ribosome interaction. The selective activity of these compounds (i.e., their preferential binding to the bacterial as opposed to the eukaryotic ribosome) is limited by the only minor sequence polymorphism present in the helix 44 drug-binding pocket. A major drawback to the clinical use of aminoglycosides has been their ototoxicity, *i.e.*, their ability to cause irreversible hearing damage, affecting ~20% of patients in brief courses of treatment. This ototoxicity is linked to the destruction of the sensory cells of the inner ear and consistently has been associated with both natural and semisynthetic aminoglycosides. Aminoglycoside ototoxicity occurs both in a sporadic dose-dependent manner in the general patient population and in an aggravated form in genetically susceptible individuals, the latter linked to mutations in mitochondrial ribosomal RNA (rRNA), in particular the transition mutation A1555G in the A-site of the mitoribosomal small subunit. Despite their toxicity, aminoglycosides are still among the most commonly used antibiotics worldwide because of their high efficacy, lack of drug-related allergy, and low cost. The mechanisms involved in aminoglycoside ototoxicity have been a matter of debate, but compelling evidence now suggests a causal involvement of reactive oxygen species (ROS). The origin of ROS generation remains unresolved, however, and both nonenzymatic and enzymatic mechanisms have been suggested. As seen from this side of the Atlantic, one might summarize the present status of streptomycin in tuberculosis as follows. This antibiotic seems more promising than any previous chemotherapeutic agent. In animals its toxicity is less than, and its effect on experimental infection better than, the best of the sulphone compounds. In man prolonged administration appears to have been free of serious and uncontrollable toxic reactions, though, as in animals, histamine-like responses, evidence of

(reversible) renal "irritation," eighth-nerve symptoms, neuritis, and dermatitis have been reported; it seems possible that some of these reactions are due to impurities in certain of the preparations used. The effect on human tuberculosis justifies cautious optimism for certain forms of the disease. A much greater possible disadvantage is that drug resistance is rather easily acquired by the tubercle bacillus and other susceptible micro-organisms, both *in vitro and in vivo* and this is particularly liable to occur during a prolonged course of treatment. This is one reason why other chemotherapeutic agents would be desirable for use in tuberculosis, even should streptomycin justify present hopes.[7-8]

3 Conventional AntiTB Second Line Drugs:

3.1 Glycopeptides: The glycopeptide agents in clinical use are vancomycin and teicoplanin. Although there have been some reported successes with vancomycin as an antimycobacterial agent in humans and animals, mycobacteria are usually considered to be resistant to this agent. Although direct studies of the action of vancomycin in mycobacteria are lacking, indirect evidence for the peptidoglycan precursors present in mycobacteria following exposure to these agents suggests that the mechanism of action of vancomycin is the same as that in other bacteria. Vancomycin binds to the D-alanine–D-alanine terminal amino acids of the peptide side chains of peptidoglycan and in doing so prevents the 4-3 cross-linking by the PBP D,L-transpeptidase and other processes in the final assembly of mature peptidoglycan.[9]

3.2 Fluoroquinolones: The primary target for the fluoroquinolones is the DNA gyrase, which relaxes the supercoiling of the DNA ahead of the DNA helicase and DNA replication complex, thus prevent DNA replication. The DNA gyrase is encoded by the *gyrA* and *gyrB* genes. As well as the DNA gyrase, the other bacterial type II topoisomerase, topoisomerase IV, is also a target of quinolones. The role of topoisomerase IV is to unlink the newly synthesized DNA strands so that the chromosomes can segregate during cell division, although this enzyme also has a function equivalent to that of the DNA gyrase. Intriguingly, pathogenic mycobacteria such as *M. abscessus*, *M. avium*, and *M. tuberculosis* appear to lack a topoisomerase IV ortholog, although the genome data for other species such as *M.*

smegmatis and *Mycobacterium vanbaalenii* suggest that they may have genes that encode this enzyme.[10]

3.3 Trimethoprim and sulfonamides: Trimethoprim and sulfonamides, such as sulfamethoxazole, are inhibitors of microbial folate metabolism. The disruption of this process leads directly to a reduction in the synthesis of the building blocks of nucleic acids and some amino acids. Although trimethoprim and sulfonamides interfere with the same process, the molecular targets of trimethoprim and sulfamethoxazole are different. Trimethoprim binds to and inhibits the dihydrofolate reductase (DHFR), inhibiting the conversion of dihydrofolate into tetrahydrofolate. The *dfrA* gene encodes the mycobacterial DHFR. Sulfonamides bind to the enzyme dihydropteroate synthetase (DHPS) and inhibit the conversion of 7,8-dihydro-6-hydroxy-methylpterin-pyrophosphate and *para*-aminobenzoic acid (PABA) to 7,8-dihydropteroate. In fact, sulfonamides are competitive antagonists of the binding of PABA to DHPS. Although chemically distinct from sulfonamides, the antileprosy agent dapsone is also an inhibitor of DHPS. Depending on the species, either the *folP1* gene or the *sulI* genes encode the mycobacterial type 1 DHPS. Although most of the data on the interaction of trimethoprim and sulfonamides come from nonmycobacterial species, several studies demonstrated that trimethoprim and sulfonamides bind to and inhibit the DHFR and DHPS of NTM.[11]

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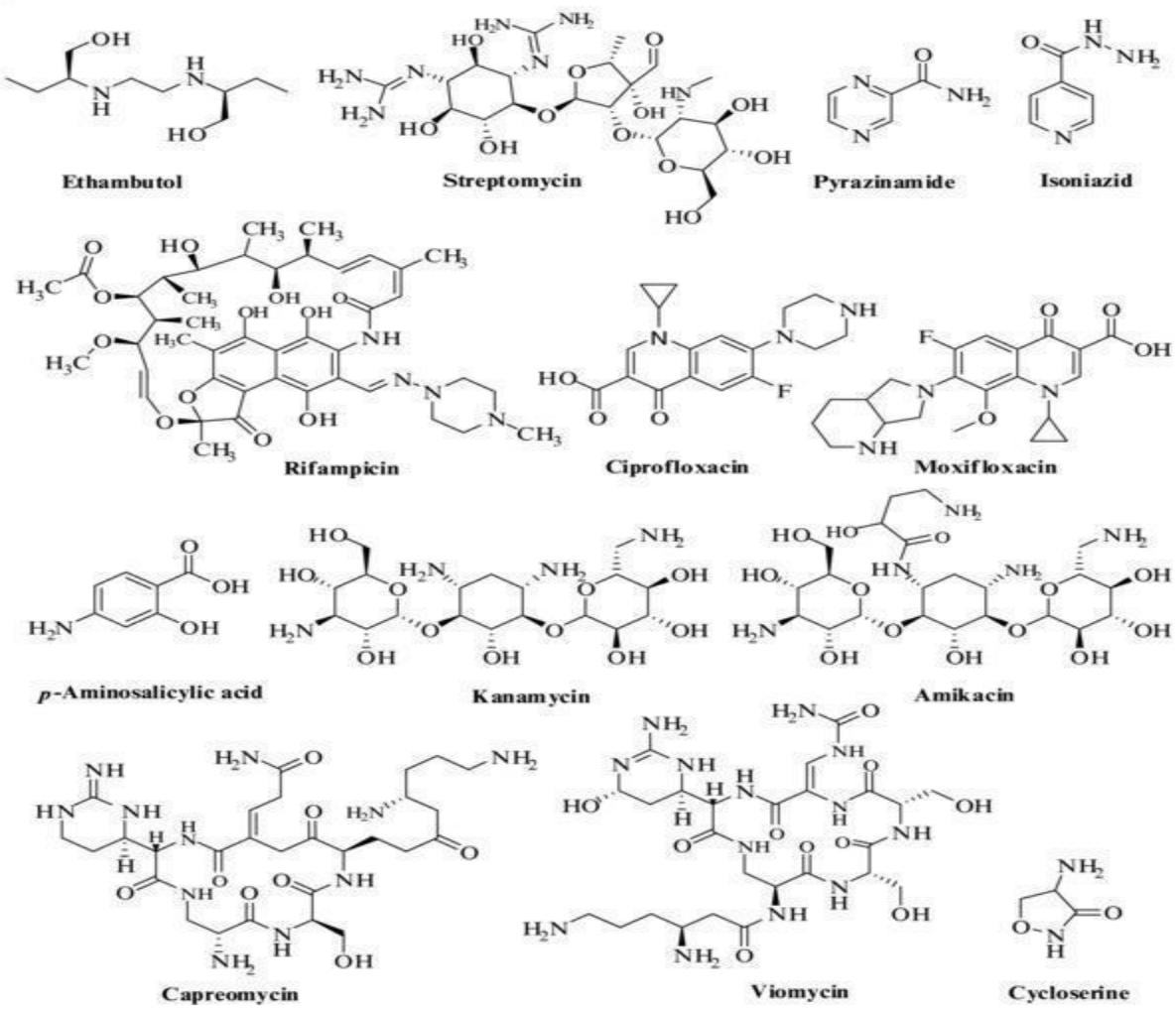

FIGURE: 1: "Chemical Structures of Some First Line and Second Line Antitubercular Agents."

3.4 Tetracyclines and glycylcyclines: The tetracyclines, such as tetracycline, doxycycline, and the glycylcyclines, such as tigecycline, bind to 30S subunit of ribosome and inhibit protein synthesis. Primarily, tetracyclines impede the access of tRNA to A site of the ribosome. Although tetracycline appears to be able to interact with ribosome at multiple points, the highest-affinity binding sites are consistent with the interference of the A site. Evidence indicates that the high-affinity binding of glycylcyclines to the 30S ribosomal subunit is the

same as, or overlaps, that of tetracycline, with additional interactions that may explain the higher affinity of tigecycline for the ribosome.[12]

3.5 Macrolides and ketolides. The macrolides and keto derivatives, ketolides, act by binding in peptide exit tunnel of the ribosome and thus preventing the growing peptide chain from vacating the peptidyltransferase center of the ribosome. This is believed to "gum up" ribosome and prevent the further elongation of the nascent peptide chain. Although the different agents may bind in slightly different places in the exit tunnel, the ultimate effect is the same.[13]

3.6 Bedaquiline: It is chemically belongs to diarylquinoline (DAR) compound and closely related to fluoroquinolones as well as chloroquine but with different side-chain moiety. Amongst all anti-TB drugs approved, Bedaquiline is the only drug, which targets the energy metabolism of mycobacteria. Adenosine triphosphate (ATP) is an essential molecule for survival of all cells including mycobacteria. Mycobacteria persisting in well-encapsulated lung cavities and in endosomes of macrophages can survive even under low oxygen molecular tension leading to resistance against standard TB drugs. Nevertheless, ATP production by ATP synthase is essential regardless the nature of mycobacteria like active or dormant, replicating or non-replicating, extracellular or intracellular and fermenting or non-fermenting. ATP synthase complex, the site of ATP production, is located in inner membrane of mycobacterial mitochondria. It is composed of two structural domains; membrane embedded F_0 domain and cytoplasmic located F_1 domain with four subunits (α, β_2 & δ_{10}, ε_{15}) and five subunits (α_3,β_3, γ,δ& ε) respectively. The F_0 domain with ε_{15} 'δ_{10}' subunits are arranged in the form of discs and the β subunit of F_1 domain has catalytic activity which combines one ADP with π to form ATP. Proton motive force generated across mitochondrial membrane by electron transfer chain, fuels the rotation of discs and results in activation of catalytic β subunit of F_1 domain resulting in ATP generation. Protonated form of Bedaquiline has high affinity for the ATP synthase complex. It specifically binds with a distinct position at the interface of 'a' and 'c' subunit in F_0 domain and blocks rotation of discs and thereby the entire process of ATP synthesis. *In vitro* studies also proved that binding affinity of

Bedaquiline is not influenced by excess proton, pH or other electrostatic interactions. Human ATP synthase complex have similar domain configuration with very narrow protein sequence similarity. This information can assure that Bedaquiline might be a safe drug in terms of pharmacodynamic aspects. Illustration of BDQ metabolism will provide valuable information to predict and prevent drug–drug interactions and adverse drug reactions associated with BDQ. CYP3A4 is involved in BDQ metabolism, leading to the formation of a dominant but less active. N-desmethyl metabolite: BDQ and its metabolites, including N-desmethyl BDQ, N-didesmethyl BDQ, and two hydroxyl metabolites, are mainly excreted via feces. However, limited information is available for bioactivation pathways of BDQ that may be related to BDQ toxicity. In addition, it is unknown whether CYP3A4 is the only cytochrome P450 (P450) that contributes to BDQ metabolism.[14-15]

3.7 The Sulphonamides and Sulphones: A more promising field was opened up by the introduction, from 1939 onwards, of sulphone compounds into anti-tuberculosis chemotherapeutic experiments; the published data on these agents up to the end of 1944 have been admirably reviewed in detail by *Tytler* (1944-5), and it will be seen that three have two phenyl rings linked by the sulphone (SO_2) group, while the fourth compound is somewhat different, having a phenyl and a thiazole group linked by the sulphone group. The starting-point of the series, diaminodiphenylsulphone, showed some inhibitory effect in infected rabbits and guineapigs, but its low water-solubility and high toxicity were bars to its clinical application. The more complex "promin" (promanide) was easily soluble and had a low enough toxicity to warrant extended trials in experimental tuberculosis. But when promin was tested in human pulmonary tuberculosis, although limited benefit was reported by some, the tolerance was found to be considerably lower than in the guinea-pig when the drug was given orally, unpleasant reactions-e g., a (reversible) anaemia occurring with some frequency; and while the toxic symptoms were much less evident after parenteral administration, so also was the clinical benefit. Experience with " diazone" in experimental tuberculosis in guinea-pigs resembled that with promin, but trials in human pulmonary disease produced conflicting reports, the majority of them unfavourable. "Promizole" also

gave encouraging results in experimental animals in the hands of Feldman, *Hinshaw, and Mann* (1944), who found it better tolerated by human beings than were the previously mentioned sulphone compounds.[16]

3.8 Carbapenems and cephalosporins. Carbapenems (e.g., imipenem and meropenem) and cephalosporins (e.g., cefoxitin and ceftriaxone), like other β-lactam antimicrobials, are inhibitors of peptidoglycan synthesis. The β-lactams bind to, and irreversibly inhibit, penicillin-binding proteins (PBP) or D,D-transpeptidases. The PBP are responsible for intermolecular peptide bridges and are required for formation of mature peptidoglycan from nascent peptidoglycan strands; these bridges are critical to structural integrity of peptidoglycan. More specifically, PBP catalyze the bridging between molecules from fourth amino acid (D-alanine) of one peptide side chain to third amino acid (*meso*-diaminopimelic acid) of an adjacent intermolecular peptide chain, forming a 4-3 cross-link. Although blocking of formation of the 4-3 cross-link leads to a disruption of the cell wall, a downstream effect of buildup of peptidoglycan fragments and precursors is activation of hydrolase autodigestion of mature peptidoglycan, further disrupting cell wall. In addition to the 4-3 cross-links, mycobacteria, like other bacteria, can also form 3-3 cross-links between the peptide side chains of the peptidoglycan. Such cross-links are catalyzed by D,L-transpeptidases and reflect a remodeling of mature peptidoglycan, and they may represent an adaptive response. As well as targeting the PBP, there is evidence that carbapenems bind to (and probably inhibit) the mycobacterial D,L-transpeptidases.[17]

3.9 Phenothiazine (PTZ), Thioridazine (THZ): PTZ/THZ has broad-spectrum antibacterial activity against Mtb. It appears to be equally active on starved Mtb, which represents the persistent state of pathogen, as it is during log phase growth. MICs for Phenothiazines are much higher than the corresponding values in macrophages, since THZ concentrates inside these host cells. The MICs in macrophages for inhibiting *M.tb.* growth have been reported as 0.23–3.6 mg/ml and 0.1 mg/ml. Equally significant is fact that at these concentrations, there were no cytotoxic effects on macrophages. Novel phenothiazine derivatives inhibited *M.tb.* in non-replicating state at MICs that were lower than those under actively growing conditions

as a 'macrophage modulator'. THZ significantly reduced number of CFU retrieved from lungs of mice infected with *Mtb* (10^6 CFU/ml, *i.p.)* within one month at a daily dose of 0.5 mg/day. Phenothiazines in general and THZ in particular, exert their anti-TB effects via calmodulin or by inhibiting $NADH_2$-menaquinoneoxidoreductase (Ndh2). *M.tb.* responds to changes in its environment by altering level of expression of critical genes that transduce such signals into metabolic changes favoring continued growth and survival. Sigma(s) factors are a class of transcription factors which control bacterial gene-expression. Typically most eubacteria encode a principal sigma factor and a variable number of alternate sigma factors, which control responses to specific environmental stimuli and adaptation to stress. The temporal expression of specific regulons controlled by one or more alternate sigma factors likely allows *M.tb.* to survive in multiple phases of TB. *M.tb.* sigma factors sH, sE and sB are well studied. The expression of latter can be triggered by either sH or sE. Thus, there exists a network of these three factors- sH, sE and sB, with overlapping functions. sH is induced after heat, redox, nitrosative and acid stress, and phagocytosis.[18] sH directly regulates transcription of genes, including these, sB, groEL/ES and thioredoxin regulons. sE is also induced upon uptake by macrophages, and upon treatment with hydrogen-peroxide, and SDS. sE regulates the expression of at least 23 genes, including sB, hsp and htpX. sE is transcribed in either a sH or an MprAB-dependent manner. sB is the minor principal sigma factor of *M.tb.* and appears to play a key role in defense against cell-envelope damage. The expression of these sigma factors is also altered by treatment with various anti-bacterial compounds, indicating that this sigma-factor network plays a role in defense against these anti-*M.tb.* mechanisms. System-wide screens can help predict specific pathways and targets that individual anti-bacterial compounds interact with. In turn, this has the potential to identify newer drug able target genes, pathways and networks. Identification of clusters of coordinately regulated genes for drug mechanism of action is a rational approach to selection of critical drug targets.[19]

Table: 1: Dosage, action and adverse reactions of first-line and selected second-line anti-TB drugs.

Drug	Dosage*	Role in the treatment strategy	Adverse reactions
First Line anti-TB drugs			
Isoniazid	5 (4–6), 300	• Bactericidal ; • Potent early bactericidal activity.	• Hepatotoxicity • Rash • Neuropathy • Lupus-like reaction • Anemia
Rifampicin	10 (8–12), 600	• Most important anti-TB drug • Bactericidal and sterilizing, rapidly rendering the patient non-infective.	• Hepatotoxicity • Rash • Hematologic
Pyrazinamide	25 (20–30), 2000	• Bactericidal early effect • Allows shortening of therapy by 3 months in DS-TB	• Hepatotoxicity • Rash • Arthralgias
Ethambutol	15 (15–20), 1600	• Bacteriostatic • The least effective drug, but protects against resistance	• Optic neuritis (can cause blindness) • Rash
Second Line anti-TB drugs			
Streptomycin	15, 1000	• Bactericidal	• Nephrotoxicity • Vestibular toxicity • Ototoxicity
Kanamycin	15, 1000	• Bactericidal	• Nephrotoxicity • Vestibular toxicity • Ototoxicity
Levofloxacin	750–1000	• Strong anti-TB activity	• Nausea • Tremulousness • Insomnia • Arthralgias • Rare QT_C prolongation, • tendon rupture
Ethionamide	15 (15–20), 1000 frequently divided	• Bacteriostatic	• Gastrointestinal upset and nausea • Hepatotoxicity • Reversible hypothyroidism

***Dosage: Daily dose and range (mg/kg bodyweight), maximum dose (mg).**
Abbreviation: DS-TB, drug-susceptible TB.

4 Newer Chemical AntiTB Agents which are under Clinical Trial/through Trials:

4.1 PA-824: It is one of two nitroimidazoles in phase II clinical trials to treat tuberculosis. In mice, it has dose-dependent early bactericidal and sterilizing activity. PA-824 inhibits synthesis of ketomycolates, an essential component of the mycobacterial cell wall. By analogy with other cell wall synthesis inhibitors, including Isoniazid and Ethionamide, this mechanism may explain bactericidal activity against actively multiplying bacilli but seems unlikely to explain activity against nonreplicating bacilli. The activity of PA-824 against slowly or nonreplicating bacilli was recently attributed to its capacity to donate nitric oxide during enzymatic nitroreduction within the *tubercle bacillus* and thereby poison the respiratory apparatus. In humans with tuberculosis, PA-824 demonstrated early bactericidal activity (EBA) at doses ranging from 200 to 1,200 mg per day, but no dose-response effect was observed.[20] When the data set was limited to regimens with dosing intervals of <72 h, both the *T*>MIC and the AUC/MIC values fit the data well. Free drug *T*>MIC of 22, 48, and 77% were associated with bacteriostasis, a 1-log kill, and a 1.59-log kill (or 80% of the maximum observed effect), respectively. Human pharmacodynamic simulations based on phase I data predict 200 mg/day produces free drug *T*>MIC values near the target for maximal observed bactericidal effect. *T*>MIC, in conjunction with AUC/MIC, is the parameter on which dose optimization of PA-824 should be based. Earlier reports regarding the *in vitro and in vivo* efficacies of the nitroimidazopyran PA-824 against *M.tb*. PA-824 was tested in vitro against a broad panel of multidrugresistant clinical isolates and was found to be highly active against all isolates (MIC < 1 µg/ml). The activity of PA-824 against *M.tb.* was also assessed grown under conditions of oxygen depletion. PA-824 showed significant activity at 2, 10, and 50 µg/ml, similar to that of metronidazole, in a dose-dependent manner.[21]

4.2 β-Sulfonyl carboxamides: Potential new targets for antimycobacterial drug development may exist among the synthetic enzymes needed to make the unique lipids produced by mycobacteria, such as mycolic acids. These high-molecular-weight, α-alkyl, β-hydroxy fatty

acids comprise the single largest component of the mycobacterial cell envelope. They are found in free lipids as trehalose mono- and dimycolate and esterified to the arabinogalactan matrix of the mycobacterial cell wall. They are vital for the growth and survival of mycobacteria, as evidenced by the bactericidal properties of mycolic acid inhibitory drugs, such as isoniazid and ethionamide. Synthesis of mycolic acids and other mycobacterial lipids requires a variety of fatty acid synthase and elongation enzymes. Although the synthesis of fatty acids is essentially the same at the primary chemical level, fatty acid synthases (FAS) are organized into two types. In Type I FAS (FAS I), most often found in eukaryotes, the individual enzymatic reactions are contained in one multienzyme complex. In Type II FAS (FAS II), commonly found in prokaryotes, the enzyme functions are carried out by seven individual proteins. Mycobacteria are known to possess both FAS I and II. Thus, inhibition of these enzymes, especially those involved in chain elongation of unique mycobacterial fatty acids, may provide novel targets for drug design.[22] It have been proposed to serve as transition-state analogues of the β ketoacyl synthase reaction involved in fatty acid elongation. The efficacy of *N*- octanesulfonylacetamide (OSA) as an inhibitor of fatty acid and mycolic acid biosynthesis in mycobacteria, using the BACTEC radiometric growth system, observed that OSA inhibits the growth of several species of slow-growing mycobacteria, including *M.tb.* (H37Rv and clinical isolates), the *M. avium* complex (MAC), *M. bovis* BCG, *M. kansasii*, and others. Nearly all species and strains tested, including isoniazid and multidrug resistant isolates of *M. tuberculosis*, were susceptible to OSA, with MICs ranging from 6.25 to 12.5 mg/ml. Only three clinical isolates of *M. tuberculosis* (CSU93, OT2724, and 401296), MAC, and *Mycobacterium paratuberculosis* required an OSA MIC higher than 25.0 mg/ml. Rapid-growing mycobacterial species, such as *Mycobacterium smegmatis, Mycobacterium fortuitum*, and others, were not susceptible at concentrations of up to 100 mg/ml. A 2-dimensional thin-layer chromatography system showed that OSA treatment resulted in a significant decrease in all species of mycolic acids present in BCG. In contrast, mycolic acids in *M. smegmatis* were relatively unaffected following exposure to OSA. Other lipids, including polar and nonpolar extractable classes,

were unchanged following exposure to OSA in both BCG and *M. smegmatis*. OSA inhibits the growth of several species of mycobacteria, including both isoniazid-resistant and multidrug resistant strains of *M. tuberculosis*. This inhibition may be the result of OSA-mediated effects on mycolic acid synthesis in slow-growing mycobacteria or inhibition via an undescribed mechanism. These indicate that OSA may serve as a promising lead compound for future antituberculous drug development.[23]

4.3 AZD5847: It is a novel oxazolidinone, demonstrates improved *in vitro* bactericidal activity against both extracellular and intracellular *M. tuberculosis* compared to that of linezolid. Killing kinetics in broth media and in macrophages indicate that, rate and extent of kill obtained with AZD5847 are superior to those obtained with linezolid. Moreover, the efficacy of AZD5847 was additive when tested along with a variety of conventional TB agents, indicating that AZD5847 may function well in combination therapies. AZD5847 appears to function similarly to linezolid through impairment of the mycobacterial 50S ribosomal subunit. Future studies should be undertaken to further characterize the pharmacodynamics and pharmacokinetics of AZD5847 in both *in vitro* and animal models as well is in human clinical trials.[24]

4.4 Pyrrolamide: Some authors have identified novel inhibitors in the pyrrolamide class which kill *M.tb.* through inhibition of ATPase activity catalyzed by the GyrB domain of DNA gyrase. A homology model of the *M.tb.* H37Rv GyrB domain was used for deciphering the structure-activity relationship and binding interactions of inhibitors with mycobacterial GyrB enzyme. Proposed binding interactions were later confirmed through cocrystal structure studies with the *M. smegmatis* GyrB ATPase domain. The most potent compound in this series inhibited supercoiling activity of DNA gyrase with a 50% inhibitory concentration (IC_{50}) of<5 nM, an MIC of 0.03 µg/ml against *M.tb.* H37Rv, and an MIC_{90} of <0.25 µg/ml against drug-resistant clinical isolates of *M.tb*. The best compound tested for *in vivo* efficacy in the mouse model showed a 1.1-log reduction in lung CFU in the acute model and a 0.7-log reduction in the chronic model. This class of GyrB inhibitors could be developed as novel anti-TB agents.[25]

4.5 Win 57273: A new quinolone, Win 57273 [1-cyclopropyl-7-(2,6-dimethyl-4-pyridinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolonecarboxylic acid], synthesized by Sterling Research Group, was tested *in vitro* against *M.tb. and M. avium* strains. The broth-determined MICs of this agent ranged from 1.0 to 4.0 µg/ml for *M.tb.* strains and from 0.25 to 8.0 µg/ml for *M. avium* strains. A distinctive feature of this agent, in comparison with ofloxacin and ciprofloxacin, is its substantially greater activity at the low pHs. For *M. avium* strains, the MICs of Win 57273 were 2.0 µg/ml or less for 54.5% of strains at pH 6.8 and 85.5% of strains at pH 5.0. Win 57273 was more active than ciprofloxacin against *M. avium* strains, and this difference was very substantial for all *M. avium* strains at pH 5.0. Taking into account that the predominant locations of these organisms *in vivo* are within the phagosomes and phagolysosomes of macrophages, *i.e.*, in acidic environments at pH 5.0 or lower, the greater activity of Win 57273 at low pH makes this quinolone especially promising for M. avium infection. The bactericidal activity of Win 57273 for M. avium strains was the same as that of ciprofloxacin, with MBCs from 4.0 to 16.0 µg/ml.[26]

4.6 1,4-di-*N*-oxide quinoxaline: Earlier reports regarding the *in vitro* efficacies of the 1,4-di-*N*-oxide quinoxaline derivatives against *M.tb.* and has led to discovery of a derivative with *in vivo* efficacy in the mouse model of tuberculosis. Quinoxaline-2-carboxylate 1,4-di-*N*-oxide derivatives were tested *in vitro* against a broad panel of single-drug-resistant *M.tb.* strains. The susceptibilities of these strains to some compounds were comparable to those of strain H37Rv, as indicated by the ratios of MICs for resistant and nonresistant strains, supporting the premise that 1,4-di-*N*-oxide quinoxaline derivatives have a novel mode of action unrelated to those of the currently used antitubercular drugs. One compound, ethyl 7-chloro-3-methylquinoxaline-2-carboxylate 1,4-dioxide, was found to be (i) active in reducing CFU counts in both lungs and spleens of infected mice following oral administration, (ii) active against PA-824-resistant *M.bovis*, indicating that the pathway of bioreduction/activation is different from that of PA-824 (a bioreduced nitroimidazole that is in clinical trials), and (iii)

very active against nonreplicating bacteria adapted to low-oxygen conditions. These data indicate that 1,4-di-*N*-oxide quinoxalines hold promise for treatment of tuberculosis.[27]

4.7 SQ641: Capuramycin (CM) is a novel nucleoside antibiotic that specifically inhibits biosynthesis of peptidoglycan (PG) by blocking the translocase I (TL1) enzyme. SQ641 is the most potent analogue in this series for *in vitro* activity against several species of mycobacteria. SQ641 is rapidly bactericidal for *M.tb.* and has a lasting postantibiotic effect (PAE). It is synergistic with *EMB* and additive with *INH*. Despite its excellent *in vitro* activity, SQ641 possesses several deficiencies that impair its therapeutic efficacy against TB. it is poorly soluble in water and is not absorbed orally. As a result, it has poor intracellular and *in vivo* activity. To overcome these deficiencies, some authors developed α-tocopheryl, polyethylene glycol 1000 succinate (TPGS) soluble and micellar formulations of SQ641. A phospholipid-based nanoemulsion formulation of SQ641 (SQ641-NE) was active against intracellular *M.tb.* in J774A.1 mouse macrophages, although SQ641 by itself was not. Intravenous (i.v.) SQ641-NE was cleared from circulation and reached peak concentrations in lung and spleen in 1 h. and were generally bacteriostatic in lungs.[28]

4.8 Isoxyl (ISO): (4,49-diisoamyloxydiphenylthiourea; 4,49-diisoamyloxythiocarbanilide; thiocarlide) is an old drug used for the clinical treatment of tuberculosis in the 1960s. *Urbancik and Titscher* demonstrated modest therapeutic efficacy of ISO monotherapy in cases of untreated pulmonary tuberculosis of various degrees of difficulty. Schmid concluded that combined INH and ISO was more effective than monotherapy with either drug. It had been noted in the early 1950s that ISO exhibited strong antimycobacterial activity in vitro. A note from Winder et al. in 1971 showed that, like INH and ETH, ISO strongly inhibited mycolic acid synthesis in *M. bovis* during 6 h of exposure to 10 mg/ml. ISO also partially inhibited the synthesis of the fatty acids of free lipids, which were stimulated by INH and ETH. Isoxyl (ISO), a thiourea (thiocarlide; 4,4*-diisoamyloxythiocarbanilide), demonstrated potent activity against *M.tb.* H37Rv (MIC, 2.5 mg/ml), *M.bovis* BCG (MIC, 0.5 mg/ml), *M.avium* (MIC, 2.0 mg/ml), and *M.aurum* (MIC, 2.0 mg/ml), resulting in complete inhibition of mycobacteria grown on solid media. Importantly, a panel of clinical isolates of *M.tb.* from

different geographical areas with various drug resistance patterns were all sensitive to ISO in the range of 1 to 10 mg/ml. In a murine macrophage model, ISO exhibited bactericidal killing of viable intracellular *M.tb.* in a dose-dependent manner (0.05 to 2.50 mg/ml). At its MIC for *M.tb.*, ISO inhibited the synthesis of both fatty acids and mycolic acids (a-mycolates by 91.6%, methoxymycolates by 94.3%, and ketomycolates by 91.1%); at its MIC in *M. bovis* BCG, ISO inhibited the synthesis of α-mycolates by 87.2% and that of ketomycolates by 88.5%; and the corresponding inhibitions for *M. aurum* A1 were 87.1% for α-mycolates, 87.2% for ketomycolates, and 86.5% for the wax-ester mycolates. Thus, these thioureas, like INH and ETH, specifically inhibit mycolic acid synthesis and show promise in counteracting a wide variety of drug-sensitive and -resistant strains of *M.tb*.[29]

4.9 Levofloxacin Derivative: LVFX is a representative new quinolone which is characterized by its potency, safety, and good pharmacokinetic profiles in humans. This agent has a unique pyridobenzoxazine structure. The synthesis of pyridobenzoxazines bearing a series of 3-aminopyrrolidinyl substituents at the C-10 positon and evaluated their activities agaisnt gram-negative and -positive bacteria. A compound with a 3-aminopyrrolidinyl group had one-half the activity of LVFX against *M. avium*, *M. intracellulare*, and *M.tb.* Mono- and dimethylation of the 3-amino moiety of the pyrrolidinyl group increased the activities against *M. avium* and *M. intracellulare* but not those against *M.tb*. On the other hand, dialkylation at the C-4 position of the 3-aminopyrrolidinyl group enhanced the activities against *M. avium*, *M. intracellulare*, and *M.tb*. Thus, introduction of an *N*-alkyl or a *C*-alkyl group(s) into the 3-aminopyrrolidinyl group may contribute to an increase in potency against *M. avium*, *M. intracellulare*, and/or *M.tb.*, probably through elevation of the lipophilicity.[30]

4.10 Clofazimine & its Derivative: The several riminophenazine compounds screened, only CFM, B746, B4100, B4101, B4154, and B4157 were found to show promising activity against tubercle bacilli. New analogs showed better *in vitro* activity than CFM; however, B4100 and B4101 were found to be less active in macrophage and animal models; consequently, they were not investigated further. The chemotherapeutic activity of B746 against MAC and *M.tb.*, which was comparable to that of CFM. The *in vitro*, intracellular,

and *in vivo* activities of CFM and its two most active analogs, B4154 and B4157. The investigated clofazimine (CFM) and two of its analogs, B4154 and B4157, for their antituberculosis activities have been tested. All of the strains were found to be susceptible to B4154 and B4157, and one strain showed moderate resistance to CFM. The MICs of B4154, B4157, and CFM at which 90% of strains were inhibited were 0.25, 0.12, and <1.0 mg/ml, respectively. The intracellular activities of CFM and B4157 were superior to that of B4154. The chemotherapeutic activities of the three compounds were evaluated in C57BL/6 mice. At a dose of 20 mg/kg of body weight, the activity of CFM was slightly superior to that of B4157; however, both compounds prevented mortality and caused a significant reduction in the numbers of CFU in the lungs and spleens. The animals treated with B4157 showed less pigmentation than animals treated with CFM. The chemotherapeutic activity of CFM was comparable to those of rifampin and isoniazid. Complete susceptibility of multidrug-resistant strains to CFM and B4157 and the therapeutic efficacies of these compounds against mouse tuberculosis make these drugs attractive agents for treatment of drug-resistant tuberculosis. **[31]**

4.11 Clarithromycin: Antituberculosis activity of clarithromycin (CLA), a macrolide antibiotic, showed high *in vitro* MICs (4 to > 16 mg/ml) for several strains of *M.tb.* and caused slight enhancement of activity of *RIF* against *H37Rv* but failed to increase the activity of either *RIF* or *INH* against other strains. However, inside J774A.1 macrophages, CLA showed high activity and was synergistic with RIF against some strains of *tubercle bacilli* susceptible or resistant to INH and RIF. The *in vivo* studies with a drug-susceptible strain (*H37Rv*), *CLA* protected mice from mortality due to tuberculosis for up to 8 weeks of observation. The CFU data for lungs and spleens revealed that the antituberculosis activity of *CLA* is inferior to those of *INH* and *STM*. However, the activity of *CLA* when used alone or in combination was comparable to that of thiacetazone, indicating its potential usefulness as a secondary drug for the treatment of tuberculosis.**[32]**

4.12 Substituted Quinolones: The screening library contained a small set of 6-fluoroquinolones and 6- fluoronaphthyridinones that are structurally related to established

inhibitors of bacterial DNA gyrases and topoisomerase IV. It is well established that *M. tb* is very sensitive to the newer generation quinolones. These compounds have all the hallmarks of newer generation DNA gyrase inhibitors including the 3-COOH group, a 6-F substitution, as well as a basic piperazine moiety at the 7-position. It also shows a number of interesting quinolones that diverge from the typical structure associated with DNA gyrase inhibition, and the basic scaffold shows high, selective activity that should be pursued. In particular, it is an example of a newer generation naphthyridone DNA gyrase inhibitor (cf. gemifloxacin and trovaloxacin). Both are alternative active amides related to inhibitors, although it is not clear if these are prodrugs for a typical 3-COOH quinolone antibiotic or whether they are acting through an alternative mechanism. Quinolone carboxamides have been reported to show antibacterial activity, although the mechanism of action was not clarified.[33]

4.13 Substituted Pyrimidines: The SMR screening set contained over 10,000 variously substituted simple and fused pyrimidines. Approximately 190 of these compounds showed good-to-modest activity in the bacterial growth assay, and only a small number of these samples were deemed both active and selective; for the most part, the active compounds also showed significant toxicity yielding. The small numbers of samples in these focused sets do not lend themselves to a through structure-activity relationship (SAR) discussion. *For example*, the combination of a 2-(3,5-dimethylpyrazol-1-yl) group with various 4-phenylamino and 4-cycloakylamino and 5-carboxyethyl substituents appears to show higher activity/selectivity ratios than closely related pyrimidines that are substituted with 2-(2-pyridyl), 4-phenylthio, and 5-methoxy groups; the latter set being less active and more toxic, yielding selectivity ratios on the order of 1.0. This trend does not hold for other similar 2-substituted quinazolines (2-methyl, 2-cyclobutyl, or 2-(thiophen-2-yl)) that show relatively similar toxicity, but have relatively poor TB IC90 activity values. Substituted pyrimidines and quinazolines have been reported to show a variety of antibacterial activities including antimycobacterial activity. Derivatives of 2-aryl-3- aminoquinazoline-4(3H)-ones have been reported that show good antibacterial and antitubercular activity.[34]

4.14 1,3-Diaryl-4-substituted Pyrazoles: The ratio of the Vero cell cytotoxicity to the anti-TB activity of >10 (IC_{90}). However, phenylpyrazoles have previously been evaluated for biological activity. *For example*, a phenylpyrazole has been evaluated as an inhibitor of indoleamine 2,3-dioxygenase, but found not to be active. Also, a series of 3-(4-phenyoxyphenyl) pyrazoles was studied as a novel class of sodium channel blocker in the rat Chung neuropathy paradigm.

4.15 1,3,4-Oxadiazoles: Of 2-substituted thio-5-aryl-1,3,4-oxadiazoles, with TB IC90 ≤10 μM. D-R testing of these compounds showed that eight had at least modest selectivity with SI >10. The most active was compound with SI of 38. There were four additional 2-alkylthio-5-aryl-1,3,4-oxadiazoles containing the general structure depicted all of which had TB IC90 < 4 μM and SI > 10. Interestingly, the two most active examples also had the same thiophene-containing 2-alkythio side chain while the two least active representatives shared the analogous furan-containing 2-alkylthio side chain. Unfortunately, since each compound differed structurally in their respective 5-aryl substituent, it is not possible to definitively conclude whether their differing activities are due to the thiophene-furan modification.[35]

4.16 2-Carboxamido-1,3,4-oxadiazoles and Related Compounds: The screening set contained acylated 2-amino-1,3,4-oxadiazoles, showed enough activity in the single-dose primary assay to warrant further evaluation in the dose-response format with a TB $IC_{90} \leq 20$ μM. Though some compounds possessed unacceptable toxicity, many displayed an SI >20. In these compounds the 5-position was invariably substituted, and good activity was observed with a diversity of alkyl, aryl, and heterocyclic groups. The closely related 2-amino-1,3,4-thiadiazoles were highly represented in the screening set, but were much less active in general. The activity cutoff for dose-response screening, but of those, were found active with an TB IC_{90} <= 20 μM. In general the thiadiazoles appeared to be more toxic than their oxygen counterparts, but some were marginally selective. The range of 5- substituents was much smaller among the thiadiazole actives, generally restricted to small alkyl or alkylthio moieties. It should be noted that similar compounds have previously been reported to possess antituberculosis and other antibacterial activities.[36]

4.17 1,2,4-Thiadiazoles: 3-aryl-5-thioacetamide-substituted 1,2,4-thiadiazoles that were tested in D-R , five possessed TB IC_{90} <10μM. Two compounds also displayed SI >10, and thus can be considered potential leads. However, similar compounds have been reported as bactericidal against *M.tb.* using a paper-disk diffusion method.

4.18 Tetrahydropyrazolo[1,5-a]pyrimidines: 5-phenyl-4,5,6,7-tetrahydro[1,5-*a*]pyrimidine contained an aryl group at the 5-position and a trifluoromethyl group at the 7-position, a carboxamide function is present at the 2 and 3 position. Of these compounds, displayed TB IC_{90} values <10 μM.[37]

5 Low Selectivity Scaffolds under Preclinical Investigation: Several classes of compounds were identified that possess good anti-TB activity, but had poor selectivity. Consequently, it is possible that through further synthetic work non-selective toxicities can be disentangled from desired activity through iterative synthesis/testing, and some of more interesting of these non-selective scaffolds as potential leads are discussed below.

5.1 3-Phenylpyrazolo[1,5-a]pyrimidines: Among compounds possessing a pyrazolo[1,5-*a*]pyrimidine template, a group of compounds possessing a phenyl substituent at 3-position and an amine or hydroxyl substituent at 7-position displayed moderate activity against *M. tb*.

5.2 2,5-Disubstituted Thiazolidin-4-ones: The literature shows numerous examples of 4-thiazolidinones being studied as antitubercular agents possessed TB IC_{90} of <10 μM. A few derivatives were reported to inhibit the growth of H37Rv at a concentration of 12.5 μg/mL.

5.3 4(5)-Phenylacetylimidazole-5(4)-carboxamides: Two compounds from this series, had TB IC90 <10 μM. Since neither had SIs >10 adequate selectivity, additional work is required before assessing the potential of this class of compounds.

5.4 Imidazo[1,2-a]pyridine-3-amines: The library contained 27 imidazo[1,2-*a*]pyridines of generic structure wherein R_1 is a phenyl or pyridyl ring system. Of these 27 compounds, 12 compounds had the 2-pyridyl ring system as the aryl substituent. In the D-R assay, seven agents from this set displayed TB IC_{90} values in the range of 1.5–4.4 μM.

5.5 5-Nitrofuran-2-carboxamides: Amides derived from 5-nitrofuran-2-carboxylic acids have emerged as a class of compounds that display potent antitubercular activity possessing TB IC90 <10 μM.

5.6 Amides of 3-(Trifluoromethyl)-4-(piperazinylmethyl)aniline: A set of amides derived from 3-(trifluoromethyl)-4-(piperazinylmethyl)aniline by acylation with 3-(acylamino)benzoic acids possessing TB IC90 values between 2.8–6.7μM.[38]

5.7 Cationic antimicrobial peptides (AMPs): It have been proposed as a potential new class of antibiotics with ability to kill target cells rapidly. Also it is thought that, development of resistance to membrane active peptides whose sole target is cytoplasmic membrane is considerably reduced. The factors important for α-helical AMPs to have the desired properties of a clinical therapeutic to treat bacterial infections include following: (1) an amphipathic nature with a non-polar face and a polar/charged face; (2) presence of high number of positively charged residues on polar face and an overall net positive charge; (3) an optimum overall hydrophobicity; (4) importance of lack of structure in aqueous conditions but inducible structure in presence of hydrophobic environment of membrane; (5) the presence of "specificity determinant(s)", that is, positively charged residue(s) in center of non-polar face which serve as determinant(s) of specificity between prokaryotic and eukaryotic cell membranes. These "specificity determinant(s)" reduce or eliminate toxicity (as measured by hemolytic activity against human red blood cells) by decreasing or eliminating transmembrane penetration into eukaryotic membranes but allowing antimicrobial peptide access to interface region of prokaryotic membranes; (6) the importance of reducing peptide self-association in aqueous environment which allows monomeric unstructured peptide to more easily pass through cell wall components to reach bacterial cytoplasmic membrane; (7) the sole target for peptide should be bacterial membrane and peptide should not be involved in any stereoselective interaction with chiral enzymes or lipids or protein receptors; (8) the peptides should be prepared in the all D-conformation to provide resistance to proteolysis; and (9) the extent of binding to serum proteins must be modulated since only unbound peptide is available to interact with bacterial target.

Antimicrobial peptides whose sole target is the cytoplasmic membrane must pass through the capsule and bacterial cell wall of *M.tb.* to reach the membrane and must be resistant to proteases in the capsule. The 37-residue peptide LL37, the only human member of the *cathelicidin* family of antimicrobial peptides, is considered to play an important role in innate immune response to *M. tuberculosis* infection. According to *Martineau et al*., synthetic peptide LL37 induced a dose dependent reduction in CFU (colony forming unit)/mL of *M. tuberculosis* H37Rv strain in iron-depleted broth (10 nM Fe, 7H9 medium). At 100 μg/mL, the CFU/mL was reduced from around 10^8 to 10^7 in 7 days. A series of 26-residue, amphipathic α-helical antimicrobial peptides consisting of all D-amino acid residues and synthetic human L-LL37 (L-enantiomer) and D-LL37 (D-enantiomer) were investigated against *M. tuberculosis* susceptible strain (H37Rv). Minimal inhibitory concentrations (MICs) were determined through a peptide killing assay. D5, the most active analog against *M. tuberculosis* had a MIC value of 11.2 μM (35.2μg/ml) against H37Rv strain and 15.6 μM (49μg/ml) against the MDR strain. Peptide D1 had similar activity as D5 against the MDR strain (57μg/mL), a 9-fold improvement in hemolytic activity and a 7.4-fold better therapeutic index compared to D5. Surprisingly, LL37 enantiomers showed little to no activity compared to the *de-novo* designed α-helical antimicrobial peptides.[39-40]

5.8 Benzimidazole Derivative: A novel benzimidazoles were synthesized by a 4-step reaction starting from 4-fluoro-3-nitrobenzoic acid under relatively mild reaction conditions. The synthesized compounds were screened for their antimycobacterial activity against *M. tuberculosis H37Rv* and displayed good activity with MIC of less than 0.2 μM. Compound ethyl-1-(2-(4-(4-(ethoxycarbonyl)-2-aminophenyl)piperazin-1-yl)ethyl)-2(4(5(4fluorophenyl) pyridin-3-ylphenyl-1H-benzo[d]imidazole-5-carboxylate was found to be most active with MIC of 0.112 μM against *H37Rv*.[41]

5.9 Thiophene Derivatives: A new class of thiophene (TP) compounds that kill *Mycobacterium tuberculosis* (*Mtb*) by the novel mechanism of Pks13 inhibition. An F79S mutation near the catalytic Ser-55 site in Pks13 conferred TP-resistance in *M.tb*. Over-expression of wild-type *pks13* resulted in TP-resistance and over-expression of the F79S *pks13* mutant conferred

high-level resistance. *In vitro*, TP inhibited fatty acyl-AMP loading onto Pks13. TP inhibited mycolic acid biosynthesis in wild-type *Mtb*, but to a much lesser extent in TP-resistant *M.tb*. TP treatment was bactericidal and equivalent to the first-line drug isoniazid, but it was less likely to permit emergent resistance. Combined isoniazid and TP treatment exhibited sterilizing activity. Computational-docking identified a possible TP-binding groove within the Pks13 ACP domain. This confirms that *Mtb* Pks13 is required for mycolic acid biosynthesis, validates it as a druggable target and demonstrates the therapeutic potential of simultaneously inhibiting multiple targets in the same biosynthetic pathway. Pks13 plays a critical role in mycolic acid biosynthesis in *M.tb.* by joining the α-alkyl C26 fatty acid branch (originating from FAS-I) and the meromycolic acid (C48–C64)13 branch (originating from FAS-II) activated by FadD3221 through Claisen-type condensation reaction to form α-alkyl β-ketoacids. Two inhibitors of this class were used to decipher the microbiological and biochemical consequences of Pks13 inhibition, to explore their potential as drug leads, and to characterize the structural requirements for activity against *M.tb.*[42]

5.10 Decaprenylphosphoryl-D-ribose 2-epimerase Inhibitors: The identification of 1,4-azaindoles, a promising class of compounds with potent antitubercular activity through noncovalent inhibition of decaprenylphosphoryl-D-ribose 2-epimerase ($DprE_1$). Further, this series was optimized to improve its physicochemical properties and pharmacokinetics in mice. A potential clinical candidate, that has potent cellular activity, drug-like properties, efficacy in mouse and rat chronic TB infection models, and minimal *in vitro* safety risks. Some compound shows synergy with PA824 and TMC207, *in vitro*, and synergy effect is translated *in vivo* with TMC207. The series is predicted to have a low clearance in humans, and predicted human dose for compound 2 is<1 g/day. Cross-resistance has not been observed between BTZ043, and azaindoles have been found to be equally active against drug-sensitive and Isoniazid/Rifampin-resistant strains. 1,4-Azaindoles possess low molecular weights, low logD values, excellent permeabilities, no CYP inhibition, good oral exposures, low *in vivo* clearance (CL) and low predicted human CL, and no major safety liabilities as assessed by a spectrum of *in vitro* assays. During lead optimization, low

solubility, high mouse-specific clearance, and weak phosphodiesterase 6 (PDE-6) inhibition were mitigated based on an understanding of the SAR of 1,4-azaindoles.[43]

5.11 Adamantyl-Imidazolo- Thiadiazoles: Evidently, hybrid obtained from the coupling of adamantylacetamide ring with 1,2,3-triazoles resulted in development of potent inhibitors against M. tuberculosis. Adamantyl urea derivatives were reported to induce antimycobacterial action against M. tuberculosis. SQ109, an adamantane based small molecule which is in phase-II clinical trials for treatment of pulmonary TB. On the other hand, Delamanid, an imidazo-oxazole based anti-tuberculosis drug was approved for treatment of multidrug-resistant tuberculosis. Thiadiazoles and imidazothiadiazoles were reported to have antitubercular activity against *M.tb.* H37Rv strains. The imidazo-thiadiazole nuclei to adamantyl ring in order to enhance bioactivity profile of the newer drug-seeds. Novel adamantanyl-imidazo-thiadiazoles for subsequent mode-of-action analysis identified that, it likely achieve this activity by targeting sterol 14α-demethylase (CYP51).[44]

5.12 Aminobenzimidazole Derivative: DNA gyrase and topoisomerase IV are two clinically validated drug targets for bacterial infections. They are highly conserved type II topoisomerases that are essential for DNA replication. Both targets are enzymes with A2B2 heterotetramers, comprising the *GyrA* and *GyrB* subunits (DNA gyrase) and the *ParC* and ParE subunits (topoisomerase IV), respectively; however, a topoisomerase IV homolog has not been identified in *M.tb*. In an enzymatic reaction that is coupled with ATP hydrolysis, these enzymes break and rejoin double-stranded DNA. A novel class of antimicrobials that target the ATPase subunits (GyrB/ParE) are aminobenzimidazoles, which were optimized using structure-guided design/SAR studies of potency against both Gram-positive bacterial species. Further optimization of the metabolic profile led to the identification of VXc-486, and its solubility was later improved by using a phosphate prodrug approach. In this study, we found that a novel aminobenzimidazole, VXc-486, which targets gyrase B, potently inhibits multiple drug-sensitive isolates and drug-resistant isolates of *M.tb. in vitro* (MICs of 0.03 to 0.30 µg/ml and 0.08 to 5.48µg/ml, respectively) and reduces mycobacterial burdens in lungs of infected mice *in vivo*. VXc-486 is active against drug-resistant isolates, has

bactericidal activity, and kills intracellular and dormant *M. tuberculosis* bacteria in a low-oxygen environment. Furthermore, VXc-486 inhibits growth of multiple strains of *M. abscessus*, *M.avium* complex, and *M.kansasii* (MICs of 0.1 to 2.0 µg/ml). VXc-486 and a phosphate prodrug of VXc-486 and showed that prodrug of VXc-486 had more potent killing of *M.tb.* than did VXc-486 *in vivo*.[45]

5.13 Oxazolidinones. The oxazolidinones are a new class of ribosome-targeting antimicrobial agents, of which only FDA-approved agent is linezolid. Oxazolidinones binding to peptidyltransferase center but overlapping the Asite pocket. In this position, linezolid interferes with the positioning of tRNA entering the A site and may also prevent the normal functioning of the peptidyltransferase center by changing its conformation.[46]

5.14 Biarylmethoxy Nicotinamide: It is one of the promising series that emerged out, initial hits were weak in terms of cellular potency, and a subsequent optimization effort resulted in about 200-fold improvement. A whole cell based screening effort on a focused library from corporate collection resulted as novel inhibitors of *M.tb.* H37Rv. The series exhibited tangible structure activity relationships, and during hit to lead exploration, a cellular potency of 100 nM was achieved, which is an improvement of >200-fold from starting point. The series is very specific to *M.tb.* and noncytotoxic up to 250 µM as measured in mammalian cell line *THP-1* based cytotoxicity assay. The data indicates good selectivity between M.tb. and eukaryotic THP-1 cell line. Some of the best compounds in the series did not show cytotoxicity up to 250 µM, thus demonstrating excellent selectivity.[46]

5.15 DC-159a: It representing a new spectrum of respiratory quinolones, was demonstrated to have potent *in vitro* and *in vivo* activity against quinolone-resistant (QR) as well as MDR *M.tb.* DC-159a exhibits notably high inhibitory activity against altered DNA gyrases with the substitution(s) Ala90Val and/or Asp94Gly in GyrA, as well as the wild-type enzyme of *M.tb*. However, the mechanism of resistance to DC-159a in *M. tuberculosis* is not yet fully understood. Mutant strains of susceptible *M. tuberculosis* H37Rv were developed by the multistep resistance selection method on 7H10 agar plates containing DC-159a at 1 µg/ml, 4 µg/ml, 16 µg/ml, and 64 µg/ml MIC.[47]

5.16 L-Aspartate-α-decarboxylase Inhibitors: L-Aspartate-α-decarboxylase (ADC) belongs to a class of pyruvoyl dependent enzymes and catalyzes conversion of aspartate to β-alanine in pantothenate pathway, which is critical for growth M.tb. A chemoinformatics-based approach to identify potential drug-like inhibitors against *M.tb.* L-aspartate a-decarboxylase (MtbADC). The structurebased high throughput virtual screening (HTVS) mode of the Glide program was used to screen molecules of the Maybridge, National Cancer Institute (NCI) and Food and Drug Administration (FDA) approved drugs databases. Ligands were rejected if they cross-reacted with S-adenosylmethionine (SAM) decarboxylase, a human pyruvoyl dependent enzyme. The lead molecules were further analyzed for physicochemical and pharmacokinetic parameters, based on Lipinski's rule of five, and ADMET (absorption, distribution, metabolism, excretion and toxicity) properties. This analysis resulted in eight small potential drug-like inhibitors that are in agreement with the binding poses of the crystallographic ADC:fumarate and ADC:isoasparagine complex structures and whose backbone scaffolds seem to be suitable for further experimental studies in therapeutic development against tuberculosis.[48] L-Aspartate a-alpha decarboxylase (ADC, EC 4.1.1.11), encoded by the panD gene, is a lyase and catalyzes the decarboxylation of aspartate to b-alanine, which is essential for D-pantothenate formation. Mutants of the panD gene are defective in β-alanine biosynthesis. β-alanine and D-pantoate condense to form pantothenate, a precursor of coenzyme A (CoA), which functions as an acyl carrier in fatty acid metabolism and provides the 49-phosphopantetheine prosthetic group in fatty acid biosynthesis. The absence of panthonate pathway in humans ensures that any inhibitor or drug against ADC would have low toxicity in patients. In an effort to discover inhibitors against ADC, b-hydroxyaspartate, L-cysteic acid, D-serine, oxaloacetate and succinic dehydrazine are reported as competitive inhibitors of ADC with *Ki* of 0.13, 0.08, 0.16, 0.81, 0.73 mM, respectively and phenylhydrazine binds to the pyruvoyl group to inactivate the protein. While D-serine, b-hydroxyaspartate, and L-cysteic acid interfere in vivo with the synthesis of pantothenic acid in bacteria, external supply of aspartic acid, b-alanine, or pantothenic acid can reverse their growth inhibitory action in M.tb.. However, to date no

selective drug-like inhibitor against MtbADC has been reported. To our knowledge, this is the first chemoinformatics-based drug design approach to propose novel and selective inhibitors of MtbADC. Eight lead molecules significantly satisfy the pharmacokinetic factors that are defined for human use and qualify as potential druglike molecules. They are: (2S,3R,4S,5S)-2,3,4,6-tetrahydroxy-5-mercaptohexanal (ZINC03871163), (2S,3S,4S,5R)-2 (hydroxymethyl) tetrahydro-2H-pyran-2,3,4,5-tetraol (ZINC03830878), 3-amino-4-(propylamino) cyclobutane-1,2-dione (LIGAND10436), (S)-thiazolidin-3-ium-4-carboxylate (ZINC00967474), (S)-5-acetoxy- 4-methylpentanoate (ZINC02036492), (2S,3S,4R,5R)-tetrahydro-2H-pyran-2,3,4,5-tetraol (ZINC03606295), (2S,3S,4R,5S)-2,5-bis(hydroxymethyl)tetrahydrofuran-2,3,4-triol (ZINC03830875) and 1H-pyrazolo [3,4-d]pyrimidin-4(7H)-one (ZINC05177572).[49]

5.17 Benzoxazinorifamycin (KRM-1648): Newly synthesized *Benzoxazinorifamycin*, KRM-1648, was studied for its in vivo anti Mycobacterium avium complex (MAC) activities. When the MICs were determined by the agar dilution method with Middlebrook 7H11 agar medium, KRM-1648 exhibited similarly potent *in vitro* antimicrobial activities against the MAC isolated from AIDS and non-AIDS patients. KRM-1648 exhibited potent therapeutic activity against experimental murine infections induced by M. *intracellulare* N-260 (virulent strain) and N-478, which has much weaker virulence. Similarly, KRM-1648 exhibited an excellent therapeutic efficacy against M. *intracellulare* infection induced in NK-cell-deficient beige mice (as a plausible model for AIDS-associated MAC infection), in which a much more progressed state of gross lesions and bacterial loads at the sites of infection were observed. When the infected beige mice were killed at weeks 4 and 8, obvious therapeutic efficacy was seen on the basis of reduction in the incidence and degree of lung lesions and bacterial loads in the lungs and spleen with infections due to M. intracelulare N-241, N-256, and N-260. In this case, the efficacy was the highest in N-260 infection, followed by strain N-241. When mice were observed until infection-induced death, survival time of the infected beige mice was found to be prolonged by KRM treatment.[50]

5.18 Pyrimidine Nucleosides: Inhibitors of a mycobacterial enzyme thymidine monophosphate kinase (TMPKmt) Pyrimidine Nucleosides Since initial report in 2005, *Kumar and colleagues* have made significant contributions in the investigation of pyrimidine nucleosides as new classes of anti-tuberculosis agents. A variety of known and unknown pyrimidine nucleosides substituted/unsubstituted at 2-, 4-, 5- and/or 6- positions of the base, and containing various deoxyribose, ribose, arabinose, dideoxyribose and acyclic sugar moieties. 5-alkynyl substituted pyrimidine nucleosides demonstrated the most potent activity against mycobacteria. The MIC90 exhibited by compounds of this series was in the range of 1–5μg/mL) against *Mtb* H37Ra. Subsequently, author reported a series of 5-acetylenic derivatives with 2',3-dideoxyuridine, and 3'-fluoro-2',3'-dideoxyuridine. Compound 2',3'-dideoxyuridine series and compound 3'-fluoro-2',3'-dideoxyuridine series exhibited excellent activity against wild-type *Mtb* H37Ra (MIC 1–2 μg/mL). Compounds with 5-arylalkynyl substituents displayed potent *in vitro* antitubercular activity against *M. bovis* and *Mtb* (MIC 0.5–5 μg/mL).Kogler *et al*. reported a series of 5-substituted -2'-deoxyuridine monophosphate analogs as potential inhibitors of mycobacterial flavin-dependent thymidylate synthase (ThyX) and displayed selective inhibition of ThyX (IC_{50} 0.91 μM) but not against the classical mycobacterial thymidylate synthase (ThyA, $IC_{50} > 50$ μM).[51]

5.19 Purine Nucleosides: Somu *et al*. reported a purine nucleoside analog (MIC_{99} = 0.19 μM) as an inhibitor of siderophore biosynthesis in *Mtb* under iron-limiting conditions. The authors mentioned that the activity was due to inhibition of adenylate-forming enzyme MbtA, which is involved in biosynthesis of mycobactins. Triazole derivatives of 5'-*O*-[*N*-(salicyl)sulfamoyl]adenosine have been investigated as inhibitors of aryl acid adenylating enzymes (AAAE) involved in siderophore biosynthesis by *Mtb* H37Rv. Enzyme assays were performed at 37 °C with recombinant MbtA expressed in M.tb. Compounds (MIC 3.13 μM) was reported as best candidate Adenosine (Ado) kinase is a purine salvage enzyme that phosphorylates adenosine to adenosine-monophosphate. A number of adenine nucleosides have been evaluated as substrates and inhibitors of adenosine (Ado) kinase from *Mtb*. The best substrates were found to be 2-aza-adenosine, 8-aza-9-deazaadenosine and 2-

fluoroadenosine, while the most potent compounds were N-1-benzyladenosine (*Ki* = 0.19 μM), 2-fluoroadenosine (*Ki* = 0.5 μM), 6-cyclopentyloxy purine riboside (*Ki* = 0.15 μM) and 7-iodo-7-deazaadenosine (*Ki* = 0.21 μM). Several of these adenosine analogs exhibited promising MICs.[52]

5.19.1 5′-*O*-[(*N*-Acyl)Sulfamoyl]Adenosines: A study of the structure–activity relationships (SAR) of 5′-*O*-[*N*(Salicyl)sulfamoyl]adenosine, a potent inhibitor of bifunctional enzyme salicyl-AMP ligase (MbtA, encoded by gene *Rv2384*) in *M.tb.*, is described, targeting salicyl moiety. A systematic series of analogues was prepared exploring the importance of substitution at the C-2 position revealing that a hydroxy group is required for optimal activity. Examination of a series of substituted salicyl derivatives indicated that substitution at C-4 was tolerated. Consequently, a series of analogues at this position provided 4-fluoro derivative, which displayed an impressive MIC_{99} of 0.098 μM against whole-cell *M.tb.* under iron-limiting conditions. Examination of other heterocyclic, cycloalkyl, alkyl, and aminoacyl replacements of the salicyl moiety demonstrated that these nonconserative modifications were poorly tolerated, a result consistent with fairly strict substrate specificities of related non-ribosomal peptide synthetase (*NRPS*) adenylation enzymes.[53] *M.tb.* produces two series of structurally related siderophores, collectively known as mycobactins, that are critical for virulence and growth. Mycobactin biosynthesis is initiated by *MbtA*, an adenylate-forming enzyme that catalyzes a two-step reaction and is responsible for incorporating salicylic acid into the mycobactins. *MbtA* first binds its substrates salicylic acid and ATP then catalyzes their condensation to afford acyladenylate and pyrophosphate. The acyladenylate remains tightly bound whereas pyrophosphate dissociates. Next, *MbtA* binds N-terminal aryl carrier domain of *MbtB* and catalyzes the transfer of salicyl moiety onto nucleophilic sulfur atom of phosphopantetheinyl cofactor of *MbtB* to afford thioester-*MbtB* that is elaborated to mycobactins by a mixed nonribosomal peptide synthetase polyketide synthase (NRPSPKS) assembly line. Acyladenylates have been shown to bind several orders of magnitude more tightly than substrate acids since they simultaneously occupy both substrate binding pockets.[54] Thus acyladenylate analogues that incorporate a

stabile linker as a bioisostere of the labile acylphosphate function provide potent adenylation enzyme inhibitors. The general inhibitor scaffold is comprised of four domains (aryl, linker, glycosyl, and base). The most crucial portion of the inhibitor scaffold is the linker domain since this must be metabolically stable and appropriately position both the aryl and nucleoside moieties in their respective binding pockets. The molecular geometry and polarity of linker pharmacophore with preparation of β-ketophosphonate, acylsulfamate, acylsulfamide, sulfamate, β-ketosulfonamide, α,α-difluoro-β-ketosulfonamide, acyltriazole, and vinylsulfonamide linkages as surrogates for labile acylphosphate linkage. Inhibitors incorporating acylsulfamate and acylsulfamide linkages were found to be most potent with low nanomolar apparent inhibition constants and possessed submicromolar antitubercular activity against whole-cell *M.tb.* rivaling first-line agent isoniazid. Next, glycosyl domain and found that both the 3′-hydroxy and 4′-ribofuranose ring oxygen were dispensable for bioactivity while modifications making the sugar either more or less flexible were detrimental.[55]

5.20 Carbohydrates: In 2005 bis-glycosylated diamino alcohols were reported by *Tripathi et al.*, where their compound showed moderate activity against *Mtb H37Ra* and against *Mtb (H37Rv)*. Derivatives of stachyose were reported by some authors. The most active compound in the series against *Mtb H37Rv* (MIC 3.13 μg/mL) which was also evaluated against various drug-sensitive and-resistant clinical isolates of *Mtb*.

5.21 Azoles: In this class, some compounds demonstrated MICs of 0.78 and 0.39 μM, against *Mtb H37Rv* respectively. Pantothenate is a key precursor of coenzyme A and acyl carrier protein, essential for many intracellular processes. *Velaparthi et al.* reported in 2008 some compounds as best inhibitors (IC_{50} of < 100 nM). *N*-Aryl-*C*-nitroazoles were investigated by Walczak *et al.* against *H37Rv (ATCC 27294)* using MABA assay. Compound exhibited an MIC of 0.39 μg/mL. A series of 2-methylbenzothiazole derivatives was described by *Huang et al*. Compounds were found to be potent inhibitors of replicating *Mtb H37Rv* (MIC 1.4 and 1.9 μM, respectively).

5.22 Thiolactomycin: Thiolactomycin (TLM), is a natural product isolated from *Nocardia* and *Streptomyces* species. TLM is an inhibitor of the β-ketoacyl-acyl carrier protein synthase (KAS) enzymes, which are part of the bacterial fatty acid synthase pathway. TLM has MIC of 62.5 μM against *Mtb*. TLM also inhibits human FAS-I enzyme, however, its lower affinity (IC_{50} 100 μM) for this enzyme can make it worthy as a selective anti-tuberculosis agent.

5.23 CPZEN-45: CPZEN-45 (MIC of 1.56 μg/mL, *Mtb H37Rv* and 6.25 μg/mL, MDR strain of *Mtb*) is a nucleoside antibiotic produced by *Streptomyces* spp. CPZEN-45 is active against both replicating and on-replicating *Mtb in vitro*.

5.24 SQ-609: *Sequella* identified a promising candidate, SQ609, as most potent among a new series of potential cell-wall inhibiting dipiperidines (MIC = 4 μg/mL). The precise mode of action of SQ 609 is unknown**.**

5.25 SQ-641: The enzyme translocase 1 (TL1), is an essential enzyme in bacteria for biosynthesis peptidoglycan in cell wall. SQ-641, which targets TL1, possesses activity against MDR clinical strains of *Mtb* (MIC = 0.5 μg/mL). It has shown efficacy in a mouse model of chronic TB by reducing the CFU in lungs of infected mice by 1.0 to 1.5 log.

5.26 Benzothiazinone (BTZ-043): BTZ-043 is highly active against *Mtb* (MIC = 1–10ng/mL). BTZ-043 also possesses activity against MDR and XDR strains. It inhibits cell wall biosynthesis, and targets the DprE1 (Rv3790) subunit of the enzyme decaprenylphosphoryl-beta-D-ribose 2'-epimerase. BTZ-043 has good oral bioavailability.

5.27 Tryptanthrin: Tryptanthrin (indolo [2,1-b]quinazolin-6,12-dione), is a natural product that was obtained from a Chinese plant, *Strobilanthes cusia*. It has broad-spectrum biological activities including anti-tuberculosis property. Tryptanthrin demonstrated MIC of 1 μg/mL against *Mtb* in BACTEC assay. It showed MIC values of 0.5–1.0 μg/mL against MDR-TB strains.[56]

Table: 2: Discovery and Validation of New Antitubercular Compounds as Potential Drug Leads and Probes through antibacterial assays.

Assay	Advantages of Assay	Disadvantages of Assay
Whole cell growth inhibition	• Compound likely enters microbial cells: the cell entry problem is less of an issue. • Can immediately move to: whole cell mode of action analysis, evaluation of microbial spectrum.	• Usually a longer assay (many hours to days). • Nonspecific compounds acting at the cell surface or intracellularly may be detected. • Compound could be unstable, sequestered or degraded by cells and thus inactive but analog could be a valid lead. • Mode of action unknown; may need to determine/validate for further development and to avoid optimizing nonspecific action or target drift.
Isolated in vitro enzyme or pathway	• Usually a rapid assay (minutes as opposed to hours or days). • Target known in advance.	• Nonspecific compounds may be detected. • Compound may not enter cells, or could be sequestered or degraded. • If compound is active on whole cells, the mode of action needs to be confirmed.
Hybrid whole cell with specific enzyme or pathway targeted readout	• Compound likely enters microbial cells: the cell entry problem is less of an issue. • Can immediately move to: whole cell mode of action analysis; evaluation of microbial spectrum. • Target known in advance.	• Usually a longer assay (many hours to days). • More difficult to design. • The mode of action needs to be confirmed to support further development and to avoid optimizing nonspecific action or target drift. • Unknown interactions can give false positives that are difficult to unravel. • Compound could be unstable, sequestered or degraded by cells and thus inactive but analog could be a valid lead.

6 Molecules in Clinical Trials:

6.1 AZD-5847: AZD-5847 is an oxazolidinone antibiotic. It possesses an MIC_{90} of 1 µg/mL against laboratory *M.tb.* strains.

6.2 PNU-100480: It is a linezolid derivative and is more active (MIC = 0.0625–0.5 µg/mL) than the parent compound and with similar efficacy to that of INH and RMP. LL-3858 or Sudoterb LL3858 (MIC90 0.25 µg/mL) in combination with current anti-TB drugs.

6.3 OPC-67683 (Delamanid): It exhibited an MIC of 0.006 µg/mL. In a mouse model, its efficacy was reported to be superior to existing anti-tuberculosis drugs without any evidence of cross-resistance. The mechanism of action of OPC-67683 is suggested to be similar to PA-824.

6.4 WQ-3034: It is a newly synthesized acidic fluoroquinolone. Its *in vitro* activity against *M.tb.* and *M. avium* complex using levofloxacin (LVFX), ciprofloxacin (CPFX), sparfloxacin (SPFX), and KRM-1648 (KRM) as reference drugs. The MICs of these agents were determined by agar dilution method with 7H11 medium. The MICs at which 50 and 90% of the test strains were inhibited (MIC50s, and MIC90s, respectively) for test quinolones for rifampin (RMP)-susceptible *M.tb.* strains were in the order SPFX < LVFX < WQ-3034 < CPFX, while those for RMP-resistant *M.tb.* strains were in order SPFX >WQ-3034 < LVFX < CPFX. (A-549 cells). When drugs were added at the concentration that achieves the maximum concentration in blood, progressive killing or inhibition of the *M. tuberculosis* organisms residing in (Mono Mac 6 macrophage (Mf)-like cell line) MM6-Mfs and (A-549 type II alveolar cell line) A-549 cells was observed in order KRM > SPFX>LVFX > WQ-3034>CPFX.[57]

6.5 HSR-903: It is a new fluoroquinolone [(*S*)-(2)-5-amino-7-(7-amino-5-azaspiro [2,4]hept-5-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid methanesulfonate], has a broad spectrum of action against gram-positive bacteria. HSR-903 has more potent activity against *M.tb.* than do other fluoroquinolones, including CPFX, SPFX, and LVFX. In pharmacological studies with mice, the levels of HSR-903 in the lungs were much higher than those in the plasma after oral administration, and concentrations of HSR-903 in lung were higher than those of CPFX and LVFX. In humans, the maximum concentration of drug in serum (*C*max) of HSR-903 at 200 mg/kg of body weight was 0.86 mg/ml at 1.3 to 2.4 h (time to *C*max [*T*max]), and the half-life (T1/2b) and area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) of HSR-903 were 18.0 h and 12.8 mg z h/ml, respectively. Author compared the *in vitro* antimycobacterial activity of a new fluoroquinolone, HSR-903, with strong activity against mycobacteria with those of

levofloxacin (LVFX), sitafloxacin (STFX), and gatifloxacin (GFLX). The MICs of the quinolones for *M.tb.* and *M.avium* complex were in the order STFX<GFLX < LVFX<HSR-903 and STFX >GFLX >HSR-903>LVFX, respectively. HSR-903 effectively eliminated intramacrophagial *M.tb.* as did LVFX, and exhibited bacteriostatic effects against *M. tuberculosis* replicating in type II alveolar cells.[58]

6.6 MetAP inhibitor: Methionine aminopeptidase (MetAP) can be established as a prominent target for developing novel inhibitor of MDR-TB pathotype**.** MetAP removes terminal N-terminal methionine from nascent proteins and is required for post translational processing and targeting of virulent protein to host body. *M.tb.* possesses two types of MetAP *i.e.* MetAP1b and MetAP1c of which the later was found to be less virulent. This enzyme belongs to the family dinuclearmetallo-hydrolases and various cofactors like Ni (II) were found to empower the protein to act. 3-ammonio-3-(4-oxido-1(H)imidazol-1-ium-5-yl) propane-1,1-bis(olate) as a potent MetAP inhibitor. Molecular docking with target showed that of all three ligands, 3-ammonio-3-(4-oxido-1H-imidazol-1-ium-5-yl) propane-1, 1-bis (olate) has highest affinity (-37.5096) and lowest IC_{50} (4.46 μM).[59]

6.7 TBA-354: While development of PA-824 continues, a potential next-generation derivative, TBA-354, has been discovered to have *in vitro* potency superior to that of PA-824 and greater metabolic stability than that of the other nitroimidazole derivative in clinical development, *Delamanid.* The combination studies revealed that TBA-354 is 2 to 4 times more potent than PA-824 when combined with Bedaquiline, and when administered at a dose equivalent to that of PA-824, TBA-354 demonstrated superior sterilizing efficacy.

6.8 Piperidin-4-imine derivatives: Various novel 1-(1H-benzimidazol-2-ylmethyl)piperidin-4-imine derivatives were developed and have showed for favorable pharmacokinetic parameters based on drug-likeness explained by Lipinski's rule of five. The title compounds were also synthesized, characterized, and tested for *ex vivo* antitubercular activity against *Mycobacterium tuberculosis H37Rv* (*ATCC27294*). The results revealed that four compounds(2-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]hydrazinecarbothioamide ,2-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]-Nhydroxyhydrazinecarbothioamide

,1-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]guanidine, and 2-[1-(1H-benzimidazol-2-ylmethyl)piperidin-4-ylidene]hydrazinecarboxamide) were the most potent (minimum inhibitory concentration 6.25 μg/mL) antitubercular agents, with less toxicity (selectivity index more than 10). The molecules were also subjected to three-dimensional molecular docking on crystal structure of enoyl-acyl carrier protein (EACP) reductase enzyme, which represents a good prediction of interactions between the molecules and EACP reductase with minimum binding energy.[60]

6.9 1-Adamantyl-3-Phenyl Urea: This series is active against Mycobacteria and previous lead compounds were found to inhibit the membrane transporter *MmpL3,* the protein responsible for mycolic acid transport across the plasma membrane. However, these compounds suffered from poor *in vitro* pharmacokinetic (PK) profiles and they have a similar structure/SAR to inhibitors of human soluble epoxide hydrolase (SEH) enzymes. Therefore, further optimization of this compound class was driven by three factors: 1) to increase selectivity for anti-TB activity over human sEH activity, 2) to optimize PK profiles including solubility and 3) to maintain target inhibition. A new series of 1-adamantyl-3-heteroaryl ureas was designed and synthesized replacing the phenyl substituent of the original series with pyridines, pyrimidines, triazines, oxazoles, isoxazoles, oxadiazoles and pyrazoles.[61]

6.10 1, 4-dihydropyridine-3, 5-dicarboxamide: In this research, new derivatives of 1, 4-dihydropyridine were designed and synthesized using *Hantzsch* condensation in which dicyclohexyl and different dicyclohexylcarbamoyl were substituted at C-3 and C-5 positions of the DHP ring. In addition, 4 (5)-chloro-2-methyl-5 (4)-imidazolyl moiety was substituted at C-4 position of DHP. The structure of synthetized compounds were characterized by TLC, IR, elemental analysis and proton NMR. Based on the *in vitro* screening data, all of the designed and synthetized compounds showed a good ability to inhibit *M.tb.* growth in terms of MIC. Aromatic carboxamide containing compounds were more potent than cyclohexyl derivative. The experimental data with computational predictions in terms of partial atomic charge of carbonyl moieties at C-3 and C-5 positions of DHP ring and partition coefficient of molecules.[62]

6.11 SQ109: SQ109 does not fit the mold of traditional pharmaceuticals, and its development has been slowed by concern that its basic structure does not fit the norms of other nonantibiotic drug classes. The goal of the SQ109 clinical program is to investigate utility of this agent in both DS and DR strains of *M.tb.* by replacing EMB in the current standard-of-care regimen to treat disease caused by DS strains and adding SQ109 to second-line regimens that are tailored to specific MDR-TB disease. The low bacterial mutation rate (2.55×10^{-11}) observed *in vitro* combined with the extensive tissue penetration observed *in vivo* suggest that the drug has significant potential clinical utility to treat pulmonary TB and to decrease relapses/ recurrences due to emergence of antibiotic resistance. Synergy between SQ109 and RIF or INH may also shorten treatment times for uncomplicated TB. The long half-life of SQ109 may facilitate combination of SQ109 with other TB drug candidates such as bedaquiline (which also has a long half-life) or sutezolid, with which it interacts well in vitro. Treatment of Mtb with SQ109 decreased the incorporation of mycolic acids into the cell wall. However, this effect was localized to incorporation into trehalose dimycolate and mycolates attached covalently to the arabinogalactan polymer. Incorporation into trehalose monomyclate (TMM) actually increased, suggesting that SQ109 did not target mycolate synthesis, but rather transport and processing. It has an MIC = 0.1–0.63 μg/mL. *In vivo* it exhibited 1 to 2.0-log reduction in CFU counts in the lungs and spleens at 25 mg/kg. However, its oral bioavailability was found to be poor (only 4%).[63]

6.12 Benzotriazine Di-*N*-Oxides: Screening of a panel of antimicrobial revealed that 1,2,4-benzotriazine di-N-oxides (BTOs) are potently bactericidal against replicating and nonreplicating *M.tb.* Medicinal chemistry optimization, guided by semi-empirical molecular orbital calculations, identified a new lead compound from this series with an MIC of 0.31 μg/mL against *H37Rv* and a cytotoxicity (CC50) against Vero cells of 25 μg/mL. It was also negative in a L5178Y MOLY assay, indicating low potential for genetic toxicity. These data along with measurements of the physiochemical properties and pharmacokinetic profile demonstrate that BTOs have the potential to be developed into a new class of antitubercular drugs.[64]

6.13 OPC-67683: It is a dihydroimidazo-oxazole agent under development by Otsuka Pharmaceuticals specifically for the treatment of tuberculosis. Cross-resistance with PA-824 occurs through mutations in Ddn, the enzyme responsible for activation. OPC-67683 is more potent than PA-824 in vitro and in vivo, with a minimum inhibitory concentration of 0.006–0.024 mg/mL and minimum bactericidal activity at 2.5 mg/kg in mice, resulting in a 2 log10 reduction in colony forming units, as compared with 50 mg/kg for PA-824 in a similar model by *Nuremberger* **(unpublished observation).** In a Phase I multidose investigation using two different formulations, OPC-67683 was administered in doses up to 400 mg. A 14-day extended early bactericidal activity trial has been performed. A Phase IIb trial has been completed in patients with multidrug-resistant tuberculosis randomized to receive.[65]

6.14 1,4-Dihydroxy-2-naphthoate Prenyltransferase Inhibitors: Since utilization of menaquinone in the electron transport system is a characteristic of Gram-positive organisms, the 1,4-dihydroxy-2-naphthoate prenyltransferase (MenA) inhibitors act as selective antibacterial agents against and *Mycobacterium* spp. On the basis of the observation of this enzymatic activity and the structure of the MenA product, dimethylmenaquinone (DMMK), we designed tertiary or secondary amine or hydrazine-containing DMMK mimics in hope that the amine moiety would interact with *Asp* residue(s) directly or through the divalent cation(s) in the active site and in which the chemically unstable 1,4-quinone system is replaced with the hydrophobicly substituted benzophenones. DMMK mimics were synthesized efficiently in four to six steps including (1) Friedel-Crafts acylation, (2) deprotection, (3) alkylation(s), (4) bromination, and (5) amination reactions. Author have synthesized 100 molecules in solution, and the library of molecules was evaluated in enzymatic assays *in vitro* (IC_{50}) against *M.tb.* MenA and in mycobacterial growth assays (MIC). More than 18 molecules exhibited MenA IC_{50} and MIC values of less than 20 μM, and in all cases MIC value was in good agreement with the IC_{50} value. From these preliminary screenings it was shown that the shorter length of linker (C_5-C_7) between the phenolic oxygen and the nitrogen atom decreased the ability to inhibit *MenA* and the efficacy of growth inhibition. In addition, the structure of amine or hydrazine significantly influences

activity; α-substituted amine or bulky tertiary amine containing molecules did not show MenA inhibitory activity at lower concentrations. Identification of effective substitution pattern (R_1, R_2, R_3, and R_4) in benzophenone moiety requires extensive SAR studies; hydroxy group on R_3 (R_3 = OH) seems to be superior to others (R_3 = H or Cl) regardless of structure of linker. Two molecules named allylaminomethanone-A and phenethylaminomethanone-A, respectively, showed MIC values of 1.5 and 12.5 μg/mL against *M.tb.* (*H37Rv*), respectively.[66]

6.15 Nitrobenzothiazole Inhibitors of ATP Phosphoribosyl Transferase (HisG): HisG represents a potential drug target for tuberculosis. HisG is an ATP-phosphoribosyl transferase (ATP-PRTasea) that catalyzes the first step in biosynthetic pathway for histidine. In order to discover more potent and diverse inhibitors, virtual screening was performed. The crystal structure of HisG has been solved in *M.tb.* as well as other organisms, and reveals a large, solvent-exposed active site with sub-sites for ATP substrates. Two docking algorithms, GOLD and FLEXX, were used to screen two large libraries — Chembridge and NCI — containing over 500,000 compounds combined. An initial subset of top-ranked compounds were selected and assayed, and seven were found to have enzyme inhibition activity at micromolar concentrations. Several of the hits contained a nitrobenzothiazole fragment which was predicted to dock into the monophosphate-binding loop, and this binding mode was confirmed by crystallographic evidence. A secondary screen was performed to identify compounds with similar structures. Several of these also exhibited micromolar inhibition in a whole-cell assay against *M. smegmatis* with several compounds having IC_{50}'s at or below 10 μM. (12-25μM MIC) The most potent compound, had an IC_{50} of 4 μM, and most interesting scaffold discovered in this effort was nitrobenzothiazole (NBT). This scaffold is reminiscent of dinitrophenol, which has weak inhibition activity against *M.tb.* HisG (0.4 mM). The compounds found by virtual screening were much more potent. A secondary virtual screen was performed to select compounds from the libraries that bore similarity to the initial hit containing NBT. Among combined set of compounds (as well as other nitro-aryl compounds) docked to the PRP protein-ligand interaction was confirmed crystallographically for one of

the hits containing NBT, which showed electron density in this position in the Fourier-difference map for a co-crystal produced by soaking.[67]

6.16 Aurachin RE: It is a strong natural products antibiotic that was recently found to possess MenA (1,4-dihydroxy-2-naphthoate prenyltransferase), bacterial electron transport inhibitory activities and has chiral center in the alkyl side chain at C9′-position. The synthesized molecules were evaluated *in vitro* assays including MenA enzyme and bacterial growth inhibitory low oxygen recovery (LORA) assay. Through asymmetric synthesis of a series of optically active molecules followed by screening, identified a series of MenA inhibitors identified as a carbamates showed significant growth inhibitory activities against non-replicating *M.tb.* (MIC LORA, 0.85 μg/mL with the MIC LORA/MIC MABA/value of 0.37, SI >10; MIC LORA/MIC MABA = 7.35 for rifampin). *In vitro* efficacy will be achieved by modifying the hydrophobic side chain and benzophenone O-methyl oxime moiety also confirmed via assays using a *modified Wayne* model. Further studies are underway to thoroughly characterize the activity against multidrug resistant.[68]

6.17 Tetrahydropyrazolo[1,5-a]Pyrimidine: It was identified as a hit series from *a M.tb.* whole cell high through-put screening (HTS) campaign. Compounds had a promising in vivo DMPK profile in mouse and exhibited potent *in vivo* activity in a mouse efficacy model, achieving a reduction of 3.5 log CFU of *M.tb.* after oral administration to infected mice once a day at 100 mg/kg for 28 days. Thus, compound is a potential candidate for inclusion in combination therapies for both drug-sensitive and drug-resistant TB.[69]

6.18 Dithiolopyrrolone (DTP): These group antibiotics are characterized by an electronically unique bicyclic structure, which contains a compact disulfide bridge between two ene-thiols. Points of diversity within the compound class occur outside of the bicyclic core, at the two amide nitrogens. Such modifications distinguish three of the most well studied members of the class, holomycin, thiolutin, and aureothricin; the DTP core has also more recently been identified in the marine antibiotic thiomarinol, in which it is linked to a marinolic acid moiety, analog of the FDA-approved topical antibiotic *Bactroban*® (GlaxoSmithKline).[70-71]

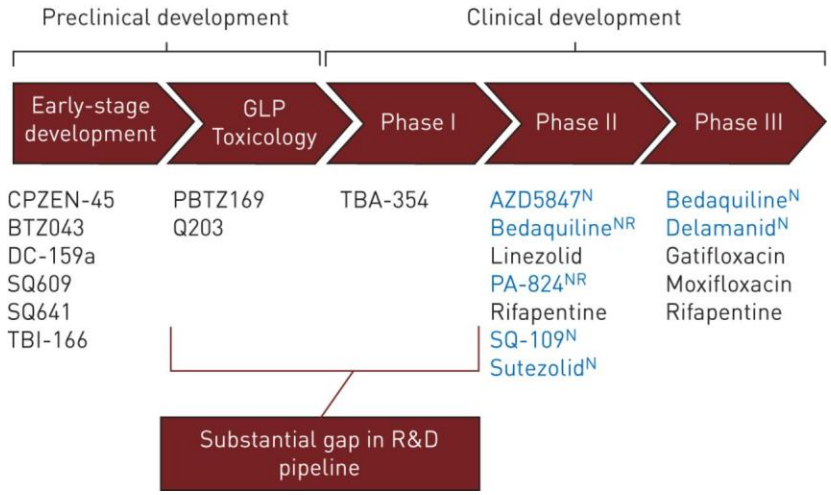

FIGURE:2: "Discovery of Some AntiTB Drug Leads Development in Clinical Trials."

6.19 *Some other studies of Mycobacterium* protein tyrosine phosphatase B (mPTPB) inhibitors: Small molecule inhibitors of *mPTPB* could be a treatment to overcome emerging TB drug resistance. Using a Diversity-Oriented Synthesis (DOS) strategy, successfully developed a salicylic acid based and drug-like *mPTPB inhibitor* with an IC_{50} of 2 μM and >20-fold specificity over many human PTPs, making it an excellent lead molecule for anti-TB drug discovery. DOS generated bicyclic salicylic acids are also promising starting points for acquiring inhibitors targeting other PTPs. Protein tyrosine phosphatases (PTPs) have emerged as the next generation drug targets due to their complementary roles to protein tyrosine kinases in controlling protein tyrosine phosphorylation levels. Salicylic acid could serve as a novel pTyr mimetic, affording PTP inhibitors with excellent *potency, specificity, and cell permeability.* Deletion of mPTPB impaired the ability of the mutant strain to survive in interferon-γ (IFN-γ) activated macrophages and severely reduced the bacterial load in a clinically-relevant guinea pig model. Given that bicyclic salicylic acid are more active than salicylic acid itself in inhibiting *PTP* activity as a result of enhanced interactions with the PTP active site.[70] Heteroatoms in adjacent positions on parent benzene ring of salicylic acid, which furnishes molecular handles for generation of the second heterocycles. The substituents can be amino, hydroxyl, halogen, and other groups that are easily functionalized. Synthesis of protected salicylic acids with substituents of 4-hydroxy-5-amino, 4,5-diamino,

4-amino-5-iodo, and 4-amino-3-iodo with including acetylation, nitration, hydrolysis, esterification, and reduction; in excellent yield, which were easily separated by flash chromatography. The reaction was proposed to play a role as ligand and phase-transfer catalyst to give structurally diverse bicyclic heterocycles with salicylic acid moiety with IC_{50} values from 20 to 30 μM, while bicycles such as did exhibit activity at 100 μM, likely due to lack of aromaticity. Compound's easy access, good activity, low molecule weight (MW = 286), coupled with prevalence of the aminothiazole motif in FDA approved drugs such as Dasatinib and Norvir, make it ideal for further elaboration to develop drug-like mPTPB inhibitors with improved potency. Thus, a focused library of aminothiazole-salicylic acids was prepared by reacting precursor with a set of aryl isothiocynates. Generally, *o*, *m*, or *p*-substituted phenyl isothiocyanates with either electron donating or withdrawing properties, as well as 1-naphthyl isothiocyanate, were tolerated to afford products in moderate to good yields, except that alkyl isothiocynates did react with same reaction conditions. All hydrolyzed products were purified by HPLC to ensure high purity with an IC_{50} of 2.0 μM, representing a 12-fold increase in comparison to that salicylic acid exhibits an IC_{50} value of 55±8 mM for mPTPB. Subsequent kinetic studies revealed that it is a noncompetitive inhibitor against pNPP with a *K*i of 2.2±0.1 μM exhibiting over 20-fold preference against these PTPs. With advantages of efficient synthesis, low molecular weight and excellent cell activity, it serves as a promising lead molecule for anti-TB drug discovery targeting mPTPB. Given the favourable pharmacological properties exhibited by the salicylic acid pharmacophore, the bicyclic scaffolds generated by the DOS strategy should also be good starting points for the development of drug-like inhibitors targeting other PTPs.[72-73]

6.20 Sulfuryl Transfer (ST) Inhibitors: Although the roles of sulfated metabolites in the mycobacterial lifecycle remain under investigation, the analogy to sulfation in higher eukaryotes is compelling. STs have also been further classified according to their functional role into estrogen STs (EST), heparin STs, tyrosyl protein ST (TPST), N-Acetyl glucosamine 6-*O*-ST and carbohydrate STs. The first crystal structure to be elucidated was that of murine estrogen sulfotransferase (mEST) in 1997 and since then, structures of nine other STs have

been characterized. These include cytosolic STs such as Phenol ST (SULT1A1), catecholamine ST (SULT1A3), mycobacterial Stf0 and Golgi-resident STs (GSTs) such as heparan *N*-deacetylase- *N*-ST-1 (NDST-1). Structures of STs in complex reveal a conserved nature of the cofactor binding site, suggesting that STs share a similar mechanisms of sulfuryl transfer. The catalytic site of each ST must also accommodate diverse substrates and these differences in specificity are reflected in the substrate-binding site of each ST.[74-75]

6.20.1 Bisubstrate Analogs: To investigate molecules that inhibit substrate–binding domains of STs synthetic bisubstrate analogs have been employed. Compounds were designed to incorporate elements from the cofactor, and the substrate, providing specificity via critical interactions within both binding pockets of enzyme. Inhibitor potency is achieved from entropic advantage of linking structures that mimic each substrate. A "*glycomimetic*" strategy was used to design inhibitors for these STs. In this approach the inhibitors retained structural and functional aspects of the natural ligands, but were designed to be synthetically more feasible. On screening a 447 member 3'-phosphoadenosine library, several bisubstrate-based compounds were identified as inhibitors of EST. The activities of these compounds were comparable to some of other compounds known to be inhibitors of EST including polychlorinated biphenols, discovered by testing a large number of hydroxylated polychlorinated biphenyl metabolites on EST activity.[76-77]

6.20.2 Kinase-Derived Inhibitors: The "*kinase inhibitor*" approach exploits similarity between reactions catalyzed by STs and kinases. Since STs and *kinases* use adenosine-based donor nucleotides to transfer an anionic moiety onto their respective substrates (STs and ATP for kinases), it was proposed that ATP derivatives might also function as ST inhibitors. Furthermore, the hydrophobic adenine binding pockets of EST and heparin N-sulfotransferase are similar to those of several kinases. A 2,6,9-trisubstituted purine library, originally designed to target cyclin dependent kinase 2, was tested for inhibitory activity with carbohydrate STs. Of the 139 compounds screened, the six most potent purines exhibited half maximal inhibitory concentrations (IC_{50s}) that ranged from 20 – 40 μM, with five of them having a common benzyl substituent at N_6. A high throughput screen of 35,000

purine and pyrimidine analogs has also identified a potent inhibitor of β–arylsulfotransferases (β-AST-IV). A **second class** of ***kinase inhibitors, Isoquinoline Sulfonamides,*** has also been tested for inhibitory activity against a panel of STs consisting of EST. ***Isoquinoline sulfonamide inhibitors*** were developed after a crystal structure of cyclic adenosine-5'-phosphate (cAMP) dependent protein kinase in complex with isoquinoline showed that the heterocycle moiety was bound in subsite occupied by the adenine ring of ATP. Of 100 *Isoquinoline and Quinoline* derivatives screened, the most active compounds inhibited single enzyme selectively with modest IC_{50} values in the range of 30 –100 μM.[78]

6.20.3 Combinatorial Target-Guided Ligand Assembly: In this strategy, a library of ligands or 'monomers' carry a common chemical handle to facilitate their combinatorial assembly. In first round, monomers were screened against the ST target at concentrations of 1 mM or higher. Compounds that demonstrated inhibitory activity were then used to construct a library of 'dimers' via an oxime linkage, and were screened for inhibitors. This approach resulted in identification of two of the first known inhibitors of Golgi-resident tyrosyl protein ST-2 (TPST-2). ST inhibitors identified in the studies above are a promising start in drug discovery efforts. However, to date the majority of ST inhibitor compounds possess fairly modest IC_{50s}, are not "drug-like", or suffer from a lack of specificity. Recent advances in structure-based drug design and high-throughput screening should greatly facilitate the discovery of new inhibitors for STs and other sulfonucleotide-binding enzymes.[79-80]

7 CONCLUSION:

Existing drugs have limited efficacy against the rising threat of drug-resistant TB, have significant side effects, and must be given in combinations of four to six drugs for at least 6 months for drug sensitive TB and up to 24 months for drug-resistant TB. The long treatment duration has led to increased patient noncompliance with therapy. This, in turn, drives the development of additional drug resistance in a spiral that has resulted in some forms of TB being currently untreatable by existing drugs. These prejudices slowed new antitubercular clinical evaluation, which has not kept pace with the rapidly expanding worldwide need for new TB

drugs. It is important to continue to explore new ideas and new chemical space to combat public health threats such as TB. New antitubercular drugs in development, particularly those with mechanisms of action that are different from existing first- and second-line TB drugs, are anticipated to be effective against both drug-sensitive and drug-resistant TB. Recent years have shown a rise in interest in application of modern drug-discovery techniques to the field of TB, leading to an unprecedented number of new TB drug candidates in clinical trials. On the downside, recent years also saw the application of modern drug development to anti-infective and antitubercular drugs. AntiTB drug discovery does not fit the mold of traditional pharmaceuticals, and its development has been slowed by concern that its basic structure does not fit norms of other nonantibiotic drug classes. Distributed computing was an essential component of methodology that enabled screening of such large databases in a reasonable amount of time, affording potential benefit of yielding more diverse hits than would be possible by more traditional approach of doing focused screens based on known *scaffolds.* However, visual inspection also played an important role in selecting compounds (among those most highly ranked) that appeared to make most favorable interactions with active site (in their docked conformations). Despite many concerns frequently-voiced about virtual screening (*e.g.* adequacy of conformation sampling, accuracy of scoring functions, etc.), the methodology has been demonstrated to work well, yielding micro-molar inhibitors (including those from a secondary screen). Pending further studies (*e.g.* target validation, ADME/T analysis, etc.), these compounds (or more potent *analogs* of them) could be considered as candidates for development into novel drugs against *tuberculosis.*

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