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Chemotherapeutic Strategies and Targets Against Resistant TB

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1. Introduction

Chemotherapeutic cure for about 40,000 years old lethal disease – TB (Callaway, 2008), was started mere ~65 years ago, with the discovery of antibiotic- streptomycin. A few effective drugs against TB have been developed since then and have been classified mainly as first-line (viz. rifampicin, isoniazid, pyrazinamide and ethambutol) and second-line drugs (e.g. ciprofloxacin, levofloxacin, cycloserine, clofazimine etc.). Drugs like rifambutin, clarithromycin and linezolid may be considered as "third line" drugs. The current course of therapy with the first-line TB drugs is more than 40 years old and is slowly becoming outdated due to emergence of multidrug-resistant tuberculosis (MDR-TB, resistant to the two first line drugs) and extensively drug-resistant tuberculosis (XDR-TB, an MDR-TB that is resistant to fluoroquinolones and also to any one of the three injectable second- line drugs: amikacin, capreomycin or kanamycin) (World Health Organisation, [WHO], 2011). Treatment with the second line drugs is limited due to the associated toxicity which halts therapy prior to cure in more than half of the patients suffering from serious side effects. The "third line" drugs have issues of proven efficacy/effectiveness and impractical cost. Longer duration of treatment, usually for six months, with complex regimens leads to poor compliance. Although poor compliance can be managed to great extent by Directly Observed Treatment, Short course (DOTS) launched by World Health Organization (WHO); but that is possible practically in developed countries only where manpower along with financial needs are met adequately. Apart from these problems, during this long treatment period, the patient and one's family suffer from socioeconomic problems, whereby psychological issues such as risk of depression come in picture. Side effect(s) of drugs, due to long treatment, is another major concern.

Researchers have been trying to find out the answer for why the TB treatment is so long and complex. McCune et al found considerable difference in the efficacy of drugs against *Mycobacterium tuberculosis* (*Mtb) in vitro* and *in vivo* (McCune & Tompsett, 1956; McCune et al 1956). However, other researchers (Barclay et al. 1953; Clark, 1985) showed that bioavailability is not a concern. It was proposed that this persistence of *Mtb* might be due to physiologic heterogeneity of bacteria in the tissues (Mitchison, 1979; Handwerger & Tomasz, 1985).

Mitchison found that the lesions have at least four different populations of *Mtb*:

a. Actively growing bacilli: can be killed by isoniazid
b. Bacilli with spurts of metabolism: can be killed by rifampicin
c. Bacilli with low metabolic activity (reside in acidic pH environment): can be killed by pyrazinamide
d. Dormant bacilli: not killed by any existing drug/regimen.

The actively multiplying bacilli are killed in the first 2 days, the remaining are dormant, which are sterilized very slowly by the existing drugs and thus the treatment period is stretched so long (Jindani et al., 2003).

Bacillus Calmette Guerin (BCG), the only approved vaccine for TB in humans, contains attenuated strain of *M. bovis*. It is generally considered safe; however this vaccination may lead to TB infection in immunocompromised individuals. Moreover, BCG only reliably protects against tuberculosis in newborns and fails in adult pulmonary tuberculosis, the most prevalent form (Kaufmann, 2011).

Due to the associated global health and socioeconomic concerns, the increasing rates of MDR-, XDR-TB, and TB-HIV coinfection, the discovery and development of potent new anti-TB agent(s), without cross-resistance with current antimycobacterial drugs, is urgently needed.

This chapter includes brief discussion on existing TB drugs and covers a comprehensive picture of the anti-TB drug discovery status heading to achieve a goal of better drugs/regimen in terms of the desired properties stated above.

2. Existing TB drugs

After the discovery of Streptomycin in 1944, 15-20 antimycobacterial drugs have been approved and used for TB therapy according to the need, availability, cost and safety profile. These existing TB drugs can be classified into first line, second line and third line drugs (also summarized in Tables 1-3).

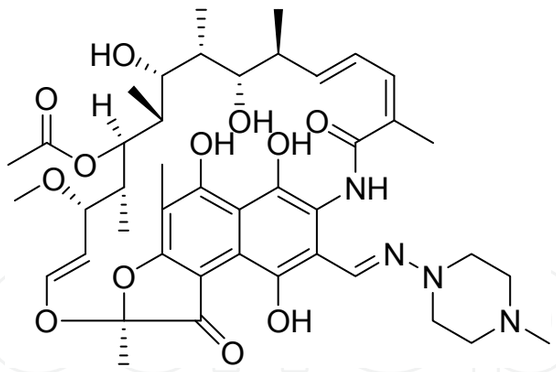
2.1 First line drugs

2.1.1 Rifampicin, RMP or R

Rifampicin was discovered in 1966. It is a semisynthetic, intensely red coloured bactericidal antibiotic (MIC 0.05-0.5 μg/mL) derived from *Amycolatopsis rifamycinica*. Its penetration to cerebrospinal fluid makes it useful to treat tuberculosis meningitis (Nan et al, 1992). RMP, should be used in combination with other antibiotics as resistance develops quickly during monotherapy. RMP may be excreted in breast milk, therefore breast feeding may be avoided during treatment. However no serious side effects have been observed in breastfed infants during RMP therapy (Peters & Nienhaus, 2008; Drobac et al 2005).

2.1.1.1 Mode of action

RMP inhibits DNA-dependent RNA polymerase in bacterial cells by binding its β-subunit, thus preventing transcription to RNA and subsequent translation to proteins (Aristoff et al, 2010; Tomioka, 2006). RMP-resistant bacteria produce RNA polymerases with subtly different β subunits which resists drug-inhibition (O'Sullivan et al, 2005)



Rifampicin

2.1.1.2 Dosing

Daily regimen 10 mg/kg (up to 600 mg/day) orally or intermittent regimen 10 mg/kg (up to 600 mg/day) orally, are prescribed. (The American Thoracic Society [ATS], 2006).

2.1.1.3 Adverse effects

The main target organs for side effects of RMP are the liver and the gastrointestinal system. Adverse effects include hepatitis with elevation of bile and bilirubin concentrations, anaemia, leucopenia, thrombocytopenia, bleeding, febrile reaction, eosinophilia, leucopenia, thrombocytopenia, purpura, haemolysis and shock, and nephrotoxicity (International Programme on Chemical Safety [INCHEM] a).

2.1.1.4 Pharmacokinetics

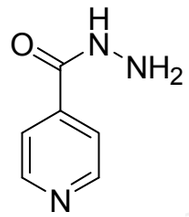
The half-life of RMP is generally 2 h (Acocella, 1978). Its absorption is not affected by antacids (Peloquin et al., 1999 a). RMP ester function is hydrolyzed in the bile by esterase catalyzed high pH. The deacetylated form of RMP can not be absorbed by the intestine and thus eliminated from the body.

2.1.1.5 Interactions

Absorption of RMP is considerably hindered when it is combined with another anti-TB drug, 4-aminosalicylic acid (PAS). Therefore, these two anti-TB drugs must be administered separately (8 to 12 hours interval). RMP affects metabolism of several known drugs, viz. warfarin, oral contraceptives, cyclosporine, itraconazole, digoxin, verapamil, nifedipine, simvastatin, midazolam and HIV protease inhibitors. Other drugs for possible interactions include clarithromycin, lorazepam atorvastatin, antiretroviral agents, rosiglitazone/pioglitazone, celecoxib, caspofungin (Baciewicz et al., 2008).

2.1.2 Isoniazid, INH or H

INH (isonicotinylhydrazine) was discovered in 1952. It is bactericidal (MIC 0.01-0.2 μg/mL) to fast replicating mycobacteria (Singh & Mitchison, 1954) but is bacteriostatic to slow-growing mycobacteria. Since the bacteria may exist in a non growing state (latent) for long periods, therapy for latent tuberculosis with INH is continued for a longer duration (6-12 months). However, INH monotherapy is never recommended to treat active tuberculosis due to the development of resistance.



Isoniazid

2.1.2.1 Mode of action

INH itself is a prodrug and is activated by mycobacterial catalase-peroxidase enzyme KatG which catalyzes the formation of isonicotinic acyl-NADH complex from isonicotinic acyl and NADH. This complex then binds to the enoyl-acyl carrier protein reductase known as InhA, consequently blocking the natural substrate enoyl-AcpM and fatty acid synthase. This results in inhibition of mycolic acid synthesis which is essential for the mycobacterial cell wall formation. A direct role for some INH-derived reactive species, such as nitric oxide, in inhibiting mycobacterial metabolic enzymes has also been shown (Timmins & Deretic, 2006; Suarez et al., 2009).

2.1.2.2 Metabolism

INH is metabolized in liver and its metabolites are excreted in the urine with 75 to 95% of the dose excreted in 24 hours (Ellard & Gammon, 1976).

2.1.2.3 Dosing

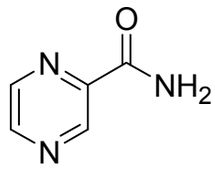
In adults, the recommended dose is 5 mg/kg/day (max 300 mg daily). For intermittent dosing (twice or thrice/week), 19-15 mg/kg/day (max 900 mg/day) is a standard dose. For patients with slow clearance of INH are put on reduced dosages. The recommended dose for children is 8 to 12 mg/kg/day (McIlleron et al., 2009; [ATS], 2006).

2.1.2.4 Adverse effects

INH causes acute toxicity in the CNS. It induces generalized convulsions, coma and metabolic acidosis. Death may occur from acute respiratory failure or hypotension. Liver, peripheral nervous and haematologic systems are the main target organs of INH chronic toxicity resulting in acute hepatitis, peripheral neuropathy, haemolytic anaemia (INCHEM, b). Vitamin B_6 (10–50 mg/day) supplements are suggested to compensate its (Vitamin B_6) depletion during treatment which may lead to peripheral neuropathy and CNS related side effects (Yamamoto et al., 2011).

2.1.3 Pyrazinamide, PZA or Z

PZA was discovered in 1952. It acts mainly as bacteriostatic agent but can be bactericidal for replicating *Mtb*. Its MIC is 20-100 μg/mL at pH 5.5 or 6.0. This drug is used in the first two months of treatment to shorten the duration of treatment, since regimens not containing PZA must be taken for nine months or more (Hong Kong Chest Service [HKCS]/ British Medical Research Council [BMRC], 1981). PZA crosses meninges and thus is effective for the treatment of tuberculous meningitis (Donald & Seifart, 1988).



Pyrazinamide

2.1.3.1 Dosing

20–25 mg/kg daily or 30–40 mg/kg thrice a week is a recommended dose. ([ATS], 2006).

2.1.3.2 Pharmacokinetics

PZA is well absorbed orally. It is metabolised by liver and the metabolic products are excreted by kidneys (Lacroix et al, 1989). The overall pharmacokinetics may differ in childrens (Arya et al., 2008).

2.1.3.3 Mode of action

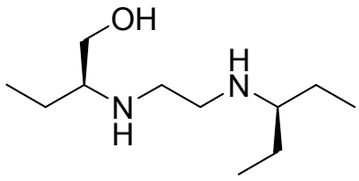
PZA is actually a prodrug. In acidic conditions, the enzyme pyrazinamidase (present in *Mtb*), converts it to the active form, pyrazinoic acid which consequently inhibits the enzyme fatty acid synthase (FAS) I, required by the bacterium to synthesise fatty acids (Zhang & Mitchison, 2003; Zimhony et al., 2007). Mutations of the pyrazinamidase gene (*pncA*) are responsible for PZA resistance in *Mtb* (Scorpio & Zhang, 1996)

2.1.3.4 Adverse effects

Some common adverse effects of PZA treatment include hepatotoxicity, joint pains (arthralgia), nausea, vomiting, anorexia, sideroblastic anemia, skin rash, hyperuricemia, dysuria, urticaria, pruritus, interstitial nephritis, malaise, porphyria and fever (rare) (Forget & Menzies, 2006).

2.1.4 Ethambutol, EMB or E

EMB was discovered in 1961 by Lederle Laboratories. It is a bacteriostatic drug. In spite of a relatively modest MIC of 10 μM like PZA, it is a useful drug for tuberculosis chemotherapy, partly because of very low toxicity and relatively few side-effects (Wilkinson et al., 1961; Thomas et al., 1961).



Ethambutol

2.1.4.1 Adverse effects

Adverse effects may include peripheral neuropathy, red-green color blindness, arthralgia, hyperuricaemia, vertical nystagmus and optic neuritis (Lim, 2006).

2.1.4.2 Mode of action

It blocks formation of *Mtb* cell wall by interfering in the synthesis of arabinogalactan (an essential component for the formation of mycolyl-arabinogalactan-peptidoglycan complex of the *Mtb* cell wall) via inhibiting the enzyme arabinosyl transferase (Belanger et al., 1996; Wiles & Jacobs Jr, 1997).

2.1.4.3 Pharmacokinetics

It is well absorbed in the gastrointestinal tract, and well distributed in body tissues and fluids. 50% of the given dose is excreted unchanged in urine (Peloquin et al., 1999 b).

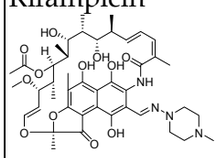
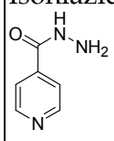
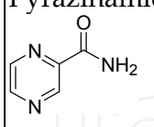
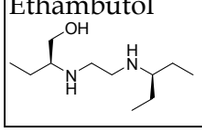
Drug	Mode of Action	Target	Daily Dose (Max. Dose)	Possible adverse reactions
Rifampicin 	Inhibits RNA synthesis	RNA polymerase beta subunit	10mg/kg (600 mg/day)	Pruritus, rash, flushing, redness and watering of eyes, breathlessness, nausea, vomiting, abdominal cramps, diarrhea, jaundice, hepatitis, liver failure (rare and in severe cases), chills, fever, headache, arthralgia, and malaise
Isoniazid 	Inhibition of cell wall formation	Acyl carrier protein reductase	5 mg/kg/ day (300 mg daily)	Rash, hepatitis, sideroblastic anemia, metabolic acidosis, peripheral neuropathy, mild central nervous system (CNS) effects, intractable seizures (status epilepticus), headache, poor concentration, weight-gain, poor memory, and depression
Pyrazinamide 	Disruption of membrane transport and energy depletion	Membrane energy metabolism	20–25 mg/kg daily (30 mg/kg)	Hepatotoxicity, joint pains (arthralgia), nausea, vomiting, anorexia, sideroblastic anemia, skin rash, hyperuricemia, dysuria, urticaria, pruritus, interstitial nephritis, malaise; porphyria
Ethambutol 	Inhibition of cell wall formation	Arabinosyl transferase	15 mg/kg daily (25 mg/kg)	Peripheral neuropathy, color blindness, arthralgia, hyperuricaemia, vertical nystagmus and optic neuritis.

Table 1. First Line Drugs

2.2 Second Line Drugs (SLDs)

A drug may be categorized as second (or as third) line if it includes one or more of the following: i. it has side-effects beyond a tolerance threshold (e.g., cycloserine), ii. its

administration is not oral and at the same time (sub)equivalent/better affordable oral medications are available, iii. it is less effective than the first-line drugs (e.g., *p*-aminosalicylic acid); iv. its cost is impractical for routine treatment.

2.2.1 Classification of SLDs

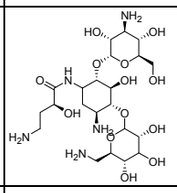
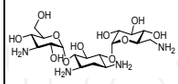
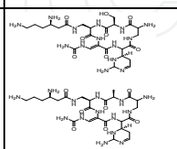
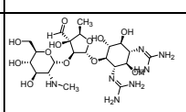
The available second-line TB drugs (SLDs) can be classified as:

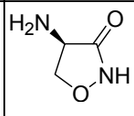
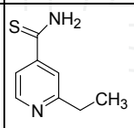
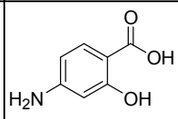
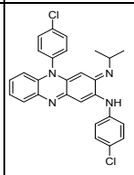
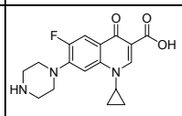
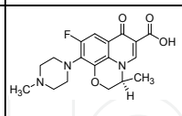
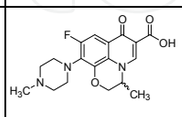
1. Aminoglycosides: e.g. amikacin (AMK), kanamycin (KM), gentamicin etc;
2. Polypeptides: e.g., capreomycin, viomycin, enviomycin;
3. Fluoroquinolones: e.g., ciprofloxacin (CIP), levofloxacin, moxifloxacin (MXF);
4. Thioamides: e.g. ethionamide, prothionamide
5. Oxazolidinone: (Cycloserine, the only antibiotic in its class);
6. *p*-Aminosalicylic acid (PAS or P).

Details of some of these SLDs are provided in the table 2.

2.3 Third line drugs

Apart from the reasons listed under second line drugs, a drug may be considered as a third line if it is useful but lacks sufficient efficacy proofs. Rifabutin, macrolides: (e.g., clarithromycin), linezolid, thioacetazone, thioridazine, arginine, vitamin D may be considered as third line antituberculosis drugs.

Drug (Discovery) Route	**Structure**	**Mode of Action**	**Daily Dose (Max. Dose)**	**Adverse effects**
Amikacin (1972) IM or IV		Inhibits protein synthesis by (binds to the bacterial 30S ribosome)	15 - 30 mg/kg (1 g) MIC 4-8 µg/mL (CDC, 1994) [a]	Auditory, vestibular, and renal toxicity, dizziness
Kanamycin (1957) IM or IV		Inhibitions protein synthesis via S12 ribosomal protein & 16 S RNA.	15 - 30 mg/kg (1 g) MIC 1-8 µg/mL	Auditory, vestibular, and renal toxicity
Capreomycin (1963) IM or IV		Inhibits protein synthesis (binds to ribosomal subunit 16S and 23S rRNA (Johansen et al., 2006)	15 - 30 mg/kg (1 g) MIC 1.25–2.5 µg/mL (Heifets, 1988; Heifets & Lindholm-Levy 1989)	Auditory, vestibular, and renal toxicity
Streptomycin (1944) IM		Same as Kanamycin	15-40 mg/kg (1 g) MIC 2-8 µg/mL	Renal, ophthalmic and respiratory toxicity

Drug (Discovery) Route	Structure	Mode of Action	Daily Dose (Max. Dose)	Adverse effects
Cycloserine (1952) Oral		Inhibition of peptidoglycan synthesis (D-alanine racemase)	15 - 20 mg/kg (1 g) MIC 5-20 μg/mL	Psychosis, Rashes, Convulsions Depression
Ethionamide (1956) Oral		Inhibition of mycolic acid synthesis	15 - 20 mg/kg (1 g) MIC 0.6-2.5 μg/mL	GI upset Hepatotoxicity Hypersensitivity Metallic taste
PAS (1946) Oral		Inhibition of folic acid and iron metabolism (unknown target)	150 mg/kg (16 g) MIC 1-8 μg/mL	GI upset Hypersensitivity Hepatotoxicity Sodium load
Clofazimine (1954) Oral		Inhibits bacterial proliferation by binding to the guanine bases of bacterial DNA	100 - 300 mg/day MIC 0.12 - 0.24 μg/mL (Lu et al. 2008)	Eosinophilic enteritis, GI irritation, discoloration of the skin (upon sun exposure)
Ciprofloxacin (1960s) Oral		Inhibition of DNA replication and transcription by inhibiting DNA gyrase	750 - 1500 mg/day MIC 0.4 to 6.2 μg/mL (Trimble et al., 1987)	GI upset Dizziness Headache Hypersensitivity Restlessness
Levofloxacin (1992) Oral		Same to Ciprofloxacin	500 mg/day MIC 0.50 to 0.75 μg/mL (Rastogi et al., 1996)	Same as for Ciprofloxacin
Ofloxacin (1980) Oral		Same to Levofloxacin	600 - 800 mg/day MIC 0.12-2 μg/mL (Vacher et al, 1999)	Same as for Ciprofloxacin

MIC (wherever not referenced) is based on Inderlied & Salfinger, 1999.
IM - intramuscular, IV – intravenous
[a]Centre for Disease Control and Prevention

Table 2. Some Second Line Drugs (Source partly from North Dakota Department of Health, 2011).

3. Drug discovery programme

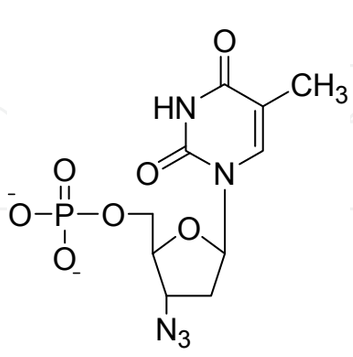
3.1 Early stage drug discovery

Tuberculosis is not only a health threat in Asian or European countries, but a serious problem globally. There is an ever increasing threat of drug-resistant TB appearing as an epidemic in many countries, particularly because no new classes of drugs have been specifically developed for the treatment of tuberculosis since the introduction of RMP in 1967. To tackle this devastating disease, continued high priority research and great efforts are being made to investigate new classes of drugs all over the world. Bill and Melinda Gates foundation has made a major financial philanthropic contribution in this regard worldwide. Governments and private sectors are also opening new avenues with significant funds to fight this disease. Apart from big industries, great roles are being played behind the curtains by basic and semi-applied researchers who start from scratch and work within financial constraints. Following are such examples of different classes of compounds from early stage screening studies.

Since research in this field gained momentum after the year 2000, selected reports published from the year 2000 onwards are included here. In view of the scope and timelines of this chapter, the focus of the literature cited is medicinal chemistry.

3.1.1 Nucleosides

Nucleosides have been of great interest as antiviral agents since decades back. Soon after the emergence of *Mtb* thymidine monophosphate kinase (TMPKmt) as a potentially attractive target for the design of a novel class of antituberculosis agents in year 2001 (Munier-Lehmann et al., 2001), several series of 2′-, 3′-, and 5-modified nucleosides and nucleotides were synthesized and evaluated for their affinities with respect to TMPKmt. Vanheusden et al, in 2002, reported monophosphates of AZT (**1**) and 2′-chloro-2′-deoxythymidine (**2**), as potent inhibitors of TMPKmt with Ki values of 10 and 19 μM, respectively.



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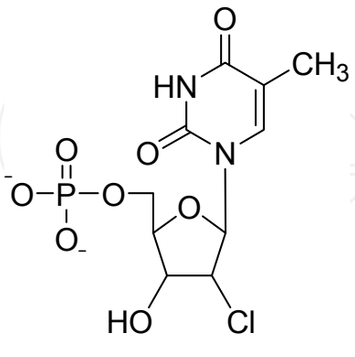

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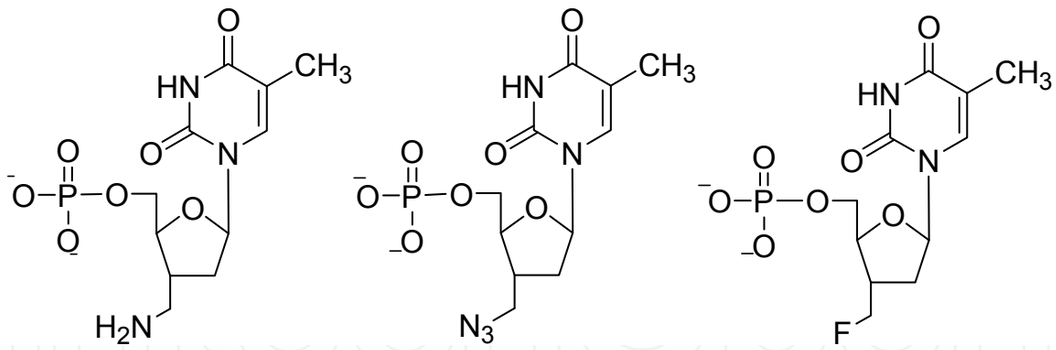
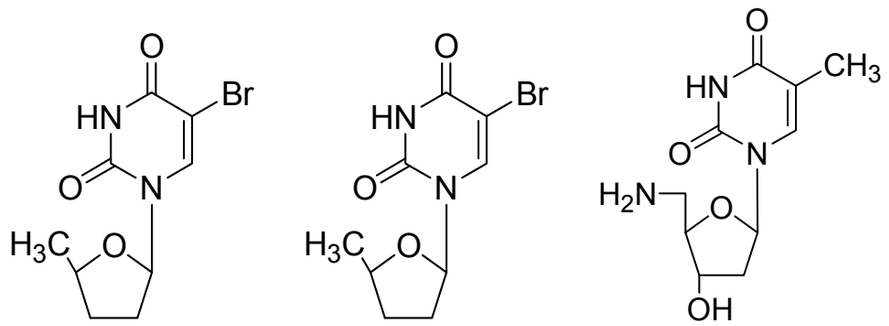
These authors in the following year (Vanheusden et al, 2003) further reported a series of 3′-*C*-branched-chain-substituted nucleosides and nucleotides for the same target. The compounds **3, 4**, and **5** were reported to exhibit *K*i values of 10.5, 12, and 15 μM, respectively, for TMPKmt.



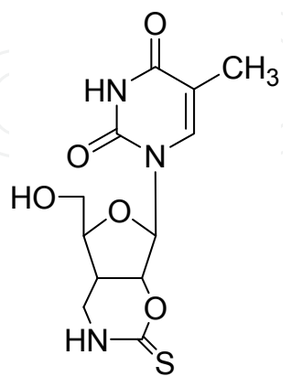
3 **4** **5**

In the year 2003, another series was reported by the same authors (Vanheusden et al., 2003) where 5-substituted-2′,3′,5′-trideoxyuridines (**6**-**8**) exhibited Ki values of 5, 7 and 12 μM, respectively, for TMPKmt.



6 **7** **8**

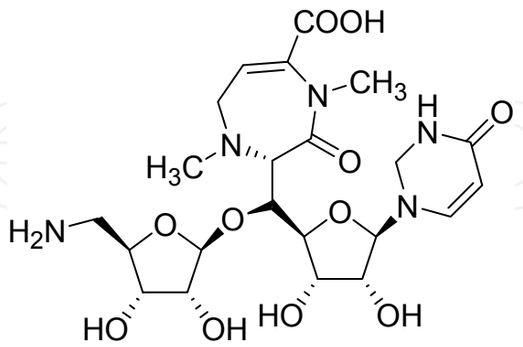
Vanheusden et al. (Vanheusden et al., 2004) also reported a series of bicyclic analogues of thymidine where compound **9** demonstrated Ki of 3.5 μM for TMPKmt with good selectivity index (SI 200) over TMPKh.



9

In all these reports, however, only enzyme inhibition was described and inhibition of mycobacterial replication was not demonstrated.

A nucleoside antibiotic (CPZEN-45) produced by Streptomyces sp., first described in 2003 by the Microbial Chemistry Research Foundation (MCRF) and Meiji Seika Kaisa Ltd. of Japan, is now undergoing preclinical studies as an anti-TB agent. Details of CPZEN-45 are provided in the preclinical section.

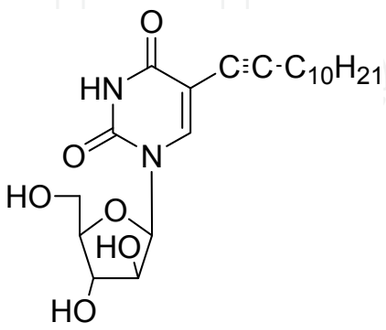


CPZEN-45

The complete genome sequence of *Mtb* has been deciphered (Cole et al., 1998). It encodes many of the enzymes required for DNA and RNA synthesis, and pyrimidine and purine biosynthesis. Our group (Johar et al, 2005) therefore hypothesized that modified nucleoside analogs could target several enzymes involved in nucleic acid metabolism. We were first to investigate and demonstrate potent antimycobacterial activity of 5-substituted pyrimidine nucleoside analogs (Johar et al., 2005). The antimycobacterial activity of test nucleosides was examined by mycobacterial growth inhibition using microplate alamar blue assay (MABA) (Franzblau et al., 1998). We observed that the most potent TMPKmt inhibitors reported earlier (Pochet et al., 2003; Vanheusden et al., 2002; Vanheusden et al., 2003) did not show antituberculosis activity in whole cell based assays. Thus the ability of a compound to function as a selective inhibitor of TMPKmt may not correlate well with its antimycobacterial activity. A cell based assay includes the steps of entry into bacterial cells and metabolism which could otherwise limit the efficacy of test molecules (Johar et al., 2005).

Since the initial report in 2005, our group (Kumar, R. and colleagues) has made a significant contribution in the evaluation of pyrimidine nucleosides as anti-tuberculosis agents. During our studies, we initially investigated the effect of a number of known antiviral and anticancer nucleosides modified in the base and/or sugar moiety against *Mtb*, *M. bovis* and *M. avium*. At concentrations upto 100 μg/ml, none of these agents showed potent inhibition of mycobacterial growth. In our subsequent studies, we designed, synthesized and examined a variety of 2-, 4-, 5- and/or 6-substituted/unsubstituted pyrimidine nucleosides containing various deoxyribose, ribose, arabinose, dideoxyribose and acyclic moieties. During our continued search of novel anti-TB agents, we found that 5-alkynyl substituted pyrimidine nucleosides were very potent inhibitors of mycobacteria (Rai et al., 2005). We (Johar et al, 2007), reported that pyrimidine nucleoside analogs 1-*β*-D-2′-arabinofuranosyl-5-dodecynyluracil (**10**), 1-(2′-deoxy-2′-fluoro-*β*-D-ribofuranosyl)-5-dodecynyluracil (**11**), and 1-(2′-deoxy-2′-fluoro-*β*-D-ribofuranosyl)-5-tetradecynyluracil (**12**) exhibited potent antimycobacterial potency in the series against *M. bovis* and *Mtb*. The MIC_{90} exhibited by compounds **10**, **11**,

and **12** (1-5 μg/mL) against *Mtb* H37Ra was close to that of the reference drug RMP (0.5-1 μg/mL). These compounds were also found to retain sensitivity against a RMP-resistant strain of *Mtb* H37Rv (American Type Culture Collection [ATCC] 35838, resistant to RMP at 2 μg/mL) at similar concentrations. No significant toxicity for these compounds was observed in MTT test *in vitro* against Vero cells and human foreskin fibroblast (HFF cells) up to a concentration of 100 μg/mL (CC_{50} >100 μg/mL).



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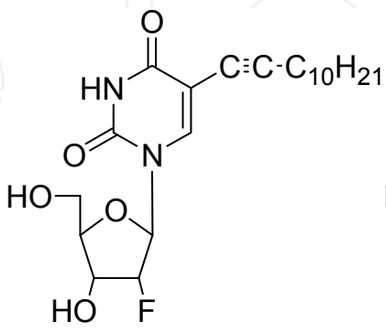

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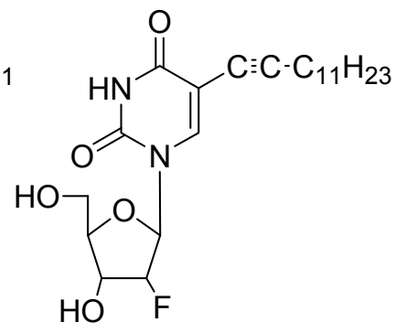
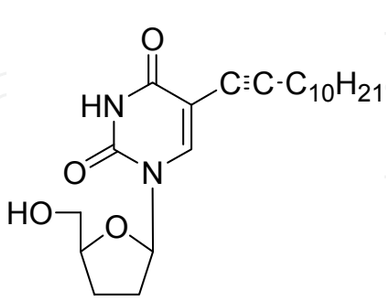
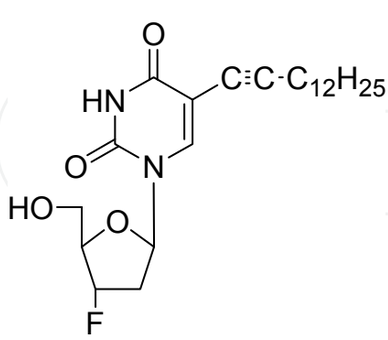


12

In the same year, we (Rai et al, 2007) further reported syntheses and evaluation of a series of 5-acetylenic derivatives of 2′,3- dideoxyuridine, and 3′-fluoro-2′,3′-dideoxyuridine for their antimycobacterial activity against *M. bovis*, *Mtb*, and *M. avium*. Compound **13** (among 2′,3′-dideoxyuridine series) and compound **14** (among 3′-fluoro-2′,3′-dideoxyuridine series) demonstrated excellent antimycobacterial activity (MIC 1-2 μg/mL) against *Mtb* H_{37}Ra. The compounds **13** and **14**, were also subjected to determine their antimycobacterial activity against a RMP-resistant H37Rv strain (ATCC 35838, resistant to RMP at 2 μg/mL) of *Mtb* using the radiometric-BACTEC assay. The drug-resistant *Mtb* strain was susceptible to the compounds **13** and **14** (MIC_{90} 1-2 μg/mL). No toxicity was observed *in vitro* against Vero cells (MTT test) up to the highest concentrations tested (CC_{50} > 100 μg/mL).



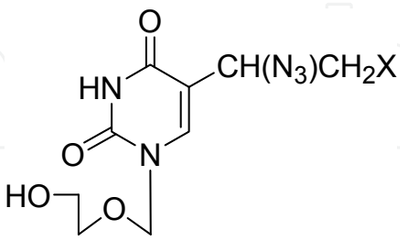
13



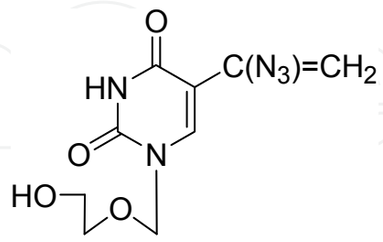
14

In a subsequent article in the same year by our group (Srivastav et al, 2007), *in vitro* anti-mycobacterial activities of several 5-substituted acyclic pyrimidine nucleosides containing 1-(2-hydroxyethoxy)methyl and 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl] acyclic moieties were investigated against *Mtb* H37Ra, *M. bovis*, and *M. avium*. In this study, 1-(2-hydroxyethoxy)methyl-5-(1-azido-2-haloethyl (**15a**), 1-azidovinyl) analog (**15b)**, 1-[(2-

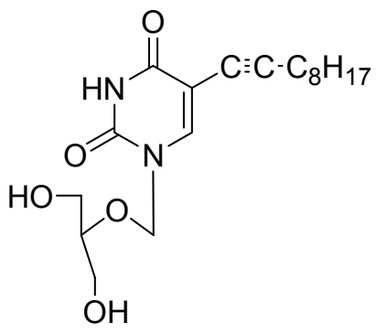
hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-decynyluracil (**16a**), and 1-[(2-hydroxy-1-(hydroxymethyl)ethoxy)methyl]-5-dodecynyluracil (**16b**) exhibited moderate *in vitro* anti-tubercular activity (100% inhibition @ 50 µg/mL) against these mycobacteria. These compounds did not show any toxicity *in vitro* against Vero cells and HepG2 cells up to a concentration of 100 µg/mL.



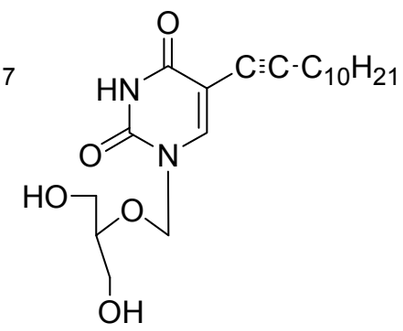
15a



15b

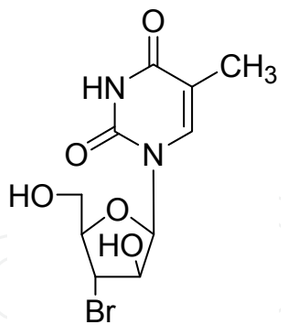


16a



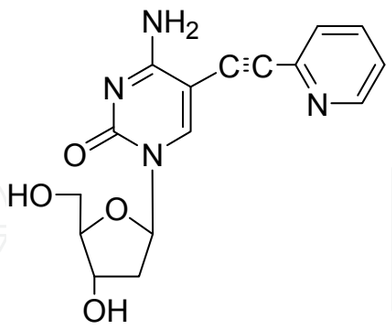
16b

In continued efforts in drug design and discovery for anti-tuberculosis agents, our group (Shakya et al, 2010) investigated various 2′- or 3′-halogeno derivatives of pyrimidine nucleosides containing uracil, 5-fluorouracil, and thymine bases. Among the compounds tested, 3′-bromo-3′-deoxy-arabinofuranosylthymine (**17**) was the most effective antituberculosis agent in the *in vitro* assays against wild-type *Mtb* strain (H37Ra) which displayed MIC_{50} = 1 µg/mL by the MABA assay. Further, it displayed MIC_{50} = 1-2 µg/mL against drug-resistant (H37Rv) (RMP-resistant and INH-resistant) strains of *Mtb* using BACTEC assay (Collins & Franzblau, 1997). The antimycobacterial effect of potent compounds was also determined against intracellular mycobacteria in a human monocytic cell-line (THP-1) infected with *Mtb* H37Ra strain using the colony-forming units (CFU) assay (Bermudez et al., 2001). Interestingly, the compound **17** demonstrated slightly better activity against intramacrophagic mycobacteria (80% reduction at 10 µg/mL concentration) than extracellular mycobacteria (75% reduction at 10 µg/mL concentration). In contrast, pyrimidine nucleosides possessing 5-fluorouracil base were weak inhibitors of *Mtb* H37Ra. The XTT and ^3H incorporation assays were performed to evaluate the toxicity of the investigated compounds *in vitro* against a human hepatoma cell line (Huh7). No cytotoxicity was found up to the highest concentration of compounds tested (CC_{50}> 100-200 µg/mL).



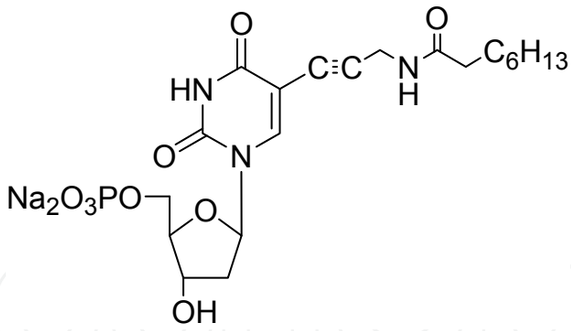
17

Our group in the same year (Srivastav et al., 2010) reported investigation of antimycobacterial activities of several 5-alkyl, 5-alkynyl, furanopyrimidines and related 2′-deoxynucleosides against *Mtb*. Compounds with 5-arylalkynyl substituents displayed potent *in vitro* antitubercular activity against *M. bovis* and *Mtb* (MIC 0.5-5 μg/mL). We found that 5-(2-pyridynylehynyl)-2'-deoxycytidine (18) exhibited potent activity against *Mtb* and showed no cytotoxicity Huh-7 cells up to a concentration of >200 μg/mL using XTT and ^{3}H-thymidine uptake assays. Therefore it was selected to test its potency in a mouse model (BALB/c) of *Mtb* (H37Ra) infection. At a dose of 50 mg/kg for 5 weeks, compound **18** showed promising *in vivo* efficacy in this mouse model. Statistically significant reduction in mycobacterial load was observed in lungs, livers and spleens of the treated mice. Our work provides first evidence of antimycobacterial potential of 5-substituted pyrimidine nucleosides in an animal model as a potential new class of antituberculosis agents.



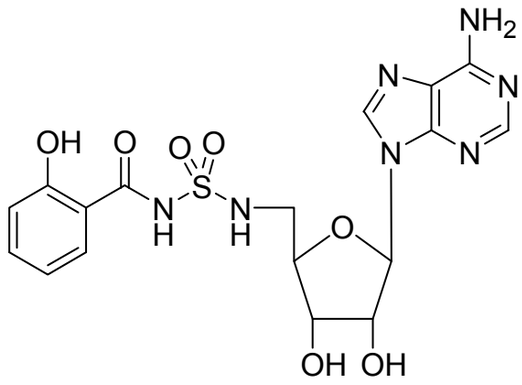
18a

Recently, Kogler et al (Kogler et al., 2011) reported a series of 5-substituted -2'-deoxyuridine monophosphate analogs as potential inhibitors of mycobacterial flavin-dependent thymidylate synthase (ThyX). Compound N-(3-(5-(2'-deoxyuridine-5'-monophosphate)) prop-2-ynyl)-octanamide displayed selective potent inhibition of ThyX with an IC_{50} value of 0.91 μM. This derivative was found to lack activity against the classical mycobacterial thymidylate synthase (ThyA, IC_{50} >50 μM).



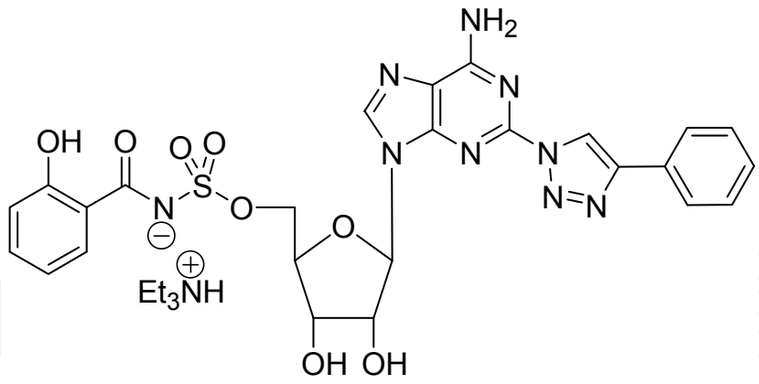
18b

Somu et al (Somu et al., 2006) reported a purine nucleoside compound **19 (**MIC_{99} = 0.19 μM) inhibiting siderophore biosynthesis of *Mtb* in H37Rv strain under iron-limiting conditions (Domenech et al., 2005, as cited in Somu et al., 2006). The activity of **19**, according to the authors, was due to inhibition of the adenylate-forming enzyme MbtA, which is involved in biosynthesis of the mycobactins. The cytotoxicity of the potent compounds in the series was evaluated against the P388 murine leukemia cell line. None of the inhibitors displayed any toxicity up to the maximum concentration tested (ED_{50} > 100 μg/mL).



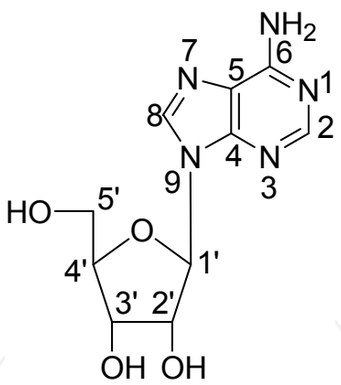
19

Gupte et al (Gupte et al., 2008) demonstrated 2-triazole derivatives of 5′-*O*-[*N*-(salicyl)sulfamoyl]adenosine 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 *E. coli*. On the basis of observed potency (MIC 3.13 μM), selectivity, lack of cytotoxicity, and enhanced lipophilicity, compound **20** was reported as the best candidate. No inhibition of cell growth was observed upto 100 μM when this class of compounds were evaluated for inhibition of cell viability against Vero cells using the MTT assay. The compound **20** was also evaluated against MEL, OCL-3, and REH human cancer cell lines. Cell proliferation of OCL-3 and REH lines were not affected at 100 μM, while in the MEL line approximately 25% inhibition was shown at 100 μM.



20a

Adenosine (Ado) kinase is a purine salvage enzyme that phosphorylates adenosine to adenosine-monophosphate. A large number of adenine modified nucleosides were evaluated as substrates and inhibitors of Ado kinase from *Mtb* (Long & Parker, 2006) The best substrates were 2-aza-adenosine, 8-aza-9-deazaadenosine and 2-fluoroadenosine and the most potent inhibitors 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 showed promising antitubercular activity when MIC studies were performed.



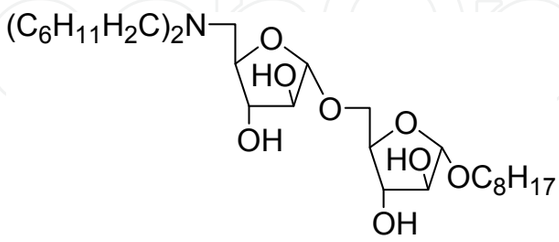
20b

In an extension of their work (Long et al, 2008) modifications to the base and ribofuranosyl moiety or modifications to the glycosidic bond positions of adenosine were analyzed against *Mtb* Ado kinase. In this study, the best substrates identified were carbocyclic adenosine, 8-aza-carbocyclic adenosine and 9-[a-L-lyxofuranosyl]-adenine.

3.1.2 Carbohydrates

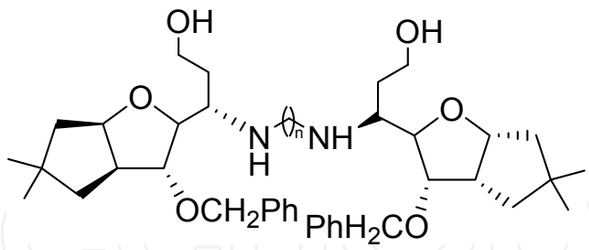
Sugar derivatives have also been examined as antimycobacterial agents. Although many reports have been published, most of them did not include toxicity data. Some representative examples of this class are summarized here.

Pathak et al (Pathak et al, 2003) synthesized several octyl 5-O-(α-D-arabinofuranosyl)-α-D-arabinofuranoside disaccharide analogs substituted at the 5-position of the non-reducing end of sugar and tested *in vitro* (Suling et al., 1998, as cited in Pathak et al, 2003) against *Mtb* (H37Ra, ATCC 25177), *M. avium* complex (MAC) as well as in a cell free assay system for arabinosyltransferase acceptor/inhibitor activity (Lee et al., 1997, as cited in Pathak et al, 2003). Compound **21** displayed IC_{50} of 1.56 mM in cell free assay and MIC 8 μg/mL against *Mtb*. No toxicity data was reported.



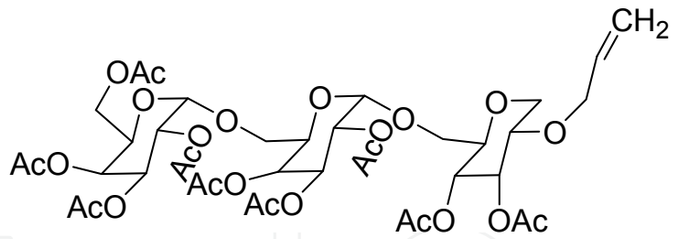
21

Tripathi et al (Tripathi et al., 2005) reported bis-glycosylated diamino alcohols with the most active compound **22a** showing MIC of 3.12 μg/mL against *Mtb* H37Ra as determined by MABA assay. But this compound displayed MIC > 50 μg/mL against *Mtb* H37Rv by Agar microdilution method (Saito et al., 19991, as cited in Tripathi et al., 2005). In this series, they discovered the next active compound **22,** exhibiting activity against *Mtb* H37Ra (MIC 12.5 μg/mL by MABA assay) and against *Mtb* H37Rv (MIC 6.25 μg/mL by Agar microdilution method) that was considered to test further. The compound **22** was also found to be active against MDR strain and showed mild protection in mice. According to the report, this compound seems to possess efficacy against *Mtb* infection in mice at non-toxic concentration (25 mg/Kg). However, at higher doses it caused toxicity.



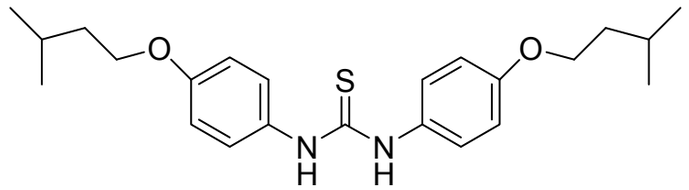
22a, n=10, 22b, n=12

Chiba et al (Chiba et al., 2007) synthesized sugar derivatives of stachyose, and evaluated them for antibacterial activity against *Mtb*, *M. avium*, and *S. aureus* using broth dilution methods (Takii et al., 2002, as cited in Chiba et al., 2007) in MiddleBrook 7H9 broth. The compound **23** (OCT359) was identified as the most active compound in the series with MIC 3.13 μg/mL against *Mtb* H37Rv. OCT359 was also tested against various drug-sensitive and -resistant clinical isolates of *Mtb*. Among them 25 clinical isolates of drug-resistant *Mtb* and 19 drug-sensitive *Mtb* were sensitive to OCT359. The MICs of OCT359 for these clinical isolates ranged from 3.13 to 25 μg/mL. No toxicity data was reported on any host cell lines.

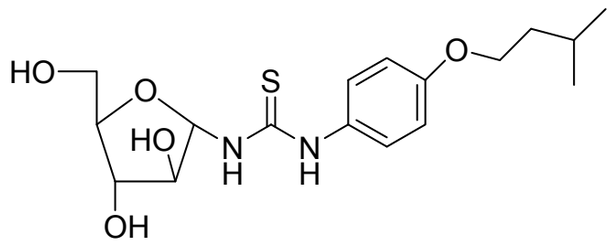


23

Liav et al (Liav et al., 2008) prepared derivatives of thiocarlide (THC), a previously known antitubercular drug, for their evaluation against *Mtb* H37Rv using MABA assay. The most active compound reported was **24** having MIC in the range of 1.56-3.12 μg/mL. No toxicity data for this compound was presented on any host cell line.

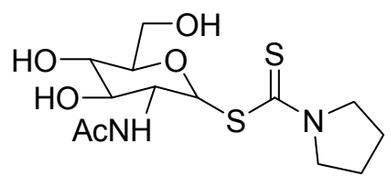


THC



24

In a recent report, Horita (Horita et al, 2011) described modification of their previously reported lead compound OCT313 (Glc-N-Ac -DMDTCB) (MIC 25 μg/mL against *Mtb* H37Rv by Broth dilution method). The resultant compound Glc-NAc-pyrrolidine dithiocarbamate (**25**, OCT313HK, Glc-NAc-PDTC) exhibited potent anti-tubercular activity with MIC of 6.25 μg/mL. OCT313HK was also effective against *Mtb* clinical isolates, including MDR and XDR strains at similar concentrations (MIC 6.25-12.5 μg/mL). No toxicity data was reported on mammalian cell lines.



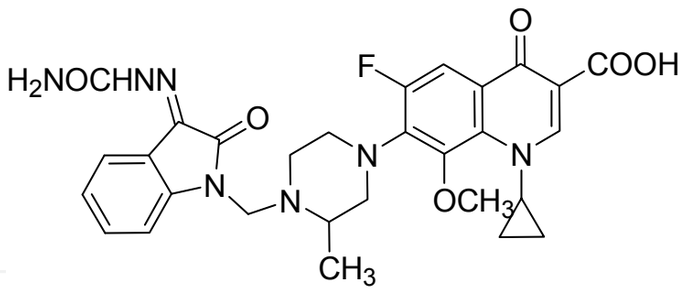
25

3.1.3 Heterocyclic compounds

3.1.3.1 Quinolines and quinoxalines

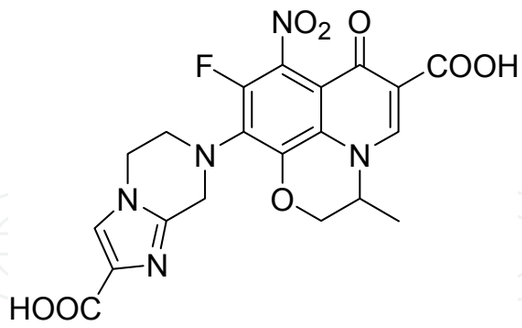
Quinolines have also been of interest for evaluation as antibacterial agents since fluoroquinolones are already used as antibiotics (e.g. ciprofloxacin, laevofloxacin, ofloxacin). Moxifloxacin and Gatifloxacin from this class are in Phase III clinical trial for tuberculosis treatment (see details in the section describing drugs in Phase III). Many research articles are available in literature on quinoline as anti-TB agents.

Sriram (Sriram et al, 2006) reported a series of 7-substituted derivatives of gatifloxacin and evaluated them for antimycobacterial activity *in vitro* and *in vivo* against *Mtb* H37Rv and MDR-TB. The compounds were also tested for their ability to inhibit the supercoiling activity of DNA gyrase from *Mtb*. Among this series, compound **26** was found to be equally active (IC_{50} of 3.0 μg/mL) as gatifloxacin in the inhibition of the supercoiling activity of wild-type *Mtb* DNA gyrase. The compound **26** was also found to be the most active *in vitro* with an MIC of 0.0125 μg/mL against *Mtb* and MDR-TB. Activity evaluation *in* animal model showed that this compound decreased the bacterial loads in lung and spleen tissues by 3.62- and 3.76-log10, respectively. After 72 h exposure with the test compounds, viability of Vero cells was assessed using MTT assay to determine their cytotoxicity. The compounds were found to be non-toxic up to a concentration of 62.5 μg/mL. The compound **26** showed selectivity index (IC_{50}/MIC) of >1250.



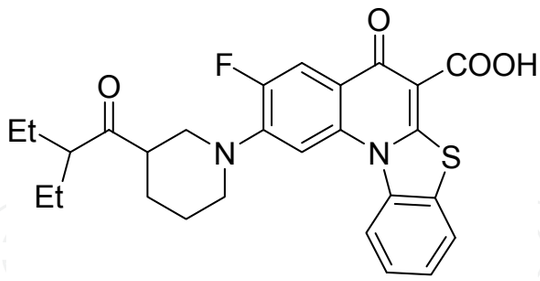
26

Sriram and coworkers (Dinakaran et al, 2008 a) also synthesized novel ofloxacin (OFX) derivatives and evaluated them for *in vitro* and *in vivo* antimycobacterial activities against *Mtb* H37Rv , MDR-TB, and *M. smegmatis* using agar dilution method. These compounds were also tested for their ability to inhibit the supercoiling activity of DNA gyrase from mycobacteria. Among the synthesized compounds, **27** exhibited most potent activity (MIC_{99} of 0.19 μM and 0.09 μM against *Mtb* and MDR-TB, respectively). The compound **27** decreased bacterial loads (strain ATCC 35801) in lung and spleen tissues by 1.91 and 2.91 -log10, respectively, at 50 mg/kg dose when evaluated in a mouse model. This compound was reported to possess a selectivity index (IC_{50}/MIC) of >1467.



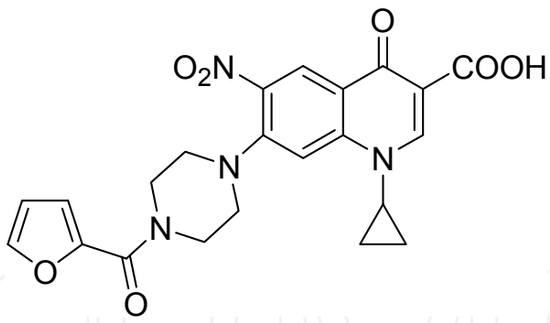
27

Another publication by the same group (Dinakaran et al, 2008 b) described various 2-(sub)-3-fluoro/nitro-5,12-dihydro-5-oxobenzothiazolo[3,2-a]quinoline-6-carboxylic acid derivatives. Among the reported compounds, **28** displayed the most potent activity *in vitro* with MICs of 0.18 and 0.08 μM against *Mtb* and MDR-TB, respectively. In a mouse model of *Mtb* infection, **28** decreased bacterial loads in lung and spleen tissues with 2.78 and 3.12 _ log10, respectively, at the dose of 50 mg/kg. The selectivity indices (IC_{50}/MIC) of the compound **28** were reported to be 1576 against MDR-TB and 700 against *Mtb*. Phototoxicity evaluation was also performed (Mayne et al., 1997, as cited in Dinakaran et al, 2008 b) and no significant phototoxicity was recorded.



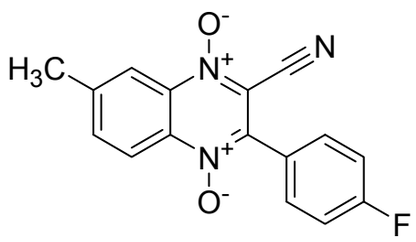
28

Senthilkumar et al, 2009, published synthesis of various 1-(substituted)-1,4-dihydro-6-nitro-4-oxo-7-(sub-secondary amino)-quinoline-3-carboxylic acids. Among the compounds investigated, **29** was found to be the most potent compound *in vitro* with MIC values of 0.08 and 0.16 μM against *Mtb* and MDR-TB, respectively. In the *in vivo* studies, **29** significantly decreased bacterial load in lung and spleen tissues, at 50 mg/kg dose. The SI (IC_{50}/MIC) of **29** was stated to be 793 against MDR-TB and 1586 against *Mtb*. No significant phototoxicity was described for **29**.



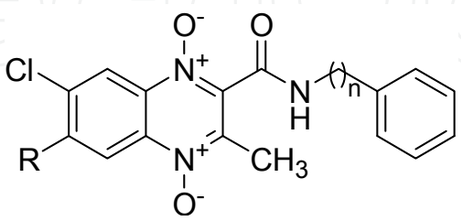
29

Other groups have also been exploring quinoline derivatives as anti-TB agents. Vicente et al (Vicente et al., 2009) published a series of 3-phenylquinoxaline 1,4-di-N-oxide against *Mtb* H37Rv using MABA assay. The compounds exhibiting fluorescence were tested in the BACTEC 460-radiometric system. The compounds affecting <90% inhibition in the primary screen (MIC >6.25 μg/mL) were not evaluated further. Thirty-four of the seventy tested compounds showed MIC values less than 0.2 μg/mL. The most active compound reported was **30** (MIC <0.2 μg/mL) with an IC_{50} >100 (SI >500).



30

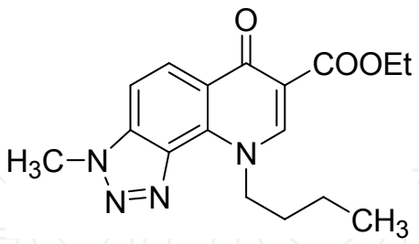
Ancizu et al (Ancizu et al., 2010) described a series of 3-methylquinoxaline-2-carboxamide 1,4-di-N-oxide derivatives. Many of the tested compounds showed MIC values less than 1 μg/mL. In this report, compounds **31** and **32** displayed most significant inhibition of *Mtb* H37Rv (MIC <0.2 μg/mL). Cytotoxicity evaluation indicated that **31** and **32** were non-toxic with IC_{50} value of >100 and SI >500.



31, R=Cl, n=1; 32, R=H, n=2

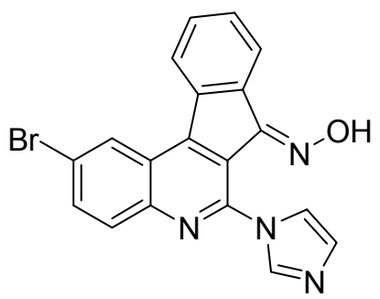
Carta et al (Carta et al., 2007) reported antimycobacterial evaluation of 3-methyl-9-substituted-6-oxo-6,9-dihydro-3H-[1,2,3]-triazolo [4,5-h]quinolone-carboxylic acids and their esters against wild-type H37Rv and 11 clinically isolated strains of *Mtb*. Several derivatives inhibited mycobacterial replication with MIC_{90} in the range of 0.5–3.2 μg/mL. The most

potent compound **33** (MIC_{90} = 0.5 μg/mL) showed no cytotoxicity (CC_{50} > 50 μg/mL), when tested against human macrophages and Hep-2 cells.



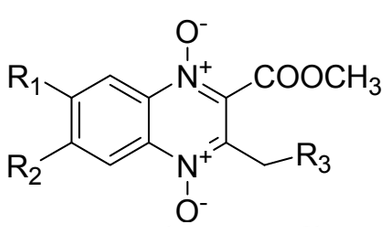
33

Upadhayaya et al (Upadhayaya et al., 2011) identified indeno[2,1-c]quinoline derivatives which were considerably active (MIC 0.39-0.78 μg/mL) but had solubility problems. Ester derivatives of the lead compound indeno[2,1-c]quinolines were synthesized, which showed 2- to 4-fold improved anti-TB activities, with increased solubility and superior selectivity index (SI) over their respective parent compounds. In this study, compound 34 was described to be the most potent agent with MIC of <0.39 μg/mL. In general, no cytotoxicity was observed in Vero cells.



34

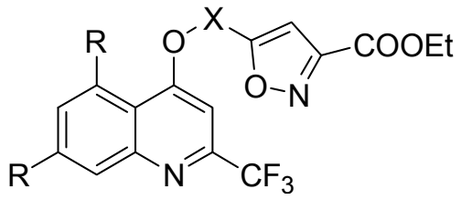
Jaso et al (Jaso et al., 2005) evaluated a series of 6(7)-substituted quinoxaline-2-carboxylate 1,4-dioxide derivatives against *Mtb* H37Rv. Fourteen compounds were selected to test for their activity against intramacrophagic mycobacteria. It was found that ethyl and benzyl 3-methylquinoxaline-2-carboxylate 1,4-dioxide derivatives with a chlorine group at position 7 of the benzene moiety (compound **35**, MIC 0.1 μg/mL, SI 470) and the unsubstituted derivative (**36**, MIC 0.1 μg/mL, SI 76) have good antitubercular activity, including activity in macrophages (EC_{90} 0.15 μg/mL and 0.0005 μg/mL, respectively). The compounds **37** and **38** of the series were also active against drug-resistant strains of *Mtb* H37Rv with MIC 0.39-1.56 and 3.13-12.5, respectively.



35, R1= H, R2 =H, R3 = Ph; **36**, R1= Cl, R2 =H, R3 = Ph;

37, R1= R2 = R3 = CH3; **38**, R1= Cl, R2 =Cl, R3 = Ph

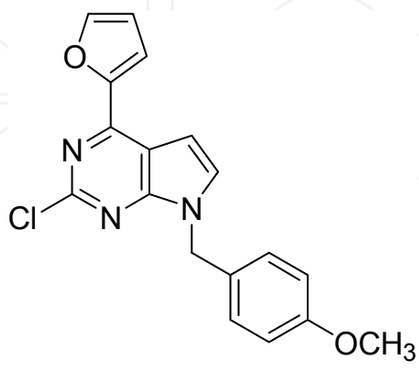
Lilienkampf et al (Lilienkampf et al., 2009) revealed several potent quinolines bearing an isoxazole containing side-chain as anti-TB compounds. These compounds were first tested for their activity against the *Mtb* strain H37Rv using MABA assay. The compounds showing good anti-TB activity were further evaluated for their potency against non replicating persistent TB (NRPTB) in a low oxygen recovery assay (LORA). The most active compounds, **39** and **40**, exhibited MICs of 0.77 μM and 0.95 μM, respectively against the replicating bacteria. These compounds, in general, also had good potency against the nonreplicating persistent bacteria without toxicity on Vero cells up to 128 μM. The compounds **39** and **40** also retained anti-TB activity against RMP-, INH-, and streptomycin resistant *Mtb* strains.



39, R=CF_3, X=CH_2; 40, R=H, X= m-Ph

3.1.3.2 Pyrimidine and purines

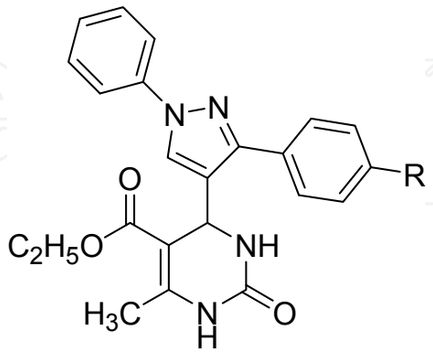
Khoje et al (Khoje et al, 2010) synthesized various purine analogs and evaluated them *in vitro* against *Mtb* H37Rv using MABA assay. The 8-aza-, 7-deaza- and 8-aza-7-deazapurine analogs displayed good antimycobacterial activities. The 7-deazapurine analogs exhibited MIC values between 0.08 and 0.35 μM; comparable or better than the reference drugs (RMP, MIC 0.09 μM; INH, MIC 0.28 μM and PA-824, MIC 0.44 μM). The most active compound among 7-deaza purines was **41** with MIC 0.11 μM and SI 1063. The 7-deazapurines were slightly more toxic towards mammalian cells, but still had good selectivity indices. In this study, five most active compounds were also evaluated against a panel of drug-resistant *Mtb* strains, where they all were found to retain activity. However, these compounds were significantly less active when tested against non-replicating persistent *Mtb*.



41

Trivedi et al (Trivedi et al., 2010) examined a series of dihydropyrimidines for their *in vitro* activity against *Mtb* H37Rv. All compounds were initially screened for their *in vitro* activity

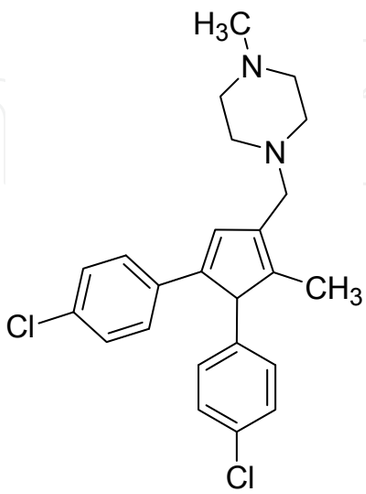
at 6.25 μg/mL. The compounds exhibiting 90% inhibition in the initial screen were re-examined at and below 6.25 μg/mL using two-fold dilutions to determine the actual MIC. Two compounds, **42** and **43** were found to be the most active agents with MIC of 0.02 μg/mL. These compounds were more potent than the reference drug INH. In Vero cells, they exhibited IC_{50} >10 μg/mL (SI >500).



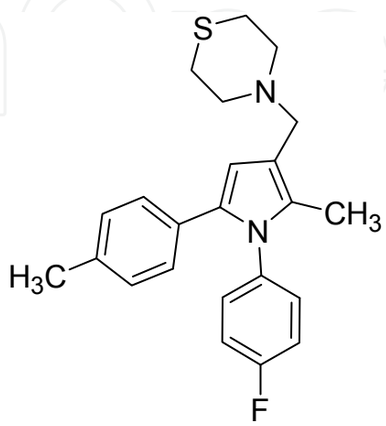
42, R=F, 43, R=NO_2

3.1.3.3 Pyrrole derivatives

Biava et al (Biava et al., 2006) reported design and synthesis of pyrrole analogues of BM212. The compounds were preliminarily screened for their activity toward *Mtb B814 and M. fortuitum CA10*. Compounds showing MIC values of 16 μg/mL or lower were further tested against *Mtb CIP 103471* and a panel of atypical mycobacteria, such as *M. marinum* CIP *6423, M. avium CIP 103317*, and *M. smegmatis* CIP *10359*. Cytotoxicity was examined in Vero cells to determine the maximum nontoxic dose ($MNTD_{50}$) defined as the drug concentration that decreased cell multiplication to less than 50% of the control. The best compound reported in this series was **44** with MIC of 0.4 μg/mL, $MNTD_{50}$ of 64 μg/mL and a high protection index (MNTD/MIC, 160) that was better than BM212, INH, and streptomycin (6, 128, and 128, respectively).

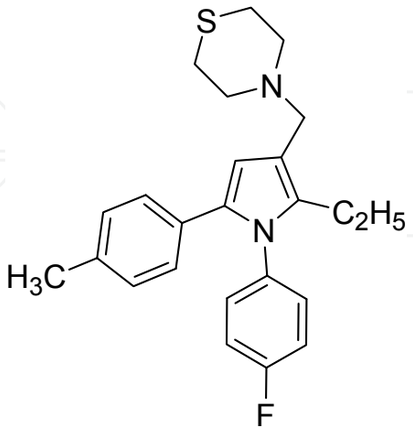


BM 212



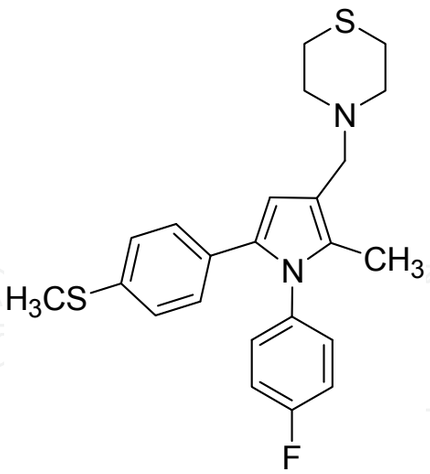
44

In the year 2009, the same group (Biava et al., 2009) further investigated new diarylpyrroles on the basis of SAR analysis of pyrroles, reported by them previously. The compound **45** emerged as the most potent agent (MIC 0.25 μg/mL) with protective index (maximum non toxic dose in Vero cells/ MIC) > 512.



45

Biava et al (Biava et al., 2010) also identified 4-((1-(4-fluorophenyl)-2-methyl-5-(4-(methylthio)phenyl)-1H-pyrrol-3-yl)methyl)thiomorpholine (**46**) as a potent antimycobacterial agent against *Mtb* 103471 and H37Rv strains (MIC values of 0.125 μg/mL comparable to streptomycin and RMP), with a cytotoxicity (CC_{50}) value of >128 μg/mL and protection index of >1000.

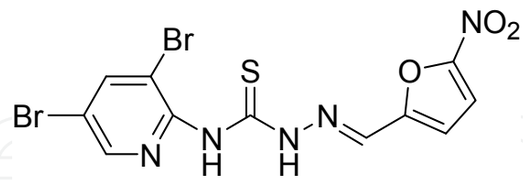


46

3.1.3.4 Furan

5-Nitrofuran-2-yl derivatives (Sriram et al. 2009) were investigated against tubercular (H37Rv) and various non-tubercular mycobacterial species in log-phase and 6-week-starved cultures. The compound **47** exhibited MIC of 0.22 μM. This compound showed 3 times more activity than INH and equal activity as RMP in log-phase culture of *Mtb*

H37Rv. It inhibited starved *Mtb* H37Rv with MIC of 13.9 μM and was 50 times more active than INH and slightly more active than RMP. It displayed an IC_{50} of 139 μM in Vero cells.

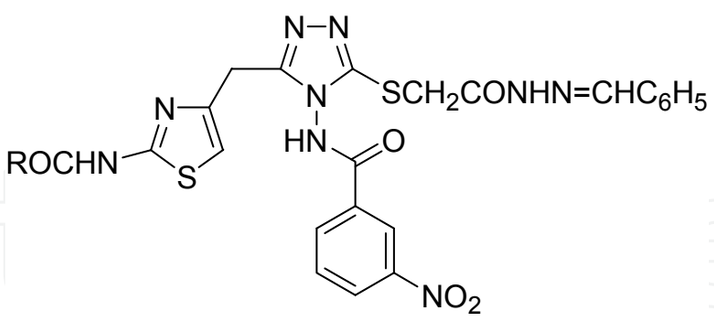


47

3.1.3.5 Azoles

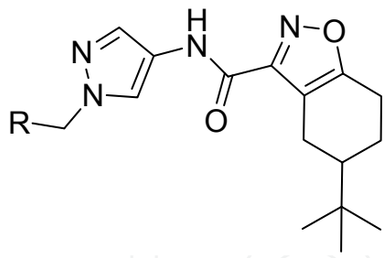
Azoles are one of the major classes of compounds which have been probed for anti-TB activity, but unfortunately, many of the publications emerging on azoles did not provide toxicity data, making it difficult to analyze their potential. Following are some of the representatives studies found worthy to summarize here.

Shiradkar et al (Shiradkar et al, 2007) published synthesis and antituberculosis activity of a series of N-{4-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-2-substituted amide derivatives against *Mtb* H37Rv (ATCC 27294) using MABA and BACTEC 460 assays where compounds **48** and **49** demonstrated MICs of 0.78 and 0.39 μM, respectively. The cytotoxicity analysis by neutral red uptake assay in Vero-C-1008 cell line showed that none of this class of compounds was toxic up to a concentration of 50 μg/mL.



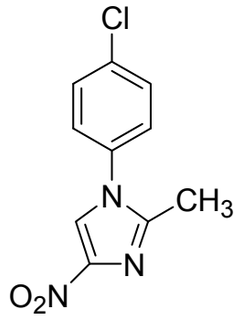
48, R = CH_3; 49, R = Ph

Velaparthi et al (Velaparthi et al., 2008) reported 5-*tert*-butyl-*N*-pyrazol-4-yl-4,5,6,7-tetrahydrobenzo[*d*]isoxazole-3-carboxamide derivatives as novel and potent inhibitors of *Mtb* pantothenate synthetase (PS). Pantothenate is a key precursor of coenzyme A and acyl carrier protein, essential for many intracellular processes including fatty acid metabolism, cell signaling, and synthesis of polyketides and nonribosomal peptides. The PS pathway is not present in humans. Compounds **50** and **51** displayed the best inhibition in terms of IC_{50} of < 100 nM.



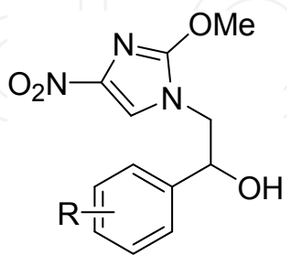
50, R = Ph; 51, R = 2-naphthyl

N-Aryl-*C*-nitroazoles were investigated by Walczak et al (Walczak et al., 2004) against H37Rv (ATCC 27294) using MABA assay. Compound **52** exhibited MIC 0.39 μg/mL with SI >160.



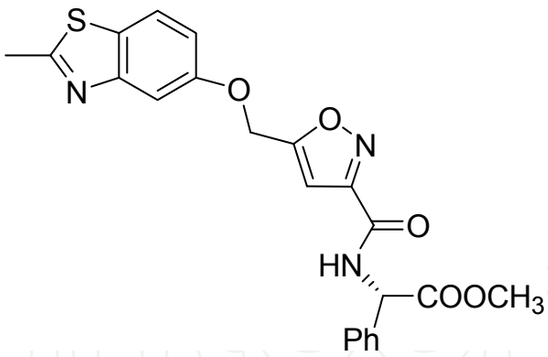
52

Lee et al (Lee et al., 2011) synthesized econazole-derived nitroimidazoles and reported their antitubercular activity against H37Rv by MABA assay. The MIC against non-replicating *Mtb* was determined by using the green fluorescent protein (GFP) expressing *Mtb* strain in the Wayne hypoxia model (anaerobic conditions) (Wayne et al. 1996, as cited in Lee at al., 2011). The MICs of the most active azoles **53** and **54** was found to be 0.5 μg/mL under aerobic conditions and 4 and 1 μg/mL, respectively, under anaerobic conditions against H37Rv. The IC_{50s} in Vero cell noted for **53** and **54** were 100 and >100, respectively.

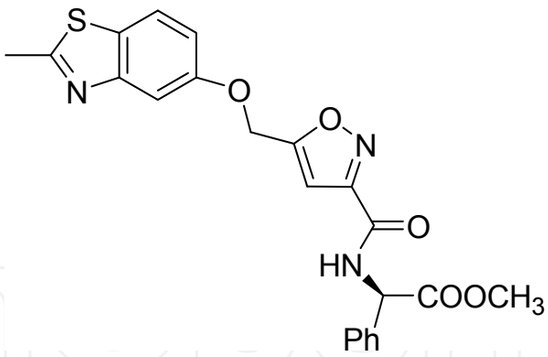


53, R=2,4-dichloro; 54, R= 4-Ph

In the year 2009, a series of 2-methylbenzothiazole derivatives was described by Huang et al (Huang et al, 2009). The most potent compounds found in this series were **55** and **56** with MIC values of 1.4 and 1.9 μM, respectively, against replicating *Mtb* H37Rv. All the active compounds in this series were nontoxic toward Vero cells (IC_{50} > 128 μM).



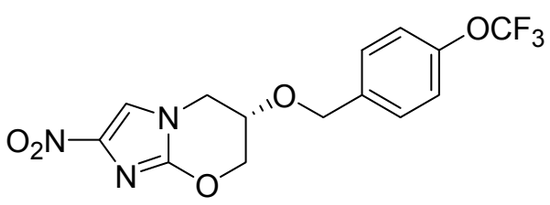
55



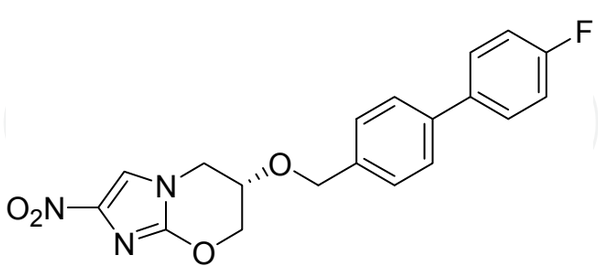
56

3.1.3.6 Azines

Palmer et al (Palmer et al., 2010) reported antitubercular activity of biphenyl analogs of PA-824, which is currently under phase II clinical trial (pl. see Phase II section), using MABA and LORA assays. Among these, several of the compounds showed potent in vitro activity with MIC values of <1 μM. The most active compound **57** had MICs of 0.015 and 1.4 μM in MABA and LORA assays, respectively. All the compounds investigated were relatively nontoxic to mammalian Vero cells, with IC_{50} >125 μM. In a mouse model of acute *Mtb* infection, seven of the compounds showed substantially (>10-fold) improved efficacies over PA-824, while three of them were >200-fold more effective than PA-824.



PA-824

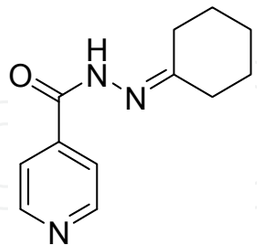


57

3.1.3.7 Pyridine hydrazides (INH analogs)

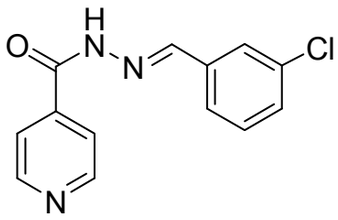
Several INH derived Schiff bases were investigated by Hearn et al (Hearn et al, 2009). These compounds showed high *in vitro* activity against *Mtb* and mycobacteria-infected macrophages. They provided strong protection in tuberculosis-infected mice with low toxicity. The mean of the MIC values determined against *Mtb* H37Rv strain Erdman for

the forty-four compounds tested was 1 μg/mL. A representative cyclohexanone derivative **58** displayed MIC of 0.03 μg/mL (SI >40,000) and exhibited log CFU reduction/lung of 4.65.



58

Lourenco et al (Lourenco et al., 2008) prepared a series of (E)-N′-(monosubstituted-benzylidene) isonicotinohydrazide derivatives and evaluated their antibacterial activity against *Mtb* H37Rv (ATCC 27294, susceptible both to rifampin and INH) *in vitro* using Alamar Blue assay. Compound **59** exhibited significant activity (MIC 0.31 μg/mL). Cellular viability of murine macrophage cells in the presence and absence of test compounds was determined by Mosmanns's MTT(3-(4,5-dimethylthylthiazol-2yl)-2,5-dimethyl tetrazolium bromide; Merck) microculture tetrazolium assay (Souza et al., 2003, as cited in Lourenco et al., 2008) and 100% cell viability was found for the compound **59** @ 100 μg/mL.

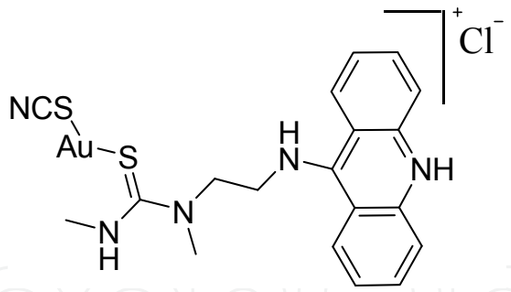


59

3.1.4 Metal complexes

Not very many reports are available on metal complexes since metal complexes are generally found to be toxic. A few representatives are summarized here.

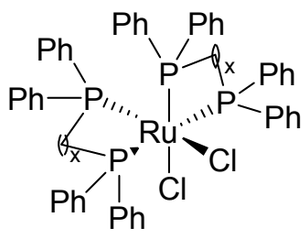
Eiter et al (Eiter et al, 2009) described Gold(I) analogues of a platinum-acridine. Compound **60** exhibited an IC_{50} of 0.652 μM and IC_{90} of 1.141 μM against *Mtb* H37Rv in a high-throughput screen. It also demonstrated inhibition of non-small-cell lung cancer cell line (IC_{50} of 3.940 ±0.38) with a selectivity index of 23.66. The compound **60** was selected to test its efficacy *in vivo* but serum samples collected from mice treated at a maximum tolerated dose (MTD) of 300 mg/kg orally did not inhibit *Mtb*. This indicated limited oral bioavailability of the complex.



60

Melnic et al (Melnic et al., 2010) investigated new hetero(Mn, Co, Ni)trinuclear iron(III) furoates where compound **61** $[Fe_2CoO(\alpha–fur)_6(THF)(H_2O)_2].H_2O$ displayed potent in vitro inhibition (MIC = 0.827 μg/mL) of *Mtb* H37Rv (ATCC 27294) with an SI of >36.2 (cytotoxicity assay was performed using Vero cell lines).

In a series of Ruthenium (II) phosphine/picolinate complexes, Pavan et al (Pavan et al, 2010) reported MIC values of 0.78 and 0.26 μg/mL for compounds **62** and **63**, respectively, against H37Rv ATCC 27294 using REMA (Resazurin Microtiter Assay) method (Palomino et al., as cited in Pavan et al., 2010). No toxicity data, however, was reported in the article.

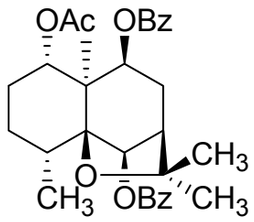


62, x=1; 63, x=2.

3.1.5 Natural products

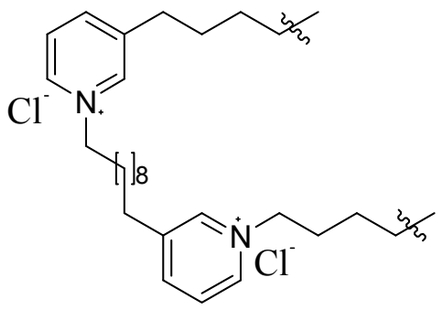
Natural product research is a tedious, labour-intensive and difficult process. Only a few publications have emerged describing significant anti-mycobacterial activity in this field. Selected reports are presented here.

Torres-Romero et al (Torres-Romero et al., 2011) evaluated new dihydro-β-agarofuran sesquiterpenes, isolated from the leaves of *Celastrus vulcanicola*, and their derivatives against H37Rv ATCC 27294 and multidrug-resistant (clinical isolate, strain 02TBDM039EP097) using the tetrazolium microplate assay (TEMA) method. (Rojas et al., 2006, as cited in Torres-Romero et al., 2011). All of the 25 compounds reported showed MIC values of >25 μg/mL against the sensitive H37Rv strain whereas 1a-acetoxy-6b,9b-dibenzoyloxy-dihydro-b-agarofuran (**64**) had MIC value of 11.9 μM against MDR TB strain, which was comparable to or better than INH or RMP. No toxicity data was included in this article.



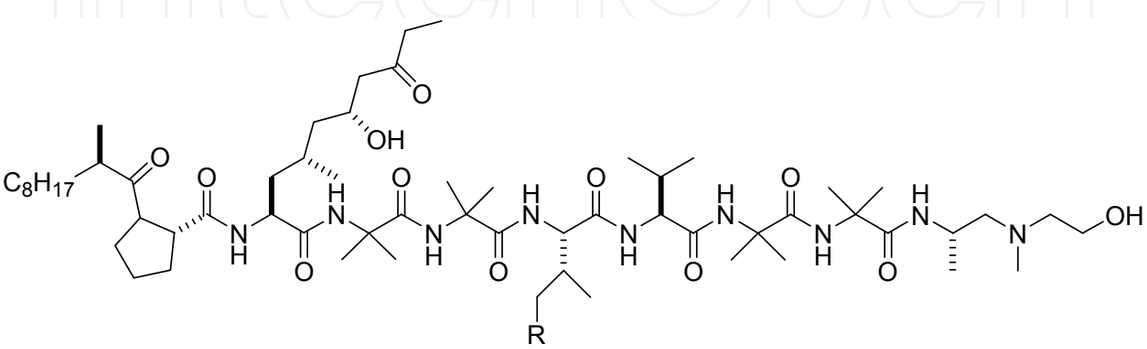
64

Nicholas et al (Nicholas et al., 2003) screened 1500 extracts derived from marine plants, invertebrates and terrestrial fungi for their ability to inhibit a newly described mycobacterial detoxification enzyme mycothiol-S-conjugate amidase (MCA) using a fluorescence-based assay that measures the extent of cleavage of the substrate mycothiol bimane by MCA (Newton et al., 2000, as cited in Nicholas et al., 2003). Only compound **65** showed inhibition of MCA (IC_{50} 0.1 μM).



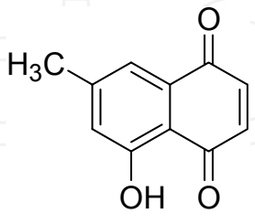
65

Three new aminolipopeptide, trichoderins were isolated by Pruksakorn et al (Pruksakorn et al., 2010) from a culture of marine sponge-derived fungus of Trichoderma sp. as anti-mycobacterial substances. Trichoderins showed potent activity against *M. smegmatis*, *M. bovis* BCG, and *Mtb* H37Rv under standard aerobic growth conditions as well as dormancy-inducing hypoxic conditions using the established methods, (Sobou et al., 2008; Arai et al., 2009, as cited in Pruksakorn et al., 2010) with MIC values in the range of 0.02–2.0 μg/mL. The best compounds **66** and **67** displayed MICs of 0.12 and 0.13 μg/mL, respectively, for aerobic and hypoxic *Mtb*. No toxicity data was included in this report.



66, R=CH3; 67, R=H

Mahapatra et al (Mahapatra et al., 2007) reported a series of synthetic and plant-derived naphthoquinone derivates of the 7-methyljuglone scaffold and their evaluation against *Mtb* H37Rv (ATCC 27294). Several of these compounds have been shown to operate as subversive substrates with mycothiol disulfide reductase. The synthesized compound **68** exhibited MIC of 0.5 μg/mL as determined by radiometric respiratory technique using the BACTEC system. The SI obtained for **68** was 30.22 (cytotoxicity evaluation was done using Vero cells).

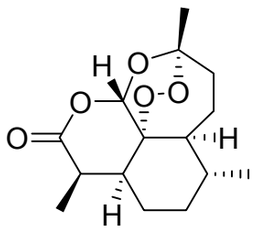


68

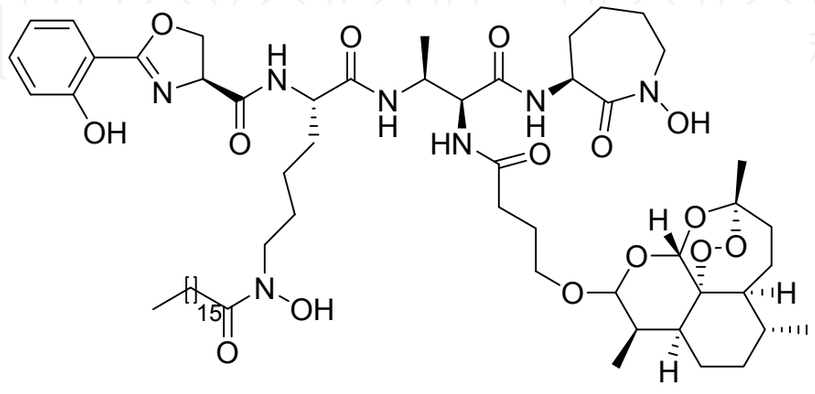
3.1.6 Miscellaneous

3.1.6.1 Artemisinin analog

Artemisinin also called qinghaosu, is a natural peroxide containing sesquiterpene based on 1,2,4-trioxane, and is a highly active and relatively nontoxic antimalarial agent (Devdutt, C. et al., 2010, as reported by Miller et al., 2011). Miller et al (Miller et al., 2011) reported Mycobactin-Artemisinin Conjugate **69** that had submicromolar activity against different clinical strains of tuberculosis. In H37Rv, it displayed MIC 0.338 μM, and in one XDR strain (HREPKOTh) it exhibited MIC of 0.078 μg/mL. No toxicity data was mentioned, however.



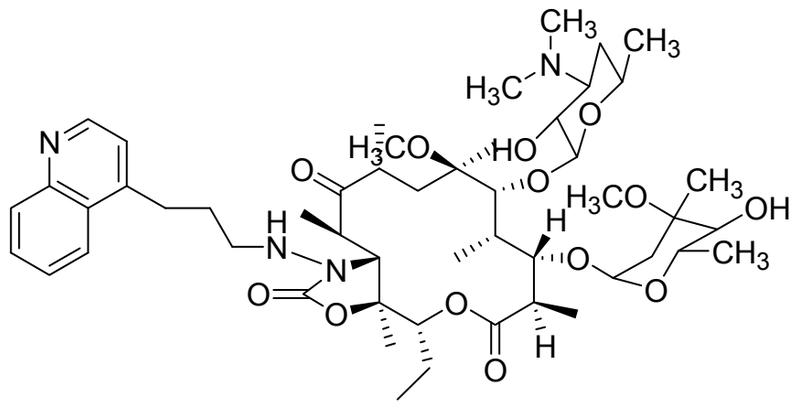
Artemisinin



69

3.1.6.2 Macrolides

Falzari et al (Falzari et al., 2005) reported macrolides and ketolides (descladinose) with substitutions at positions 9, 11, 12, and 6, which were assessed for activity against *Mtb*. Several compounds with 9-oxime substitutions or aryl substitutions at position 6 or on 11, 12 carbamates or carbazates demonstrated submicromolar MICs. Four compounds possessing low MICs also effected significant reductions in CFU in infected macrophages. The active compounds were assessed for tolerance and the ability to reduce CFU in the lungs of BALB/c mice in an aerosol infection model. A substituted 11,12 carbazate macrolide demonstrated significant dose-dependent inhibition of *Mtb* growth in mice, with a 10- to 20-fold reduction of CFU in lung tissue. The compound **70** (RU66252) was found to be a promising compound having MIC of 0.25 μM with SI 99.52.



70

3.1.6.3 Peptides

Jiang et al (Jiang et al, 2011) reported evaluation of a series of α-helical peptides consisting of all D-amino acid residues and synthetic human L-LL37 (L-enantiomer) and D-LL37 (D-enantiomer), against *Mtb* H37Rv and a clinical MDR strain. Not very good activity was observed. The most active analog had MIC of 11.2 and 15.6 μM, against H37Rv and MDR strains, respectively.

3.2 Molecules in pipeline

(Source: Working Group on New Drugs [WGND] and TB Alliance, and Tuberculosis Trial Consortium [TBTC])

After years of vacuum, TB drug development pipeline has begun to enrich during the past decade. The major credit goes to the Global Alliance for TB Drug Development (TB Alliance) which is largely funded by Bill & Melinda Gates Foundation as a philanthropic effort and Working Group on New Drugs (WGND). It is also to be noted in regard of this pipeline that many of the compounds here are either derivatives of existing drugs or are working on the same target as existing drugs. This is obviously a shorter and a quicker method for new drug development, however, this approach may pose a risk of cross-resistance in these future drugs. This risk may be neglected, however, in view of urgent need of effective drugs

to halt TB associated mortalities. Following are the compounds that are at various stages of preclinical and clinical development (summarized in tables 3-5).

3.2.1 Hit to lead

Sponsor/Developer	Compounds	Target	Remarks
The Lilly TB Drug Discovery	Novel synthetic compounds	Unknown	Not much information is available
FAPESP/Brazil	Ruthenium(II)phosphine/picolinate complexes, synthetic (>100).	Unknown	MIC less than 1 µM against H37Rv and resistant strains. *In vivo* assays are underway
AstraZeneca R & D Bangalore	200,000 Synthetic, novel compounds	Not mentioned	Target against H37Rv strain
GlaxoSmithKline, TB Alliance:	Synthetic compounds	Not mentioned	Whole cell microorganism screen
University of Illinois, TB Alliance	Total 1,21,0000 compounds. 66,000 synthetic and semisynthetic	Whole cell	Approximately 1500 hits have been identified and confirmed
Shaw Environmental and University of Illinois at Chicago	30 Indole-based combinatorial biosynthetic compounds (Several compounds showed activity comparable to first line drugs).	Under investigation	Whole cell microorganism screening against replicating and nonreplicating *Mtb*.
Mycosynthetix, University of Illinois at Chicago	15,000 Natural product extracts as fungal metabolites	Not mentioned	Not much information is available
University of Illinois at Chicago, Myongji University	Actinomycete metabolites purified and derived from 70,000 natural products extract	Not mentioned	Several samples showing MIC of less than 0.5 µg/mL.
Vertex Pharmaceuticals, Incorporated	315,000+ Compounds	*Mtb* Protein Kinase Inhibitors	The screening assay uses a basic protein kinase assay.

Sponsor/Developer	Compounds	Target	Remarks
AstraZeneca R & D Bangalore, India	500,000 Synthetic compounds.	Enzyme(s) involved in DNA synthesis	Not much information is available
AstraZeneca, TB Alliance	Synthetic compounds are under screening via high throughput assay	Folate Biosynthesis Inhibitors	Not much information is available
GlaxoSmithKline, Texas A & M University, TB Alliance	Library of 1.4 million synthetic compounds has been screened via High throughput screening	Malate Synthase Inhibitors	Hits have been identified.
Colorado State University, TB Alliance	Synthetic, known already and evaluated as cholesterol synthesis inhibitors.	Menaquinone Synthase (MenA) Inhibitors	Project aims to "retro-design" and evaluate derivatives of the known compounds
UPenn and TB Alliance	110,000 Synthetic compounds derived from natural products	Inhibitors of *Mtb* energy metabolism, electron transport chain	About 100 hits have been identified

Table 3. Various Compounds at Lead Identification stage

3.2.2 Lead optimization

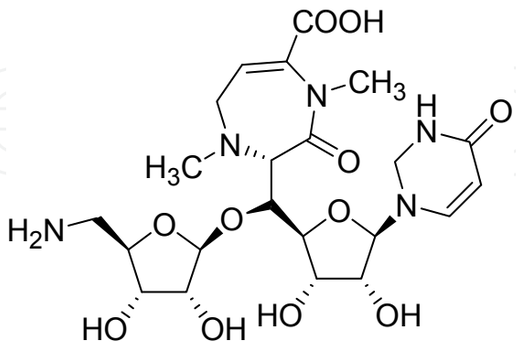
Sponsor/Developer	Compounds	Target	Remarks
TB Alliance, Institute of Materia Medica, The Beijing Tuberculosis and Thoracic Tumor Research Institute and University of Illinois.	Riminophenazine (clofazimine) derivatives >500 synthetic compounds	Considered to inhibit energy metabolism in *Mtb* (*Mtb*	Riminophenazines (clofazimine) have been employed to treat leprosy. *In vivo* studies are underway
GlaxoSmithKline, TB Alliance	>2 Million synthetic compounds.	InhA Inhibitors	------
Anacor Pharmaceuticals	>1000 Synthetic boron-containing compounds	LeuRS inhibitors, Protein synthesis	------
TB Alliance, GlaxoSmithKline	>1 Million synthetic compounds	Mycobacterial Gyrase Inhibitors	Several lead compounds have been identified and are being evaluated further.
AstraZeneca, TB Alliance	Synthetic	Mycobacterial Gyrase Inhibitors	------

Table 4. Various Compounds at Lead Optimization stage

3.2.3 Pre clinical

3.2.3.1 CPZEN-45

Sponsor/developer: Microbial Chemistry Research Foundation, Tokyo, Japan Lilly TB Drug Discovery Initiative NIAID, IDRI, Lilly, YourEncore.



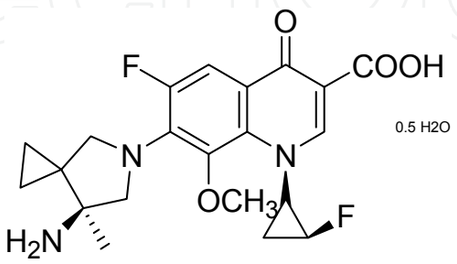
CPZEN-45

Synonyms: Caprazene, caprazamycin, nucleoside antibiotic

Summary: CPZEN-45 is a nucleoside antibiotic produced by Streptomyces sp. first described in 2003 by investigators at the Microbial Chemistry Research Foundation (MCRF) and Meiji Seika Kaisa, Ltd of Japan. CPZEN-45 possesses MIC of 1.56 µg/mL against *Mtb* H37Rv and 6.25 µg/mL against a MDR strain of *Mtb*. This compound is active against both replicating and non-replicating *Mtb in vitro*, suggesting it could be efficacious against latent organisms *in vivo*. CPZEN-45 has shown efficacy against both drug sensitive and XDR *Mtb* in a mouse model of acute tuberculosis (TB). Recent data by NIAID using the gamma interferon gene-disrupted (GKO) mouse model of acute tuberculosis in which infection was achieved by aerosol exposure to *Mtb* (Erdman) also demonstrated efficacy of CPZEN-45 with 1-1.5 log CFU reduction in lungs of infected mice. Its mode of action is not specified (Hirano et al., 2008; WGND)

3.2.3.2 Quinolone DC-159a

Sponsor or developer: Japan Anti-Tuberculosis Association, JATA Daiichi-Sankyo Pharmaceutical Co.



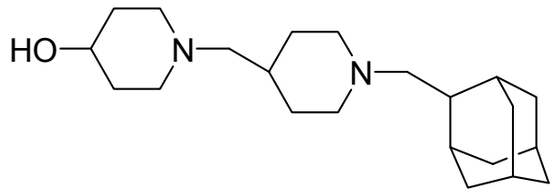
DC-159a

Summary: DC-159a exhibited the highest activity against drug-susceptible (MIC = 0.03 µg/mL), quinolone-resistant (QR) MDR-TB and non-tuberculous mycobacteria isolates

compared to that of moxifloxacin, gatifloxacin, levofloxacin and RMP. The potent activity of DC-159a is ascribed to the inhibition of DNA gyrase from wild-type and MDR-*Mtb*. In the drug-susceptible-*Mtb* infection model, it exhibited better early bactericidal activity (EBA) and higher log reduction of CFU in lungs, compared to moxifloxacin, levofloxacin, INH and RMP. In the QR MDR-TB infection model, it showed 2~3 times longer "mean survival days" which was superior to moxifloxacin, levofloxacin, INH and RMP. Pharmacokinetic study of DC-159a in a monkey model after an oral dose of 5 mg/kg of body weight, showed that it achieved a higher peak concentration (C_{max}; 2.20 μg/ml) and area under the concentration-time curve from 0 to 24 h (AUC 0–24; 16.9 μg.h/ml) than the MIC against *Mtb*, and showed better pharmacokinetic properties than levofloxacin (C_{max}, 1.68 μg/ml; AUC 0–24, 15.3 μg.h/ml). DC159a lacked interaction with cytochrome P450 3A4 (WGND; Disratthakit, & Doi, 2010; Sekiguchi et al., 2011), suggesting a better safety profile.

3.2.3.3 SQ-609

Sponsor/developer: Sequella



SQ-609

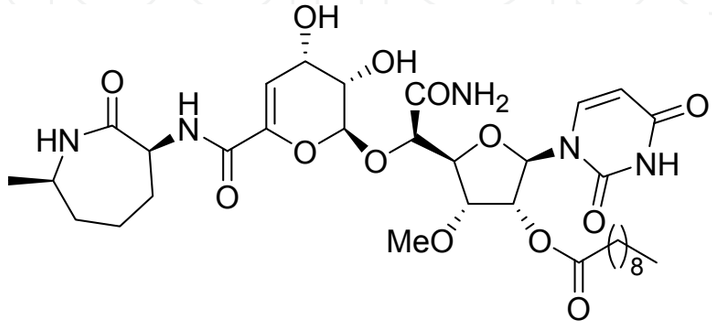
Summary: Sequella screened >100,000 molecules for anti-mycobacterial activity and identified SQ609 as the most potent (MIC = 4 μg/mL) and promising candidate among a new series of potential cell-wall inhibiting dipiperidines that are structurally different than any existing antitubercular drugs/candidates. Precise mode of action of SQ 609 is unknown (WGND; Bogatcheva et al., 2011).

3.2.3.4 SQ-641

Sponsor/developer: Sequella

Target**:** Translocase 1 (TL1) enzyme Inhibitors

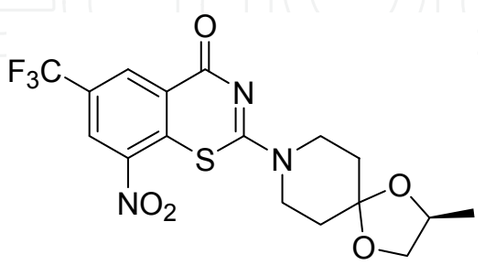
Compounds: **>**7000 compounds synthetic compounds derived from natural products



SQ-641

Summary: Translocase 1 (TL1) enzyme, which is absent in eukaryotic cells, is an essential enzyme in bacteria for the biosynthesis of the peptidoglycan layer of the cell wall. The semi-synthetic nucleoside Capuramycin has been studied as inhibitor of TL1 enzyme. The lead candidate SQ-641 (MIC = 0.5 μg/mL) is under preclinical development for the treatment of TB. Its mycobactericidal rate is faster than any existing TB drugs. SQ-641 possesses activity against MDR clinical strains of *Mtb*. It has shown efficacy in a mouse model of chronic TB by reducing CFU in lungs of infected mice by 1.0 to 1.5 log (WGND; Bogatcheva et al., 2011).

3.2.3.5 Benzothiazinone (BTZ-043)



BTZ-043

Summary: BTZ-043 belongs to a new class of antimycobacterial agents. It is highly active against *Mtb* (MIC = 1-10 ng/mL) and other actinobacteria. It also possesses activity against MDR- and XDR-TB strains. It showed *in vitro* bactericidal activity comparable to INH. It is non-mutagenic and has good oral bioavailability. BTZ-043 inhibits cell wall biosynthesis, and targets the DprE1 (Rv3790) subunit of the enzyme decaprenylphosphoryl-beta-D-ribose 2'-epimerase.

3.2.3.6 Q-201

Sponsor/developer: Quro Science, Inc.

It is an imidazopyridine compound. Not much detail is available about this compound.

Phase	Compound	Sponsor/developer	Mode of action
I	AZD5847	Astrazeneca	Protein synthesis inhibitor
II	PNU-100480	Pfizer	Protein synthesis inhibitor
	LL3858	Lupin Pharmaceuticals Inc.	Not yet known
	SQ-109	Sequella, NIH	Not yet known
	PA-824	TB Alliance	Protein synthesis and cell wall lipids inhibitor
	OPC67683	Otsuka Pharmaceutical Co. Ltd.	Protein synthesis and cell wall lipids inhibitor
	TMC 207	Tibotec	Affects proton pump of ATP synthase
	Linezolid	Tuberculosis Trials Consortium (TBTC), Pfizer	Protein synthesis inhibitor Novel unique
II/III	Rifapentine	CDC, Sanofi-aventis	Inhibits DNA dependent RNA polymerase

Phase	Compound	Sponsor/developer	Mode of action
III	Moxifloxacin	University College London	Inhibits bacterial replication
	Gatifloxacin	Institut de Recherche pour le Développement, WHO, European Commission (primary developers)	Inhibits bacterial replication

Table 5. Compounds in phase I-III clinical trials

3.2.4 Phase I

3.2.4.1 AZD-5847

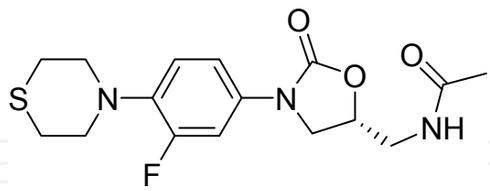
Sponsor/developer: Astrazeneca

Summary: AZD-5847, an oxazolidinone antibiotic (structure is not disclosed), originally developed for staphylococcal infections, is currently in Phase 1 clinical trials. It possesses MIC_{90} of 1 μg/mL against laboratory *Mtb* strains and clinical isolates resistant to INH, RMP, streptomycin, EMB or OFX (Abstract Balasubramanian et al., 2011). Studies to examine safety, tolerability and blood levels of AZD-5847 in healthy volunteers are underway.

3.2.5 Phase II

3.2.5.1 PNU-100480

Sponsor/developer: Pfizer

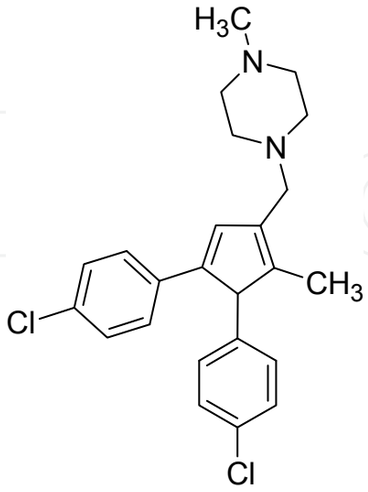


PNU-100480

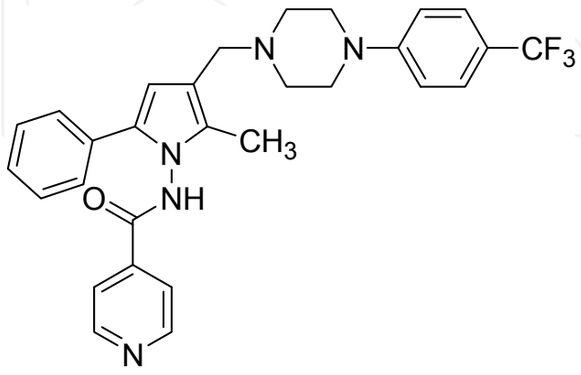
Summary: PNU-100480 is a structural analogue of linezolid (see details in Phase II section). It is more active than linezolid against TB (Williams, et al., 2009 as cited in Alffenaar et al., 2011) and possesses similar efficacy to that of INH and RMP (Cynamon et al., 1999, as cited in Alffenaar et al., 2011). Its MIC was found in the range of .0625-0.5 μg/mL in drug-susceptible and drug-resistant clinical strains of *Mtb* (Alffenaar et al., 2011). When added to a first-line regimen in a murine model, PNU-100480 had a synergistic bactericidal effect, while linezolid had an antagonistic effect (Williams, et al., 2009 as cited in Alffenaar et al., 2011). 14 day dose-escalation and 28 day dose study in healthy volunteers have been completed (WGND, 2011).

3.2.5.2 Pyrrole (LL-3858) or Sudoterb.

Sponsor/developer: Lupin Pharmaceutical Inc.



BM 212

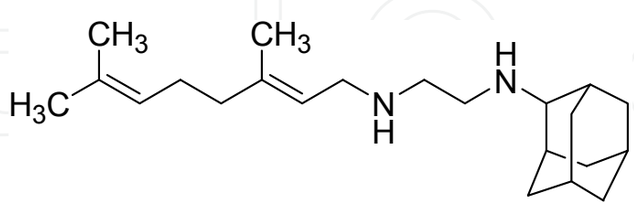


LL3858

Summary: Deidda et al. (Deidda et al., 1998) first reported the activity of the pyrroles against *Mtb*. The most potent compound identified was BM212 (MICs = 0.7 to 1.5 µg/mL against several strains of *Mtb*). This work by Deidda et al. later on inspired Lupin to synthesize a series of pyrroles and one of their leads LL3858 is currently in clinical development for the treatment of TB (Arora et al., 2004). The MIC90 of LL3858 for *Mtb* is reported to be 0.25 µg/mL (Tuberculosis**.** 2008. Ll-3858, as cited in van den Boogaard et al., 2009). LL3858, in combination with current anti-TB drugs, is reported to sterilize the lungs and spleens in lesser time than the conventional therapy (Sinha et al., 2004). The mechanism of action for this class of compounds has not yet been established.

3.2.5.3 Diamine (SQ-109)

Sponsor/developer: Sequella, NIH



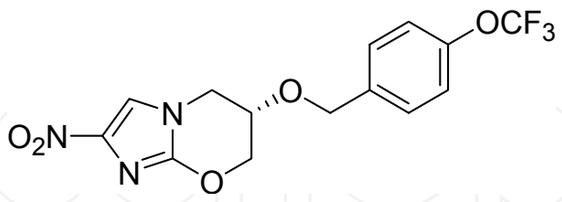
SQ-109

Summary: SQ109, or N-adamantan-2-yl-N′-(3,7-dimethylocta-2,6-dienyl)-ethane-1,2-diamine, is being developed by Sequella. It was the most potent compound (MIC = 0.1–0.63 µg/mL) in the series (Lee et al., 2003). *In vivo* studies showed 1 to 2.0-log reduction in CFU counts in the lung and spleen at 25 mg/kg. Its oral bioavailability is only 4% (Jia et al., 2005). Preclinical toxicology studies have been completed and further phase 2 clinical studies are underway.

3.2.5.4 Nitroimidazoles (PA824 AND OPC67683)

3.2.5.4.1 PA-824

Sponsor/developer: TB Alliance

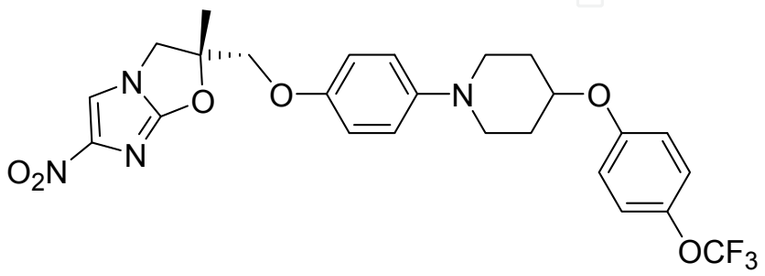


PA-824

In 1970s Ciba-Geigy in India screened a series of nitroimidazoles as radiosensitizers. Many of them were later found to possess antimicrobial activity, (including anti-*Mtb* activity). However, further development was discontinued after the lead molecule CGI-17341 was found to be mutagenic. In 1995 a pharmaceutical company, PathoGenesis, modified Ciba-Geigy's molecules and screened around 700 compounds against *Mtb* and found PA824 as the most active (Stover et al., 2000) and non mutagenic (Ginsberg & Spigelman, 2006). After PathoGenesis, Chiron Corporation obtained the rights and finally the Global Alliance for TB Drug Development acquired its rights for its clinical development. It has potent *in vitro* activity against *Mtb*, as evidenced by an MIC range of 0.015 to 0.25 mg/ml, and retains this activity against isolates resistant to a variety of commonly used anti-TB drugs. PA-824 kills *Mtb* bacilli by inhibiting the synthesis of protein and cell wall lipids (Stover et al., 2000). In mouse model it was highly active for latent TB in combination with moxifloxacin (Nuermberger et al.; 2005). It is suggested, however, that PA-824 is a prodrug and requires reductive activation of the aromatic nitro group (Manjunatha et al., 2006). PA-824 showed good tissue permeability in rat studies. Its minimum bactericidal dose (to reduce the lung CFU count by 99%) was found to be 100 mg/kg/day in murine studies. PA-824 in combination with INH prevents selection of TB mutants resistant to INH. It is effective against replicating and persistent TB bacilli. It is also effective against MDR strains and *Mtb* grown under oxygen depletion (Tyagi et al., 2005; Lenaerts et al., 2005). It has completed phase 1 studies in healthy volunteers (Spigelman, 2005).

3.2.5.4.2 OPC-67683 (Delamanid)

Sponsor/developer: Otsuka Pharmaceutical Co. Ltd.

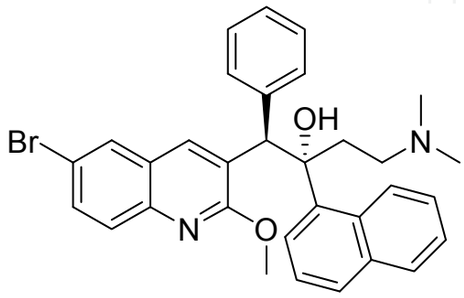


OPC-67683

Another nitroimidazole compound, OPC-67683 (MICs 0.006 µg/mL) is being developed by Otsuka Pharmaceutical. It was found to be potent against *Mtb in vitro* and *in vivo* (Matsumoto et al., 2005). In a mouse model, its efficacy was reported to be superior to that of currently used TB drugs. The effective plasma concentration of OPC-67683 was 0.100 µg/mL (achieved with an oral dose of 0.625 mg/kg). It showed no cross-resistance with the current anti-TB drugs. The mechanism of action of OPC-67683 is suggested to be similar to PA-824 (Kawasaki et al., 2005).

3.2.5.5 Diarylquinoline (TMC-207 or R-207910 or Bedaquiline)

Sponsor/developer: Tibotec

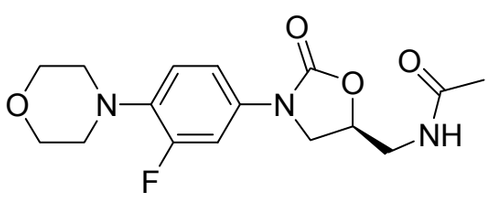


TMC-207

TMC-207 is owned by Johnson & Johnson (J&J) and is being developed at its research subsidiary Tibotec. TMC-207 not only showed very potent *in vitro* activity against both MDR and drug-susceptible strains of *Mtb* but also has potent activity against other Mycobacterial species (*M. avium*, *M. marinum*, *M. fortuitum*, and *M. abscessus M. smegmatis*). Its MIC ranges from 0.002 to 0.06 µg/mL for drug susceptible and drug resistant strains (Andries et al., 2005; Huitric et al., 2007). It is active *in vitro* against TB organisms resistant to INH, RMP, streptomycin, EMB, PZA, and moxifloxacin. It has no cross-resistance with current anti-TB medications (Andries 2004). In mice, a single dose had bactericidal potency for about eight days. When used as monotherapy, a single dose of TMC-207 was as potent as the triple combination of RMP, INH, and PZA and was more active than RMP alone. It works on the proton pump of ATP synthase (Andries et al., 2005). The effective half-life was found was ~24 h. Single ascending dose and 14-day multiple ascending dose studies in healthy human males showed no severe adverse effects. Further clinical trials are underway.

3.2.5.6 Linezolid for the Treatment of Multi-Drug Resistant Tuberculosis

Sponsor/developer: Tuberculosis Trials Consortium (TBTC), Pfizer

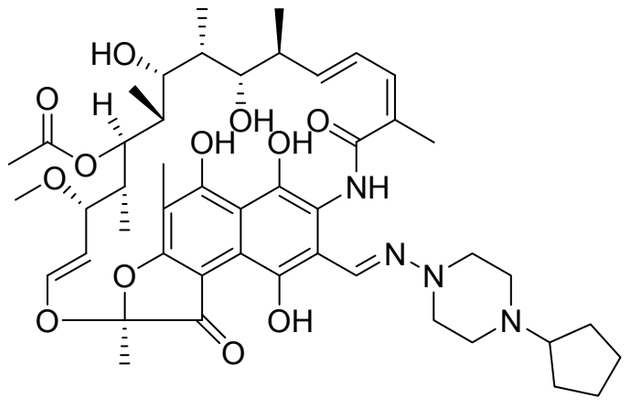


Linezolid

Linezolid is an approved antibacterial drug without a TB indication. It was discovered in 1990s and approved in 2000 for the treatment of Gram positive bacterial infections. It is active against most Gram-positive bacteria with MIC_{90} 1-2 μg/mL (Alcalá et al., 2003). It works as a protein synthesis inhibitor. Lack of information on its efficacy is one of the major concerns for its use as anti-TB agents (Migliori et al., 2009). Long-term use has been associated with thrombocytopenia, neuropathy and haematopoietic suppression (Gerson et al., 2009).

3.2.5.7 Rifapentine (TBTC study)

Sponsor/developer: CDC, Sanofi-aventis



Rifapentine

Rifapentine is a cyclopentyl derivative of the first-line TB drug RMP. Its MIC was found to be 0.03 μg/mL by 7H12 broth radiometric assay (Heifets et al., 1999). Its mechanism of action is the same as of RMP (Williams et al., 1998). It induces the CY450 system to a lesser extent than RMP (Weiner et al., 2004). It can also be used for latent TB as a part of regimen with either moxifloxacin or INH (Nuermberger et al., 2005). The aim of the clinical trial is to examine antimycobacterial activity and safety of an experimental intensive phase (first 8 weeks of treatment) tuberculosis treatment regimen in which RMP is substituted by rifapentine.

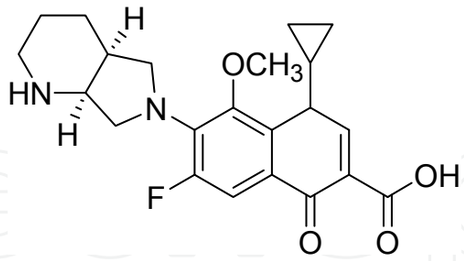
3.2.6 Phase III

3.2.6.1 Fluoroquinolones

In the past few years, attention has been focused on the use of fluoroquinolones for shortening the treatment duration of *Mtb*. Most of the credit for the use of fluoroquinolones goes to a clinical trial by the Tuberculosis Research Centre, Chennai, India (Tuberculosis Research Centre [TRC], 2002). In this trial, newly diagnosed pulmonary TB patients were randomly divided to receive one of four regimens containing a fluoroquinolone – ofloxacin (OFX). The rates of sputum conversion by this treatment at 2 months ranged from 92%-98% (superior to ~80% conversion rate by conventional therapy) (Tuberculosis Trials Consortium [TBTC], 2002).

3.2.6.1.1 Moxifloxacin

Sponsor/developer: University College London

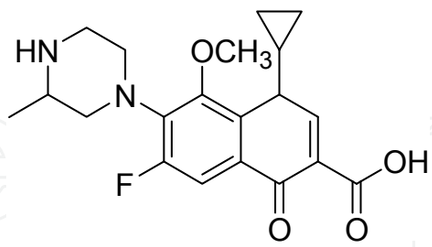


Moxifloxacin

Moxifloxacin ("Avelox" by Bayer) is a broad-spectrum antibiotic (400 mg/day dose) and is active against both gram positive and gram negative bacteria. It exhibits MIC of 0.5 μg/mL against *Mtb* (Shandil et al., 2007). It displayed early bactericidal activity comparable to INH and rifampin in humans (Pletz 2004; Gosling 2003). It affects bacteria by binding to the DNA gyrase and topisomerase IV, which are involved in bacterial replication. It has no cross-resistance to other antituberculosis drug classes; therefore, it might be useful against MDR-TB and XDR-TB. Further, it has been shown to display good activity profile against MDR strains (Tortoli et al., 2004). However, it has CNS side effects and drug interactions with other fluoroquinolones. Moxifloxacin has not been reported to be safe or effective in children younger than 18 or in pregnant or lactating women (Bayer, n.d.). Nuermberger et al. (2004) found that substituting moxifloxacin for INH shortens the duration of therapy for active disease much better than does substituting moxifloxacin for EMB.

3.2.6.1.2 Gatifloxacin

Sponsor/developer: Institut de Recherche pour le Developpement, WHO, European Commission (primary developers)



Gatifloxacin

Gatifloxacin ("Tequin" by Bristol-Myers Squibb) is also a broad-spectrum antibiotic (dosage of 400 mg/day). It works by the same mechanism as moxifloxacin. It is active against occasionally dividing *Mtb*, but not for dormant bacteria (Paramasivan et al., 2005). Gatiflozacin in combination with ethionamide and PZA was most effective to sterilize the lungs and prevent relapse (Cynamon & Sklaney, 2003). Gatifloacin can cause CNS toxicity and has been associated with increases in insulin levels among diabetics. It has not been shown to be safe or effective in children younger than 18 or in pregnant or lactating women. Gatifloxacin has completed a phase 2 study on randomized patients receiving 8 weeks of

therapy with either conventional treatment or the combination of INH, PZA, and RMP with either OFX or moxifloxacin, or gatifloxacin. In this study, serial sputum colony count measurements indicated that the patients in the moxifloxacin and gatifloxacin arms cleared their sputum more quickly than the patients receiving conventional therapy or the regimen containing OFX (Lienhardt et al., 2005).

3.2.7 Experimental compounds

The following experimental compounds are not commercially available. Their efficacy and safety are unknown.

3.2.7.1 A herbal product from Ukraine has been subjected to many open label clinical trials, with promising results in TB and TB/HIV coinfected patients (Zaitzeva et al., 2009; Nikolaeva et al., 2008a, 2008b). Open label trials with adjuvant Dzherelo (Immunoxel) have also been positive in MDR-TB and XDR-TB patients (Prihoda et al., 2007).

3.2.7.2 V-5 Immunitor or "V5", is an oral vaccine available in tablets for hepatitis B and hepatitis C treatment. TB sputum clearance was unexpectedly noted within a month, in hepatitis C-TB co-infected patients. Blinded studies suggest that V5 is also effective against MDR-TB (Olga et al., 2010; Butov et al., 2011).

4. Conclusion

After decades of reluctance in the TB drug discovery, several groups/institutions such as TB Alliance, Working Group on New Drugs (WGND) and New Medicines for Tuberculosis (NM4TB) have rekindled hope for new anti-tuberculosis drug(s) which may offer promise against MDR- and XDR-TB, and HIV-TB co-infection. The new drugs may also have capability of shortening the treatment duration of drug susceptible TB. Apart from the above big organizations, smaller research teams worldwide including our laboratory are actively involved in the search of new classes of potent and safe anti-tuberculosis drug(s).

The current TB drug pipeline (Table 6), no doubt, is the richest we have ever seen, but still it will take a long before any new drug hits market with approval. There are hurdles on the way ahead. Fund constraints, slow pace trial designs, insufficient infrastructure to validate the drug(s), validation and approval mechanism of Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA), and most importantly, the strong political will power, are the crucial issues ahead. The TB Trials Consortium (TBTC) (funded by The Centers for Disease Control and Prevention), National Institutes of Health (NIH) and European and Developing Country Clinical Trials Program (EDCTP) have to play better and expanded roles along with the ongoing efforts to accelerate the drug development. Governments, regulatory agencies, pharmaceutical and biotechnology companies, involved international agencies and communities, and basic and applied researchers worldwide all have to work together to achieve the goal of eradicating TB, like other big burden disease such as HIV.

It is worth to mention here lastly, that not only cure by drugs, but prevention measures and awareness steps by Governments and social bodies are also crucial and play very important role to stop any such infectious devil. Particular area on alert which need drastic improvements are imprisonment, health care systems, sex workers, travel and transportations,

and mass gathering activities such as festivals and events. The most important but neglected part of prevention program, which might be addressed and implemented urgently and effectively, is a separate and intense educational program designed for families having a member with diagnosed active TB. Together with a successful drug hunt and preventive measures, we can soon hope of the world without fear of millions of yearly deaths from tuberculosis.

Discovery Classes, (Sponsor/developer)			**Preclinical**	**Clinical**			**Existing Drugs**
Screening	**Lead Identification**	**Lead Optimization**		**Phase I**	**Phase II**	**Phase III**	
Natural Products (IMCAS)	Whole-Cell Hit to Lead Program (GSK)	Mycobacterial Gyrase Inhibitors (GSK)	Nitroimidazoles (U. of Auckland/ U. Ill Chicago)	AZD5847	PNU-100480	Moxifloxacin (Bayer)	**First Line** Rifampicin Isoniazid Pyrazinamide Ethambutol
Topoisomerase I Inhibitors (AZ/NYMC)	Folate Biosynthesis Inhibitors (AZ)				PA-824 (Nova-rtis)		
	RNA Polymerase Inhibitors (AZ) Energy Metabolism Inhibitors (AZ/U. Penn)	InhA Inhibitors	CPZEN-45				
Nucleosides	Ruthenium(II)pho-sphine/picolinate complexes	Pyrazinamide Analogs (Yonsei)	Preclinical TB Regimen Develo-pment (JHU/U. Ill Chicago)		Lnezolid; low dose	Gatifloxacin	**Second Line** Amikacin Kanamycin Capreomycin Streptomycin Cycloserine Ethionamide, PAS Clofazimine Ciprofloxacin Levofloxacin Ofloxacin
Carbo-hydrates	Whole-Cell Hit to Lead Program (AZ)						
Metal Complexes	Folate Biosynthesis Inhibitors	Diarylquinolines Tibotec/U. of Auckland	DC-159a		TMC 207 (Tibotec)		
Hydrazides and hydrazones	Menaquinone Synthase (MenA) Inhibitors						
Hetero-cyclics Quinolines, Quinoxalines, Pyrimidines, Purines, Pyrroles, Azines	Protein Kinase Inhibitors	Riminophenazines (IMM/BTTTRI)	SQ-641		LL3858	Rifape-ntine	
	Enzyme(s) involved in DNA synthesis		SQ-609		SQ-109 OPC67683		
Chalcones	Malate Synthase Inhibitors		BTZ-043				**"Third Line"** Rifabutin, Macrolides: (e.g., clarithromycin); Linezolid, R207910, Thioacetazone; Thioridazine; Arginine; vitamin D; .
Artemisinin derivatives	Actinomycete metabolites				Rifapentine		
	Fungal metabolites						
Macrolids			Q-201 (Quro Science Inc.)				
Peptides							

Table 6. Drug discovery: Screening to Existing Drugs.

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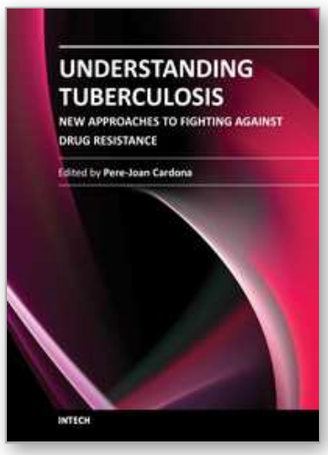

Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance

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In 1957, a Streptomyces strain, the ME/83 (S.mediterranei), was isolated in the Lepetit Research Laboratories from a soil sample collected at a pine arboretum near Saint Raphael, France. This drug was the base for the chemotherapy with Streptomicine. The euphoria generated by the success of this regimen lead to the idea that TB eradication would be possible by the year 2000. Thus, any further drug development against TB was stopped. Unfortunately, the lack of an accurate administration of these drugs originated the irruption of the drug resistance in Mycobacterium tuberculosis. Once the global emergency was declared in 1993, seeking out new drugs became urgent. In this book, diverse authors focus on the development and the activity of the new drug families.

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