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Review Paper

Tuberculosis chemotherapy: An overview in perspective of recent developments

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Abstract: Tuberculosis is one of the leading causes of morbidity and mortality among human populations. Current TB chemotherapy that takes 6 to 9 months is difficult to administer and has many side effects. The emergence of new problems like co-infection with HIV, multidrug resistant TB (MDR-TB), extensively drug resistant TB (XDR-TB) and totally drug resistant TB (TDR-TB) have made tuberculosis control even more difficult. Thus, there is an urgent need for the development of new antituberculosis drugs to effectively combat the menace of TB in its present manifestation. At present ten molecules are in clinical development for tuberculosis, among them four are being redeveloped or repurposed for tuberculosis and the remaining six are new molecules which are explicitly being developed for tuberculosis. This review article summarizes an account of the existing chemotherapeutics and highlights the latest information about emerging drugs in preclinical and clinical trial studies and their mechanism of action.

Introduction

Tuberculosis (TB) is a contagious, deadly disease caused predominantly by infection with *Mycobacterium tuberculosis* and it spreads through the air when a person with active TB disease of the lungs or airways exhales (while coughing, sneezing, singing, playing a wind instrument or to a lesser extent, talking).^[1] It affects the world's poorest population^[2] and remains one of the biggest public health problem in the 21st century.^[3] According to World Health Organization (WHO), there were almost 9

million new cases of TB in 2011 and 1.4 million TB deaths occurred including 990,000 HIV negative people and 430,000 persons co-infected with HIV. The largest number of TB deaths in 2011 occurred in Asia (59%) and Africa (26%), smaller proportions of cases occurred in the Eastern Mediterranean Region (7.7%), the European Region (4.3%) and the Region of the Americas (3%).^[4] The provision of diagnosis and treatment according to the DOTS/Stop TB strategy has resulted in major improvements in TB care and control. Between 1995 and 2011, 51 million people were successfully treated for TB in countries

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that had adopted the DOTS/Stop TB strategy and saved at least 20 million lives.^[4]

The cell-wall of mycobacteria consists of a wide array of complex fatty acids, such as mycocerosic acid, mycolic acid, arabinogalactans and peptidoglycans.^[5] The unique structure of the cell wall of *M. tuberculosis* allows it to lie dormant for many years as a latent infection, particularly as it can grow readily inside macrophages, hiding it from the host's immune system.^[6] It is estimated that only in 5-10% immunocompetent hosts, *M. tuberculosis* infection progresses to active pulmonary disease. The remaining 90-95% of the infected individuals are asymptomatic and generally believed to harbour latent bacilli that can reactivate to cause tuberculosis, sometimes decades after the initial infection. Other factors that contribute to the rise in TB and responsible for the increased death rates are HIV/AIDS, multidrug resistant TB (MDR-TB), extensively drug resistant TB (XDR-TB) and totally drug resistant TB (TDR-TB).^[7] Infection by the HIV/AIDS markedly enhances the rate of both new *M. tuberculosis* infection and activation of latent infection. The overall incidence of TB in HIV-positive patients is 50 times higher than HIV-negative individuals.

Multidrug resistant TB (MDR-TB) is a form of TB caused by *Mycobacterium tuberculosis* which becomes resistant to the most effective antitubercular drugs i.e. isoniazid and rifampin.^[8] Extensively drug resistant TB (XDR-TB) caused by *Mycobacterium tuberculosis* which becomes resistant to at least isoniazid and rifampin in addition to being resistant to one of the fluoroquinolones, as well as resistant to at least one of the second line injectable TB drugs i.e. amikacin, kanamycin or capreomycin.^[9] Totally drug resistant TB (XXDR-TB or TDR-TB) is a dangerous

form of the disease caused by *Mycobacterium tuberculosis* which becomes resistant to all the first and second line TB drugs. It is very difficult although not always impossible to treat.^[10]

The chemotherapy of tuberculosis largely becomes ineffective due to (i) administration of sub-standard drugs, inadequate or irregular drug supply and lack of supervision (ii) ignorance of health care workers in epidemiology, treatment and control (iii) improper prescription of regimens (iv) interruption of chemotherapy due to various side effects (v) laboratory delays in identification and susceptibility testing of *M. tuberculosis* isolates, quick appearance of drug resistant tubercle bacilli (vi) failure on the part of patients to complete the full course of long treatment and inappropriate monotherapy.^[11,12] The research on *M. tuberculosis* has progressed in the last decade^[13,14] and the regimens were optimized in conjunction with directly observed therapy short course (DOTS) for drug resistant tuberculosis.^[15,16] The number of antitubercular agents in preclinical and clinical development is higher today than any other period in the past 50 years. This review provides a brief discussion of the existing antitubercular chemotherapeutics and an overview of the chemical entities against tuberculosis currently in clinical trials.

Present Antitubercular Drugs and their Limitations:

Streptomycin: Streptomycin, an aminoglycoside derivative was discovered in 1943 by Selman Waksman.^[17] Chemically they are aminocyclitols (cyclohexane with hydroxyl and amino or guanidine substituents) with glycosyl substituents at one or more hydroxyl group and made up of three components: streptidine, streptose and

N-methyl-L-glucosamine. Streptomycin is both bacteriostatic and bactericidal against *M. tuberculosis* according to the concentration of the antibiotic. Concentrations of streptomycin approximately 1µg/mL inhibits the growth of *M. tuberculosis* H₃₇Rv.^[18] It disrupts protein biosynthesis in *M. tuberculosis*, through an interaction with the small 30S subunit of the ribosome.^[19] This antibiotic cannot be taken orally, as it is poorly absorbed in gastrointestinal tract. It is introduced in to the body via intramuscular injections. Various adverse side effects like loss of hearing, kidney damage, dizziness, vertigo, impaired coordination, rashes, fevers, yeast infections and oral thrush have been reported.

Para aminosalicylic Acid (PAS): In 1943, p-aminosalicylic acid (PAS) was discovered by Jorgen Lehmann and its antitubercular activity was established after two successful clinical trials conducted in 1944 and 1949.^[20,21] The mechanism of action of PAS is still not clear but it is presumed that it inhibits the formation of mycobactins (ionophore for iron transport) and the bacteriostatic activity might be caused by the inhibition of metabolic pathway for iron uptake.^[22,23] PAS has bacteriostatic *in vitro* activity against *M. tuberculosis* at 1µg/mL and it has also been used for many years in combination with streptomycin for all forms of TB. PAS is easily absorbed by the gastrointestinal tract and well distributed throughout the body. Side effects include gastrointestinal irritation, rashes, fever, pruritus rarely followed by exfoliative dermatitis or hepatitis of allergic nature. It is rarely given to patients with renal diseases because it is largely excreted in the urine.

Isoniazid: Isonicotinic acid hydrazide, Isonicotinyl hydrazide or INH (Hydrazid) was introduced in 1951 for the treatment of

tuberculosis and it is a more potent drug than streptomycin or p-aminosalicylic acid. Isoniazid has *in vitro* activity against *M. tuberculosis* in the range of 0.01–0.2 µg/mL.^[24] It is a prodrug activated by the mycobacterial catalase peroxidase enzyme (*kat G*), which catalyzes the formation of the isonicotinic acyl-NADH complex. This complex binds to the enoyl-acyl carrier protein reductase InhA, leading to the blockage of the synthesis of mycolic acid.^[25] Isoniazid is well tolerated with few side effects. Hepatic side effects occur in patients with previous hepatobiliary diseases. Neurological and gastrointestinal side effects are rare.

Pyrazinamide (PZA): Pyrazinamide is a synthetic derivative which was discovered in 1952. It is a structural analogue of nicotinamide; possess a MIC of 6.25-50 µg/mL at pH 5.5. Its introduction in TB treatment was a great success as its use shortened the duration of TB therapy from 9 months to 6 months.^[26] Despite its importance in shortening TB therapy, the mechanism of action of PZA is poorly understood. PZA enters *M. tuberculosis* by passive diffusion, where it is converted in to its active derivative pyrazinoic acid (POA) by the enzyme pyrazinamidase (PZase). Pyrazinoic acid (POA) with a pKa (where Ka is the acid dissociation constant) of 2.9 is trapped within the cell as the carboxylate ion and excreted by a weak efflux pump and passive diffusion. Small amounts of protonated pyrazinoic acid (HPOA) capable of diffusion across the cell membrane, leads to inhibition of fatty acid synthase (FAS) I, which is required by the bacterium to synthesize fatty acids.^[27,28] Hepatotoxicity is the most common and serious side effect of pyrazinamide and the severity of side effect depends on the dose and length of treatment. Another side effect observed with

pyrazinamide is arthralgia, caused by elevation of plasma uric acid levels.

Cycloserine: D-Cycloserine was first isolated in 1955 from cultures of *Streptomyces garyphalus*, *Streptomyces orchidaceus* and *Streptomyces lavendulae*. D-cycloserine or D-4-amino-3-isoxazolidone is a structural analog of the amino acid D-serine. It inhibits the incorporation of D-alanine into peptidoglycan biosynthesis through inhibition of D-alanine racemase and D-alanyl alanine synthetase.^[29] The MIC against *M. tuberculosis* is 5-20 µg/mL. The side effects produced by cycloserine are severe in the central nervous system, which creates psychotic states with suicidal tendencies and epileptic convulsions.

Ethionamide: Ethionamide or 2-Ethylthioisonicotinamide is isoniazid analogue which was discovered in 1956. It is converted into ethionamide sulfoxide via oxidation, blocks the synthesis of mycolic acids.^[30] It inhibits most tubercle bacilli at concentrations of 0.6-2.5 µg/mL. Gastrointestinal side effects are common and considered as one of the major reasons for discontinuation of this drug from tuberculosis chemotherapy. Neurotoxicity with sign of mental disturbances is also common.

Kanamycin: Kanamycin is another aminoglycoside derivative, isolated in 1957 from *Streptomyces kanamyceticus* and consists of three components: Kanamycin A, B and C. Amongst them Kanamycin A is the largest. The kanamycin molecule consists of deoxystreptamine and two amino sugars: kanosamine and 6-glucosamine. Its MIC is 1-8 µg/mL and it is given intramuscularly. The mechanism of action is similar to that of streptomycin, as it interacts with the 30s ribosomal subunit in more than one site (whereas streptomycin binds to only one

site) and inhibits protein synthesis via S12 ribosomal protein and 16S RNA. The major side effects are auditory toxicity, vestibular toxicity and ototoxicity, however auditory toxicity is more pronounced than vestibular toxicity and ototoxicity. Nephrotoxicity is the other side effect caused by Kanamycin.

Ethambutol (EMB): Ethambutol was discovered in 1961. Chemically it is ethylene diamino-di-1-butanol. It disrupts cell wall synthesis by specifically targeting the polymerization of arabinogalactans and lipoarabinomannan. It indirectly inhibits mycolic acid synthesis (by limiting the availability of arabinan for the mycolic acids to attach to) and activate a cascade of changes in the lipid metabolism of *M. tuberculosis*, leading to the disaggregation of bacteria clumps into smaller clusters.^[31,32] The *in vitro* activity of ethambutol against *M. tuberculosis* is 0.5 µg/mL.^[33] Ethambutol is rather well tolerated. The main side effect of concern is ocular toxicity, consisting of retrobulbar neuritis with various symptoms, including reduced visual acuity, constriction of visual fields and color blindness. Ocular toxicity seems to be dose-related. At a daily dose of 25 mg/kg, visual impairment occurs in about 3% of patients, rising to 20% at doses higher than 30 mg/kg per day.

Rifamycin (RIF) and their derivatives:

The rifamycin and their derivatives belong to a class of antibiotics called ansamycin (macrocyclic ring bridged across two nonadjacent positions of an aromatic nucleus). They were obtained by fermentations from cultures of *Streptomyces mediterranei* and discovered in 1959. The rifamycins and their semisynthetic derivatives have a broad spectrum of antimicrobial activity. Rifamycins bind to the β-subunit of bacterial DNA-dependent RNA polymerases to prevent chain initiations.^[34] Rifampin was discovered in

1966 as a semisynthetic derivative of rifamycin B.^[35] Chemically it is 3-[(4-methyl-1-piperazinyl) iminomethyl] 1-rifamycin. It was found to be active against *M. tuberculosis* at concentrations below 1 µg/mL in semisynthetic media. The most frequent adverse effects of rifampin are cutaneous reactions and gastrointestinal disturbances. It can also disturb liver function, but the risk of it causing serious or permanent liver damage is limited, particularly among patients with no previous history of liver disease. Another semisynthetic derivative Rifabutin was approved in 1992 for the treatment of atypical mycobacterial infections.

Amikacin: Amikacin is a semi-synthetic aminoglycoside derivative of kanamycin in which C-1 of aminogroup is amidated with 2-hydroxy-4-amino-butirric acid moiety. Amikacin was discovered in 1972 and like other aminoglycosides it is given intramuscularly or intravenously and its MIC against *M. tuberculosis* is 4-8 µg/mL. It binds to the bacterial 30S ribosomal subunit, leading to inhibition of protein synthesis. Amikacin affects the neuromuscular junction and may lead to neuromuscular blockade.^[33]

Emerging Compounds in Clinical Development:

Before discussing the various chemical entities in clinical development a brief description of the various stages of clinical development has been presented.

Clinical development of a lead molecule into a drug involves preclinical studies (*in vitro* as well as *in vivo* evaluation) and clinical studies, which is further classified into three phases.

Clinical Studies

Phase I Studies: The first stage is known as clinical Phase I trials, in which single dose of the drug is given to a small number of human subjects (10 to 15) for assessing drug safety, tolerability and pharmacokinetics.

Phase II Studies: The second stage consists of two phases, Phase II A and Phase II B. In Phase II A, the drug is usually evaluated for its activity as monotherapy in the first 1-2 weeks of TB treatment. Sputum samples are collected daily and then early bactericidal activity (EBA) is analyzed.^[36] Activity during the first 2 days of monotherapy was correlated with the activity of the drug to prevent drug resistance. In this phase possible ranges of doses are also evaluated.^[37] Phase IIB studies include the effect of a new drug, in context of multidrug therapy on a large number of participants (approximately 200-500). The most common end point of phase IIB studies is sputum culture status after 2 months of therapy and the results predicts the probability for shortened duration of treatment.

Phase III Studies: The third stage is known as clinical Phase III trials. It assesses treatment failure (continued positive culture results during treatment) and recurrent (or relapsed) TB (positive culture results after treatment completion). Only 2-5% of patients with drug-susceptible TB experience therapy failure or relapse. Therefore the most common phase 3 trial is to evaluate whether addition of new agent will shorten the treatment while retaining low risk of treatment failure or relapse. Such trials are known as noninferiority design. Depending on the desired statistical power, a noninferiority trial for shortened treatment duration will require approximately 500–900 patients for 18–30 months. Also this phase includes study of a drug in the treatment of multidrug-resistant TB (MDR-TB) and such studies can also use a noninferiority design.

There are currently ten molecules in clinical trials for TB treatment. Out of which four existing drugs: **gatifloxacin**, **moxifloxacin**, **rifapentine** (Phase III) and **linezolid** (Phase II), are being redeveloped for tuberculosis and six new molecules: **OPC-67683** (Phase III), **PA-824** (Phase II), **TMC-207**, **PNU-100480**, **AZD-5847** (Phase II), and **SQ-109** (Phase II) are specifically being developed for tuberculosis (Table1).^[38, 39]

Brief discussion of compounds in clinical trials:

Fluoroquinolones

The fluoroquinolones such as ciprofloxacin, lomefloxacin, norfloxacin, pefloxacin, sparfloxacin, gatifloxacin and moxifloxacin are broad-spectrum antimicrobial agents. Several members of this class have been used as second-line drugs in the treatment of MDR-tuberculosis. Among these fluoroquinolones, gatifloxacin and moxifloxacin are currently in Phase III clinical trial for the treatment of tuberculosis.

Gatifloxacin and Moxifloxacin: Gatifloxacin and Moxifloxacin are 8-methoxy fluoroquinolone. Both of them are members of fourth generation class of antibiotics. Moxifloxacin is widely known for the treatment of respiratory tract infections. Also in 1999 US FDA recommended it for the treatment of various infections like skin and soft tissue infections, acute sinusitis, community acquired pneumonia and acute exacerbation and chronic bronchitis.

Synthesis: The synthesis of gatifloxacin was initiated^[40,41] with the highly fluorinated compound 2,4,5-trifluoro-3-methoxybenzoic acid (**1**) which was treated with thionyl chloride at reflux temperature to

afford 2,4,5-trifluoro-3-methoxy-benzoyl chloride **2**. Base catalyzed condensation of the compound **2** with diethyl malonate in presence of magnesium ethoxide as base and toluene as solvent gave tricarbonyl derivative **3** which on heating with aqueous para toluene sulphonic acid underwent partial hydrolysis and decarboxylation to yield β -ketoester **4**. Reaction of intermediate **4** with ethylorthoformate then added a carbon atom to the activated methylene yields **5**, which was further condensed with cyclopropylamine in ethanol leading to the exchange of the ethoxy group with the amine to afford enamine **6**. Treatment of **6** with sodium fluoride in anhydrous DMF as solvent led to displacement of one of the ring fluorine by the basic nitrogen on the side chain leading to the formation of the quinolone **7**. Reaction of **7** with 2-methylpiperazine in anhydrous DMSO, led to the displacement of second fluorine, in this case by the less hindered basic nitrogen atom of the 2-methyl piperazine. Saponification of the ester finally afforded gatifloxacin (**8**) (Scheme 1).

Reaction of **7** with pure cis-(S,S)-octahydropyrrolopyridine on the other hand led to the formation of moxifloxacin (**9**) (Scheme 2).

Mechanism of Action: Gatifloxacin and Moxifloxacin exert their effects by inhibiting *Mycobacterium tuberculosis* topoisomerase II DNA gyrase, an enzyme which is essential for the maintenance of DNA supercoils and necessary for chromosomal replication.

Pre clinical studies: Gatifloxacin (*in vitro* MIC 0.12-0.25 $\mu\text{g/mL}$) and Moxifloxacin (*in vitro* MIC 0.18-0.5 $\mu\text{g/mL}$) have shown 8-16 fold better *in vitro* activity against *M. tuberculosis* than ofloxacin and ciprofloxacin.^[42,43,44] In a mouse model of

tuberculosis infection, moxifloxacin and gatifloxacin containing regimens have the ability to shorten treatment of drug-susceptible tuberculosis from 6 months to 4 months.^[45,46]

Clinical Trial Studies: Phase II trials in which gatifloxacin or moxifloxacin were substituted for ethambutol or isoniazid in the control regimen gave better results.^[47,48] Phase III trials are in progress to determine whether tuberculosis treatment can be shortened to 4 months by substitution of gatifloxacin for ethambutol, or moxifloxacin for ethambutol or isoniazid.

Oxazolidinones

The oxazolidinones consists of 2-oxazolidinone as a pharmacophore and are protein synthesis inhibitors which belong to a new class of synthetic antibacterial agents. Linezolid was developed by Pharmacia and Upjohn (The company has now merged with Pfizer). PNU-100480 was also developed by Pfizer while AZD-5847 was developed by AstraZeneca. All three of them are currently in Phase II clinical trials for the treatment of tuberculosis.^[49]

Linezolid (LZD, Zyvox): Linezolid is the first member of the oxazolidinone antibiotic class. It is a direct analogue of two derivatives - DuP105 and DuP721 and their MIC against *M. tuberculosis* were reported in the range of 0.3-1.25 µg/mL^[50] but their development was discontinued in Phase I trials due to toxicity issues.

Synthesis: The synthesis of linezolid begins with commercially available 3,4-difluoronitrobenzene (**10**) (Scheme 3). The nucleophilic aromatic substitution with morpholine using diisopropylethylamine as base and ethyl acetate as solvent at reflux temperature in nitrogen atmosphere for four hours resulted in compound **11**. It was

then reduced with ammonium formate in presence of 10% palladium on carbon and a mixture of THF and methanol as solvent at ambient temperature resulting in intermediate **12**. The amino group in compound **12** was protected by using benzyl chloroformate in presence of sodium bicarbonate as base in acetone and water at lower temperatures to give **13**, which was further condensed with (R)-glycidyl butyrate in presence of n-butyl lithium as base and THF as solvent resulting into **14**. Compound **14** was further reacted with methanesulfonyl chloride (MsCl) in presence of triethylamine (Et₃N) as base and DCM as solvent to give **15**. Compound **15** was treated with sodium azide in DMF as solvent at high temperature to give **16**. Finally hydrogenation of **16** was accomplished using hydrogen gas in presence of 10% palladium on carbon in ethyl acetate as solvent at ambient temperature to afford the amino intermediate, which was further treated with acetic anhydride in presence of pyridine as base to obtain Linezolid (**17**).^[51]

Mechanism of Action: Linezolid inhibits protein synthesis by a unique mechanism which is not shown by other antibiotics. It binds to the 23S ribosomal RNA, inhibits translation in the early phase preventing the proper binding of formyl-methionine tRNA.^[52]

Pre clinical studies: Against *M. tuberculosis*, linezolid possess *in vitro* activity in the range of 0.25-1 µg/mL.^[53] In a mouse model of tuberculosis infection, linezolid possess comparable activity to that of isoniazid. Also no side effects were reported when 50 mg/kg of the linezolid was administered for 29 days.

Clinical Trial studies: Very few Phase I and II clinical studies data are available so far. Linezolid at 600 mg/day showed weak

early and extended bactericidal activities when given once or twice daily to patients with pulmonary TB. Doses of linezolid was administered at 600 mg or 1200 mg daily and in all cases the cultures from respiratory samples were sterile after 6 weeks of treatment and 3 patients demonstrated clinical and microbiological cure after 5-24 months of treatment. Long-term use results in toxic side effects such as peripheral and optic neuropathy. Nowadays linezolid is used as a third line agent in combination regimens to treat MDR-TB or XDR-TB.^[54]

PNU-100480 (Sutezolid): Another oxazolidinone derivative PNU-100480, which is a linezolid analogue, was reported in 1996 for the treatment of tuberculosis.

Synthesis: The synthetic strategy for the synthesis of Sutezolid (**18**) similar to linezolid with only one exception, that thiomorpholine is used instead of morpholine in the initial nucleophilic aromatic substitution step.

Mechanism of Action: Its mechanism of action is similar to that of linezolid.

Pre clinical studies: PNU-100480 exhibits better *in vitro* activity against *M. tuberculosis* in the range of 0.03-0.50 µg/mL than linezolid.^[52,53] Also it showed more potency than linezolid in the murine model of tuberculosis.^[55,56] The combination regimen of PNU-100480, moxifloxacin, and pyrazinamide enhanced the bactericidal activities as compared to the standard regimen containing rifampin, isoniazid & pyrazinamide which indicates that PNU-100480 has the potential to shorten the treatment of drug-susceptible and drug-resistant tuberculosis by 1-2 months.^[53,56]

Clinical Trial studies: Phase I trials with PNU-100480 have been completed and

published. Phase I studies were designed to assess safety, tolerability, pharmacokinetics and pharmacodynamics in healthy volunteers by giving different doses such as 100, 300, 600 mg twice daily or 1200 mg once daily for 14 days or 600 mg twice daily for 28 days to which pyrazinamide was added on 27th and 28th day of regimen. Also a cohort of PNU-100480 was given at 600 mg twice a day for 28 days to better understand the risk of toxicity. All the doses were safe and well tolerated.^[57] Phase IIA study which is underway includes an EBA analysis which suggests that 600 mg and 1200 mg doses were well tolerated for 14 days.^[58]

AZD-5847 (Posizolid): AZD-5847 is a new analogue of oxazolidinone originally developed for staphylococcal infections. Its *in vitro* activity against *M. tuberculosis* is almost comparable to that of linezolid and PNU-100480.

Synthesis: The synthesis of AZD-5847 (Posizolid) initiated with the condensation of the protected 3,5-difluoroaniline (**19**) and 1-benzyl-4-piperidone in presence of butyl lithium in THF, which resulted in 4-(1-benzyl-4-hydroxypiperidin-4-yl)-3,5-difluoroaniline **20**. It was dehydrated with refluxing conc. HCl to furnish the tetrahydropyridine **21**. The treatment of **21** with benzyl chloroformate in acetone/water led to the carbamate **22**, which was cyclized with (R)-glycidyl butyrate in presence of butyl lithium in THF to provide the oxazolidinone **23**. The condensation of **23** with isoxazol-3-ol using PPh₃ and DIAD in THF led to the expected ether adduct **24**, which was debenzylated by treatment with 1-chloroethyl chloroformate in DCM, yielding free tetrahydropyridine derivative **25**. The condensation of **25** with (S)-2,3-O-isopropylidene-glyceric acid by DEC or DCC and TEA in DCM afforded the

corresponding acyl tetrahydropyridine **26**, which was finally deprotected using HCl in THF to furnish the target dihydroxy compound (**27**) (Scheme 4).^[59]

Mechanism of Action: Its mechanism of action is similar as that of linezolid.

Pre clinical studies: AZD-5847 possesses an MIC of 1 µg/mL against *Mycobacterium tuberculosis* strain and demonstrated efficacy in the murine model of tuberculosis.^[60]

Clinical Trial studies: Phase I study includes pharmacokinetic parameters in which fasting volunteers were compared with fed volunteers. A single ascending dose includes 600 mg oral dose of AZD-5847. The C_{max} in fasting volunteers was 2.60 µg/mL while in fed volunteers it was 5.66 µg/mL with approximately t_{1/2} of 8 hours. A multiple ascending doses of 800, 1600 and 2400 mg administered for 14 days in healthy volunteers were well tolerated and resulted in increased C_{max} up to 10 µg/mL.^[60] AZD-5847 was well tolerated in both the studies and no major side effects were identified. The common side effects associated with multiple ascending dose system are gastrointestinal disorders, reversible and dose-related changes in white blood cells and mildly increased reticulocyte counts. The Phase II clinical trials are in progress to determine whether the compound is developed for drug-susceptible and drug-resistant tuberculosis.

Diarylquinolones

Diarylquinolines have been identified in a process of screening various compounds for potential antitubercular activity. Among them TMC-207 or R-207910 or Bedaquiline has been identified as the lead compound

and it is currently in Phase II clinical trials.^[61]

TMC-207(Bedaquiline): TMC-207 is the first compound from a new class diarylquinoline. It was discovered by Johnson & Johnson through a high-throughput screening against *Mycobacterium smegmatis*^[62] and it is currently being clinically developed by Janssen Infectious Diseases (formely Tibotec) in collaboration with the GATB.

Synthesis: TMC-207 synthesis begins from the reaction of 3-phenylpropanoyl chloride **29** and 4-bromoaniline **28** (Scheme 5).Vilsmeier-Haack reaction was used to obtain the quinoline ring and the final condensation step leads to four stereoisomers that were separated by column chromatography over silica gel and then by chiral chromatography to yield the desired (R,S) compound.^[63]

Mechanism of Action: TMC-207 inhibits c subunit of ATP synthase enzyme, which is vital for the production of energy or ATP in *Mycobacterium tuberculosis*. ATP synthase is divided into F₀ and F₁ multi-subunit complexes; F₁ is cytoplasmic whereas F₀ is membrane associated and consists of a multimeric complex of proteins of the configuration a, b₂, c₉ - 12. TMC-207 is not active on human mitochondrial ATP synthase.^[64,65]

Pre clinical studies: TMC-207 exhibits an *in vitro* potency of 0.06 µg/mL against *M. tuberculosis* and 0.03-0.12 µg/mL against a range of *mycobacterium* clinical isolates such as *M. bovis*, *M. avium*, *M. kansasii*, *M. smegmatis* and *M. ulcerans*. It is also active against dormant bacteria.^[62]

TMC possess potent early and late bactericidal activity. Oral administration

achieved high *in vivo* activity. In the non established infectious mouse model of TB, a single dose of TMC-207 at 50 mg/kg was more efficacious than 25 mg/kg isoniazid which established its excellent EBA. In the same model a minimal effective dose (MED) was more effective after 4 weeks of TMC-207 (6.25 mg/kg) than isoniazid (12.5 mg/kg). In the established infectious mouse model of TB, TMC-207 (25 mg/kg) was more efficacious than rifampin alone (10 mg/kg). It is as active like the first-line regimen (RIF/INH/PZA) and addition of TMC-207 (25 mg/kg) to this regimen improved culture conversion after 4 and 8 weeks of treatment. It was also demonstrated that addition of TMC-207 (25 mg/kg) to the first-line regimen or substitution of TMC-207 for isoniazid in this regimen shortens the treatment duration from 6 months to 4 months.^[66]

Clinical Trial studies: Phase I trial includes pharmacokinetic, safety and tolerability study in healthy volunteers in a double-blind, randomized and placebo-controlled design. The maximum plasma concentration (C_{max}) was reached within 5 hours after oral administration of TMC-207 from 10 to 700 mg. Both C_{max} and area under curve (AUC) increased proportionally with the administered dose. The multiple-ascending dose included 50, 150 and 400 mg/day which showed an increase by a factor of ~2 in the AUC from the dose time to 24 hours and later AUC determined between day 1 and day 14. Not considerable variation was seen, and this suggests effective half-life is 24 hours. The mean AUC_{0-24h} values were 7.91, 24 and 52 $\mu\text{g hour/mL}$ at steady state with 50, 150 and 400 mg/day dose, respectively.^[62]

Phase IIA trial examined the EBA on patients with pulmonary TB, in which various doses of TMC-207, rifampin or

isoniazid is given for 7 days. The EBA of both rifampin and isoniazid was better than that of TMC-207. Only 400 mg dose of TMC-207 showed an EBA from 5-7 days as that of rifampin and isoniazid. In addition, the drug was well tolerated with no serious side effects.^[67,68] Phase IIB trial showed a reduced time to culture conversion and a higher number of culture conversion after 8 weeks of a standard background regimen plus TMC-207, in patients with multidrug-resistant tuberculosis. The compound was safe and well tolerated over an 8-week treatment period with only the common side effect, nausea occurring frequently among patients in the TMC-207 group than among those in the placebo group. Also it has received conditional approval by the US FDA for the treatment of MDR-TB, in addition to the current second-line treatment regimen. Provisional guidance for its use has also been issued by the WHO. TMC-207 is a potential candidate with novel mechanism of action for novel TB regimen. It can create greater impact on TB disease through inclusion in a first line regimen and/or as a component of a simpler more affordable drug-resistant TB regimen.

Nitroimidazopyrans

The nitroimidazopyrans PA-824 and OPC-67683 are derived from the bicyclic nitroimidazofurans. PA-824 (a nitroimidazo-oxazine) developed by TB Alliance, is in Phase II clinical trial while OPC-67683 (a dihydroimidazo-oxazole) developed by Otsuka Pharmaceuticals and it is currently in Phase III clinical trials.

PA-824: During 1970's Ciba-Geigy in India investigated bicyclic nitroimidazofurans as potential radiosensitizing agents for use in cancer radiotherapy.^[69] It was later discovered that many of these compounds possessed antimicrobial activity, including

activity against *M. tuberculosis*. However, the lead compound CGI 17341 was found to be mutagenic in the Ames assay and further research was discontinued. Nevertheless, a series of 3-substituted nitroimidazopyrans (NAPs) were synthesized and evaluated against *M. tuberculosis*.^[70] The strong activities obtained from these compounds suggested that the bicyclic nitroimidazole moiety might be an interesting pharmacophore. Based on this observation, a chemical library of 328 nitroimidazopyrans was designed and evaluated on *M. tuberculosis*. Out of all these compounds, PA-824 was identified as a promising antitubercular agent.^[71]

Synthesis: The synthesis of PA-824 required five linear steps, starting from 2,4-dinitroimidazole, which was first alkylated on N-1. Then cyclization afforded the nitroimidazopyran fused ring which was converted to PA-824 after alkylation with 1-(bromomethyl)-4-(trifluoromethoxy) benzene (Scheme 6).^[72]

Mechanism of Action: PA-824 is a prodrug, which means it needs to be activated before it becomes effective against *M. tuberculosis*. It is activated by the reduction of an aromatic nitro group in the parent drug. The reduction requires cofactor (F-420)-dependent glucose-6-phosphate dehydrogenase (FGD1). It does not act on human cells as they lack the bacterial enzymes and cofactors, which are needed to convert PA-824 in to its active form. Reactive chemical species generated through the bioreduction are presumed to be responsible for the bactericidal activity. PA-824 kills *M. tuberculosis* by inhibiting the synthesis of certain proteins and cell wall lipids that are necessary for its survival. It is believed that PA-824 acts in this way against replicating, aerobic mycobacteria only.^[71] PA-824 is also active against latent TB

bacteria. In a latent state, bacteria are anaerobic and either non-replicating or replicating very slowly. It is reported in literature that PA-824 kills latent bacteria by releasing a gas called nitric oxide (NO) which poisons the bacteria. NO gas is produced naturally by specific immune cells of human body after they engulf TB bacteria. But this immune response is sometimes not sufficient to eliminate an infection.^[73] PA-824 mimics the body's natural immune response and helps in releasing the gas upon entering the *M. tuberculosis*.

Pre clinical studies: The MIC value against *M. tuberculosis* ranges from 0.15-0.3 µg/mL. The MIC against drug-sensitive *M. tuberculosis* (12 clinical isolates) is 0.06-0.25 µg/mL and against mono, poly or multidrug resistant *M. tuberculosis* (21 clinical isolates) the MIC is 0.015-0.531 µg/mL.^[71,74] In a mouse model of acute TB infection, the novel combination of PA-824, moxifloxacin and pyrazinamide cured mice more rapidly than the first-line combination regimen of rifampin, isoniazid and pyrazinamide.^[74,75] PA-824, moxifloxacin and pyrazinamide in a combination regimen had potent sterilizing activity that accelerated the rate of conversion to sputum-negative. This suggested that PA-824 may substitute well for rifampin during intensive phase therapy.^[76] Researchers concluded that if these results are replicated in humans, regimens containing this combination therapy may radically shorten the treatment of multidrug-resistant tuberculosis.^[77]

Clinical Trial studies: Phase I trials evaluated the safety and efficacy of PA-824 in single oral administration ranges from 50 to 1500 mg and multiple-dose administration from 200 to 600 mg in healthy volunteers. The C_{max} was reached after 4-5 hours and ranged from 0.3 to 2.9

mg/mL. AUC was also dose dependent and ranged from 7.5 to 101.8 mg hour/mL with a plateau reached with the dose of 1 g. The mean half-life was around 18 hours. The drug was well-tolerated in 58 healthy individuals who were given PA-824 for a period of 7 days and no adverse effect occurred. The pharmacokinetic properties of the drug supported a regimen of one dose per day.^[77] Phase II trials was initiated to examine the EBA study. It involved oral dosage of PA-824 at 200, 600, 1000 or 1200 mg from day1 to day 14.^[79] All doses were well tolerated but exhibited equivalent activity. This activity was significant, though limited (daily log₁₀ cfu decline of 0.098 ± 0.072), similar to that observed in mice.^[80] These results indicated that PA-824 can be incorporated in to a regimen to treat drug-susceptible and drug-resistant TB more quickly and effectively.

OPC-67683 (Delamanid): OPC-6783 is a nitro-dihydro-imidazooxazole and it is closely related to PA-824. The synthesis and evaluation of a number of 6-nitro-2,3-dihydroimidazo[2,1-b] oxazoles resulted in its discovery.

Synthesis: OPC-67683 is synthesized on the basis of convergent synthesis. It is a strategy that aims to improve the efficiency of multistep chemical synthesis. The first synthon **42** was achieved by the Buchwald palladium-catalyzed arylation of 4-(4-(trifluoromethoxy) phenoxy) piperidine. Elimination of the tetrahydropyran protecting group afforded the desired phenol. The second synthon **46** was prepared from 2-chloro-4-nitroimidazole which was mono-alkylated with an epoxide. De-esterification of the product gave diol which was converted to an epoxide after selective mesylation and cyclization. Coupling of the two synthons (**42** and **46**) followed by

intramolecular ring closure led to OPC-67683 (Scheme 7).^[81]

Mechanism of Action: OPC-67683 kills *M. tuberculosis* by disrupting the cell wall. It inhibits the synthesis of methoxy-mycolic and keto-mycolic acid. Like PA-824, it is also a prodrug. *M. tuberculosis* metabolizes the drug and produces one main metabolite—a desnitro-imidazooxazole.^[82]

Pre clinical studies: The *in vitro* activity against *M. tuberculosis* is in the range of 0.006–0.024 µg/mL, approximately 10 times lower than the MIC of PA-824. The *in vivo* activity against mouse model was found to be effective at low doses. It reduced the number of bacteria in the lungs of normal and immunocompromised mice at lower concentrations than the standard first-line drugs. The combinations of OPC-67683 with rifampin and pyrazinamide eradicated TB bacteria easily in two months than the current intensive phase regimen of rifampin, isoniazid, ethambutol and pyrazinamide.^[82]

Clinical Trial studies: The phase I study includes pharmacokinetic study in which OPC-67683 was well tolerated at a dose of 100, 200, 300 or 400 mg daily for 14 days. After an oral dose of 200 mg, its C_{max} reached 0.22 µg/mL with an (AUC)_{0–24} (µg.hour/mL) of 3.551. The results revealed that EBA of OPC-67683 did not differ significantly between dosages, although patients who received a dose of either 200 or 300 mg experienced a slightly greater decline in the number of TB bacilli in their sputum than those who received 100 mg or 400 mg of the drug. Also 300 mg dose revealed its maximum absorption and overall the medication was well tolerated in patients, with no serious side effects.^[83] A phase II study was carried out by Otsuka pharmaceuticals in which randomized, double-blind trial of OPC-67683 in MDR-

TB patients was conducted. A dose of 100 mg or 200 mg twice daily was administered in addition to standard second-line drugs. Results are not yet available. It is now in clinical Phase III trial in which 200 mg dose will be given to MDR-TB patients.

Ethylenediamines

A library of more than 60,000 combinatorial compounds was generated, based on 1,2-ethylenediamine pharmacophore, as structural analogue of ethambutol and screened against *Mycobacterium tuberculosis*. Among them, SQ-109 is the most promising analogue for tuberculosis treatment which is being developed by Sequella and it is currently in Phase II clinical trials.^[89]

SQ109: SQ-109 is a new orally active diamine antibiotic for the treatment of tuberculosis.

Synthesis: The synthesis of SQ109 begins with trans, trans-farnesyl bromide (Scheme 8). Trans, trans-farnesyl bromide **48** is substituted by ethylenediamine to yield the corresponding mono substituted amine **49**. Reductive amination performed with 2-adamantanone yielded the desired 2-adamantylidiamine SQ109.^[84]

Mechanism of Action: SQ109 inhibits mycobacterial cell wall synthesis, targeting trehalose monophosphate transferase whereas ethambutol targets arabinosyl transferase.^[85]

Pre clinical studies: The *in vitro* activity of SQ-109 against *M. tuberculosis* ranges from 0.1-0.63 µg/mL. The *in vivo* activity against mouse model was found to be more effective than ethambutol. SQ-109 was given to mice for one month, resulting in a reduction of mycobacterial load in spleen and lungs that

was comparable to the effect of treatment with ethambutol but less than that of treatment with isoniazid.^[86, 87]

Substitution of SQ-109 for ethambutol in the standard regimen RHZE improved efficacy for the tuberculosis treatment.^[88]

Clinical Trial studies: Phase I included a double-blind, placebo controlled study performed on 62 healthy volunteers. The results revealed that oral dosage of 300 mg of SQ-109 were safe and well tolerated. It was rapidly distributed to tissues and presented a long half-life of 61 hours. Multiple dose healthy volunteer studies have been completed but the results have not been published. Phase II A study objective was to evaluate the extended early bactericidal activity (EBA), safety, tolerability, and pharmacokinetics of several doses of SQ109 with or without Rifampin (RIF) for 14 days in adults with newly diagnosed, uncomplicated, smear positive, pulmonary TB patients. The results have not yet been published.^[89, 90]

Rifamycins

The rifamycins were discovered in 1959 as metabolites of a microorganism *Amycolatopsis mediterranea*. Three semisynthetic rifamycins such as rifampin, rifapentine and rifabutin have excellent potential to kill all *M. tuberculosis* organisms present in an infection (as long as those organisms are susceptible to rifamycins). Rifampin is the most commonly used rifamycin in tuberculosis treatment. Rifapentine is currently being developed for the treatment of tuberculosis by GATB and it is currently in Phase III clinical trials.

Rifapentine: Rifapentine **42** is the potent derivative of 3-(4-cyclopentyl-1-piperazinyl-iminomethyl)-rifamycin. The cyclopentyl

ring is substituted by methyl ring on the piperazine ring of rifampin.

Mechanism of Action: Rifapentine inhibits mycobacterial RNA synthesis by binding to the β -subunit of DNA-dependent RNA polymerase.

Pre clinical studies: The minimum inhibition concentration against *M. tuberculosis* H37Rv is $0.06\mu\text{g/mL}$ ^[91] while in the mouse model of tuberculosis infection, administration of rifapentine, isoniazid and pyrazinamide for 5 days/week was more effective than the standard regimen containing rifampicin, isoniazid, pyrazinamide and ethambutol. The regimen containing rifapentine is safe and well tolerated but surprisingly the 2-month sputum culture conversion rate is similar.^[92]

Clinical Trial studies: Rifapentine is more potent analogue with a ($t_{1/2}$) of 10-15 hours, i.e., five times more than the 2-3 hours $t_{1/2}$ of rifampin. However, as with rifampin, rifapentine induces the expression of P450 enzymes. A combination of rifapentine (600 mg) and isoniazid (900 mg) once a week in the continuation phase of treatment compared with rifampin (600 mg) and isoniazid (900 mg) twice a week seems suboptimum, especially in patients with advanced disease or HIV coinfection who are at high risk of acquiring rifamycin

resistance.^[93] Clinical studies are in progress to assess the effects of several doses of rifapentine which is substituted for rifampin and daily rifapentine in the first line regimen to shorten treatment.

Conclusion

Several major problems like multidrug resistance (MDR), extensive drug resistance (XDR), totally drug resistance (TDR) and latency are associated with the current tuberculosis chemotherapy. There is a requirement of novel TB drugs to combat these problems. Moreover, it is also a time of great opportunities that many new potential anti-TB drugs with novel mechanism of action are in the pipeline as discussed above which may sustain global commitment towards eradication of TB. Possibly there will be major improvements in TB treatment as well as in the duration of treatment in drug susceptible, drug resistant and latent TB over the next 10 years. Progress will require a joint effort of industry and publicly funded agencies.

Acknowledgments

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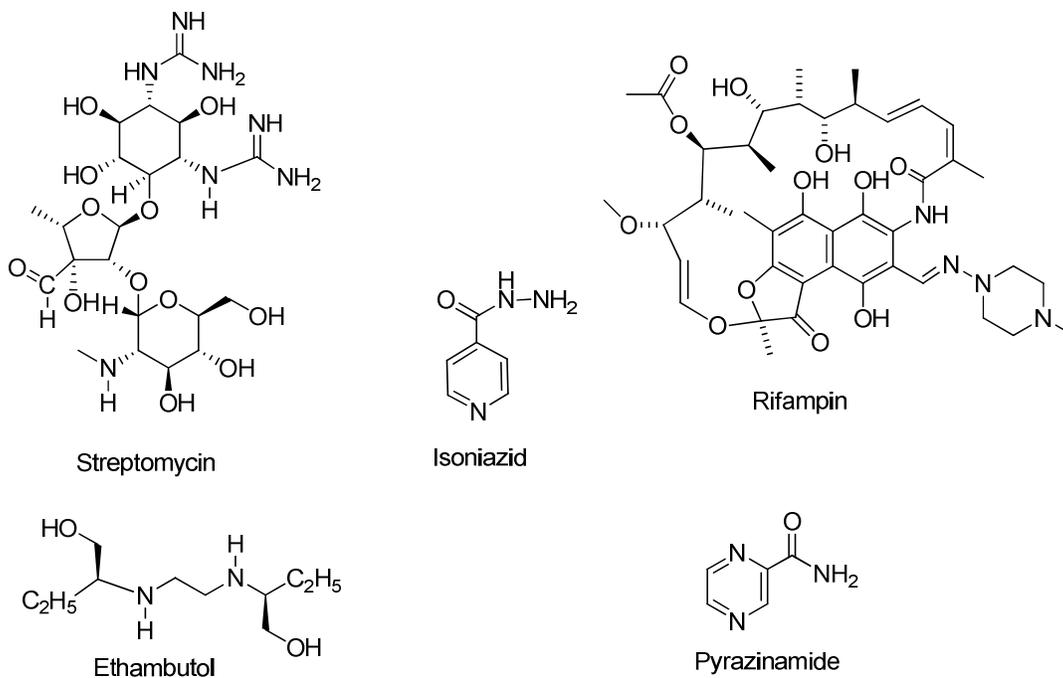


Figure 1: First Line Antitubercular Drugs

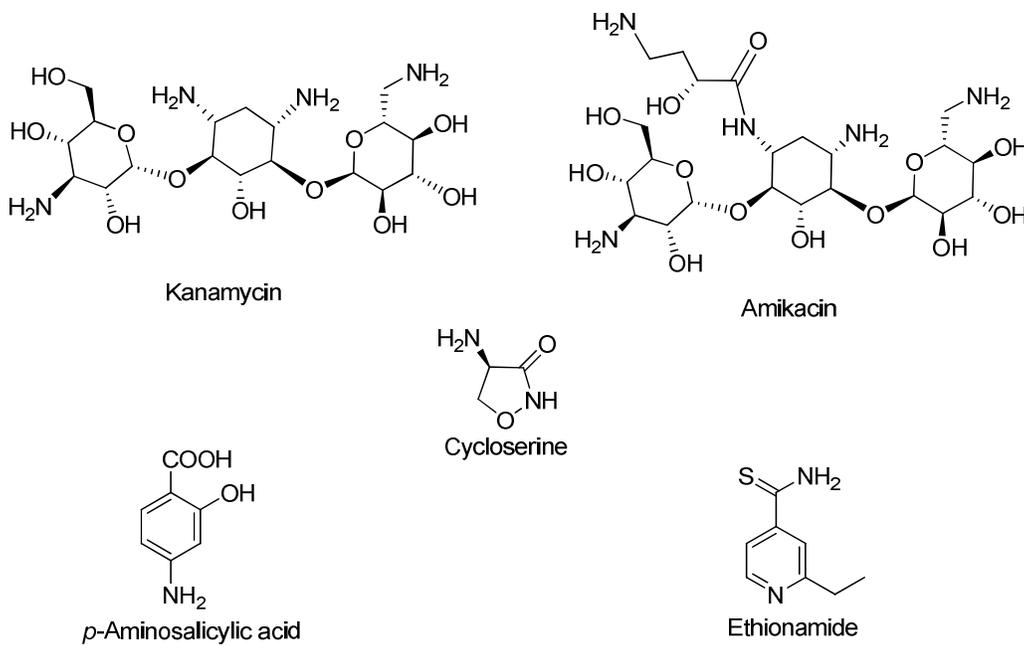
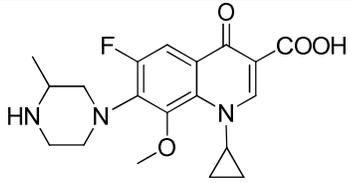
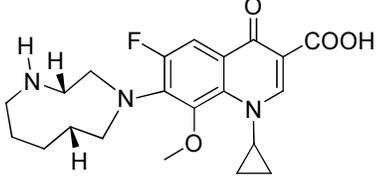
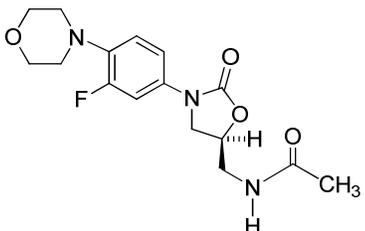
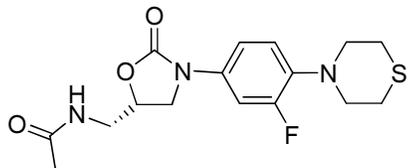
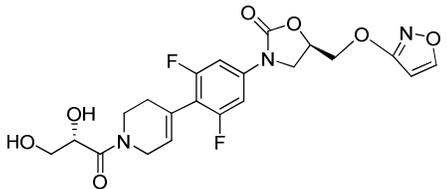
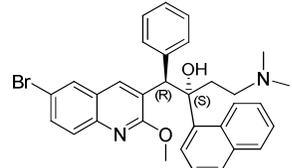
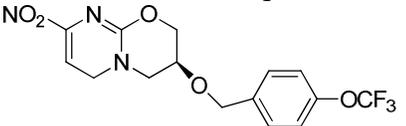
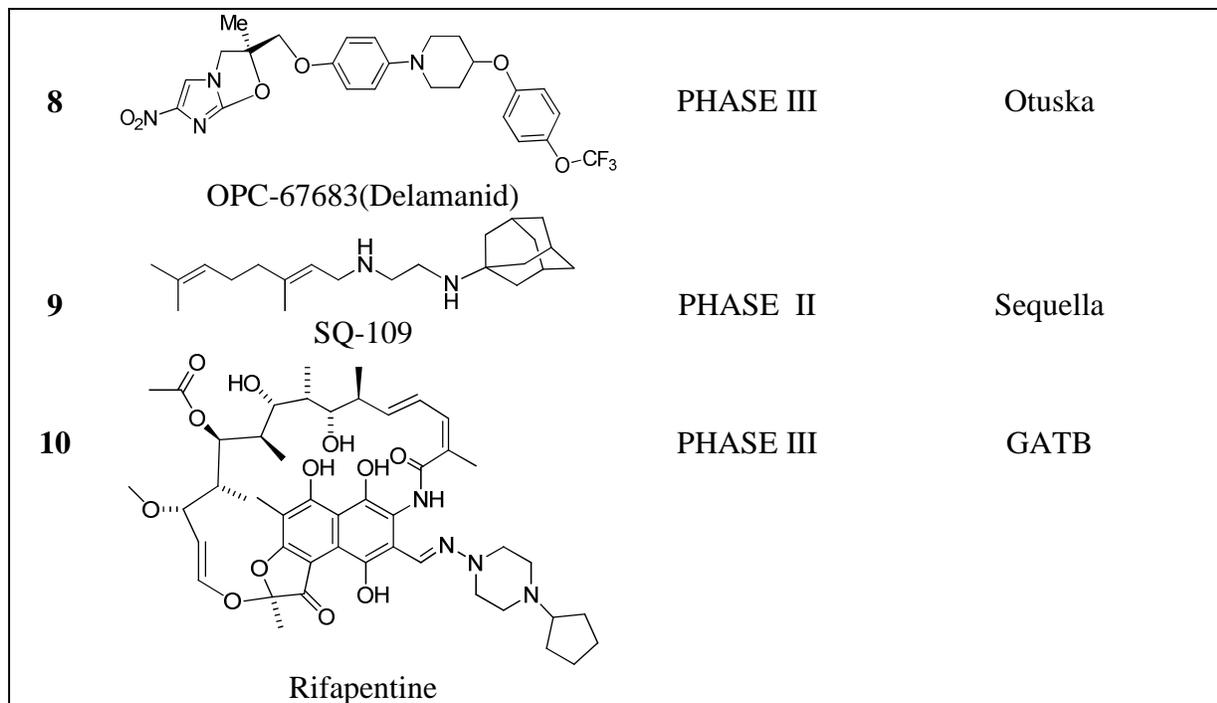


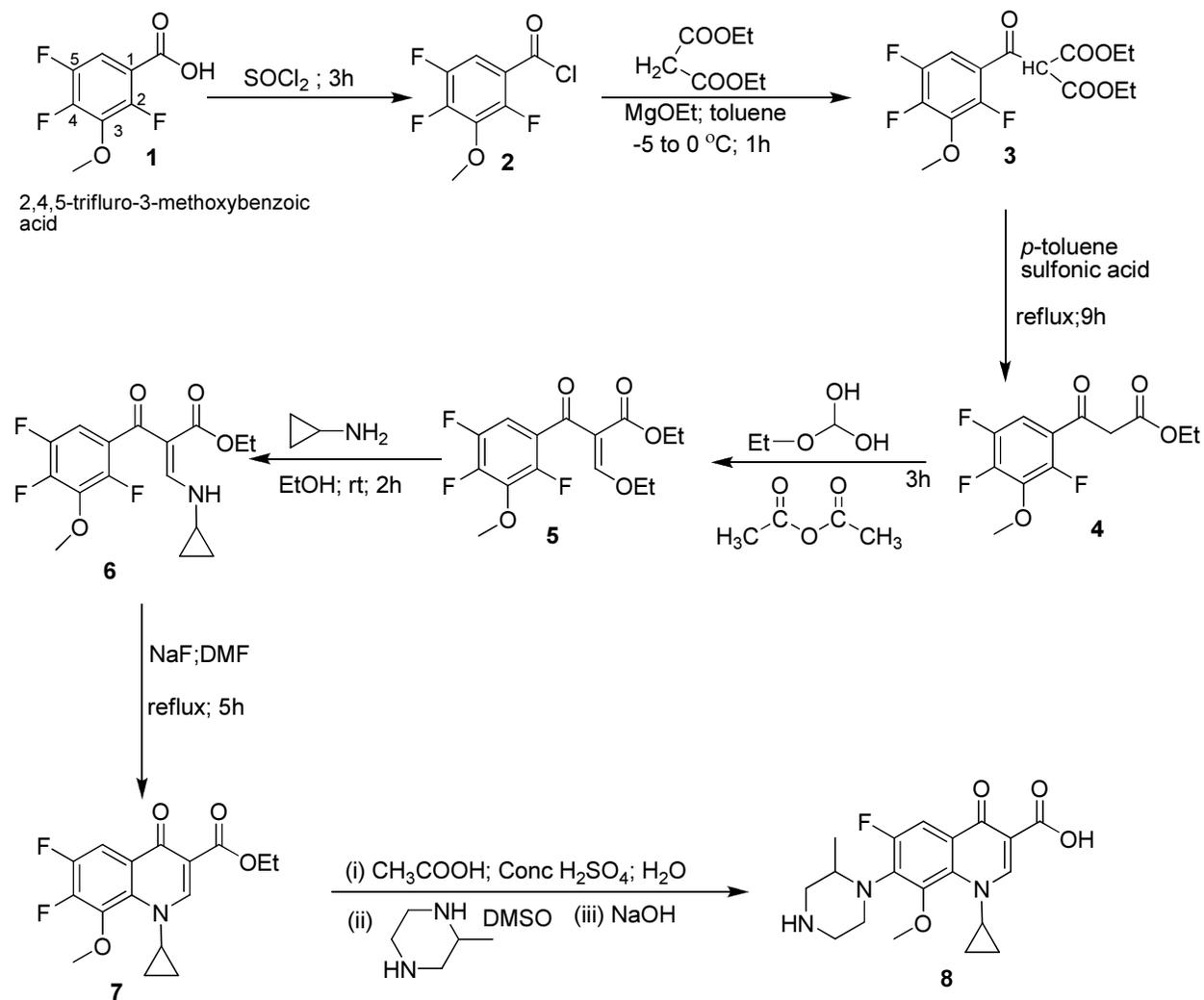
Figure 2: Second Line Antitubercular Drugs

Table 1: Antitubercular Molecules in Clinical Developmental Stage ^[38, 39]

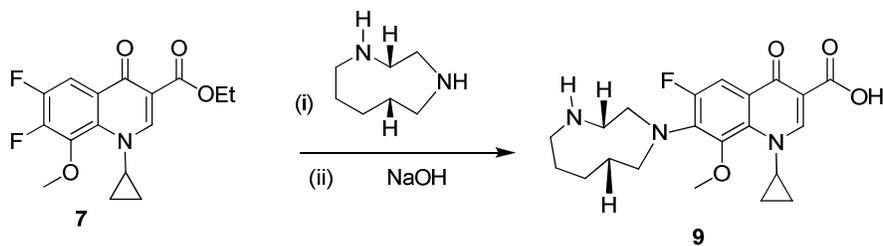
S.No	Compound	Development Stage	Sponsor/Coordinator
1	 Gatifloxacin	PHASE III	OFLOTUB; NIH
2	 Moxifloxacin	PHASE III	Bayer; GATB; CDC; NIH; FDA
3	 Linezolid	PHASE II	NIH; UPJHON (Pfizer)
4	 PNU-100480 (Sutezolid)	PHASE II	Astra Zeneca
5	 AZD-5847 (Posizolid)	PHASE II	Astra Zeneca
6	 TMC-207 (Bedaquiline)	PHASE II	Janssen; GATB
7	 PA-824	PHASE II	GATB



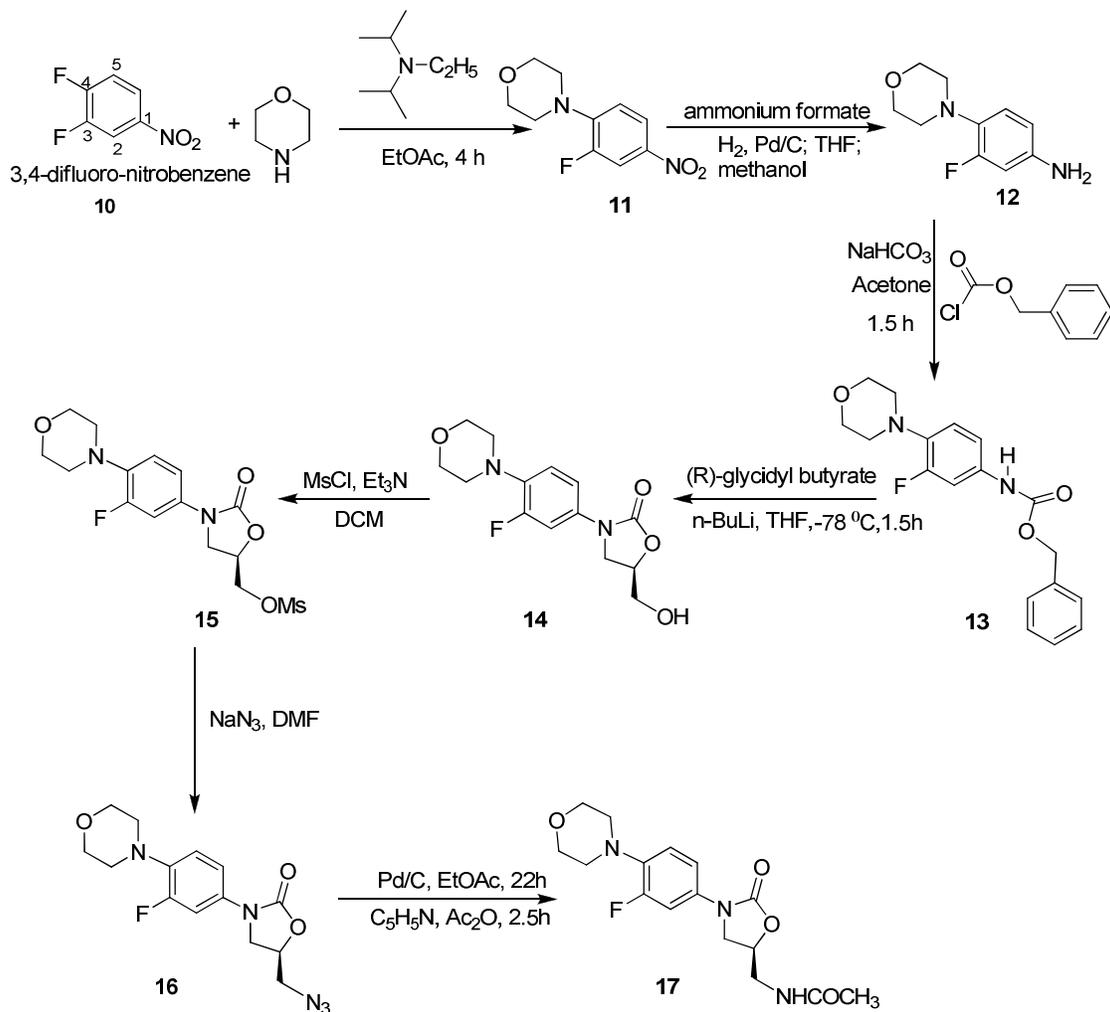
NIH - US National Institutes of Allergy and infectious diseases TB Research unit, TB research centre (Chennai); **GATB** -Global Alliance for TB drug development; **CDC** - US centers for disease control and prevention TB consortium; **FDA** - US Food and Drug Administration.



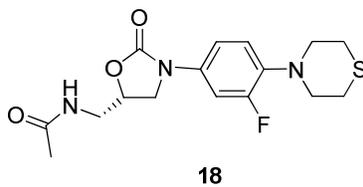
Scheme 1: Synthesis of Gatifloxacin

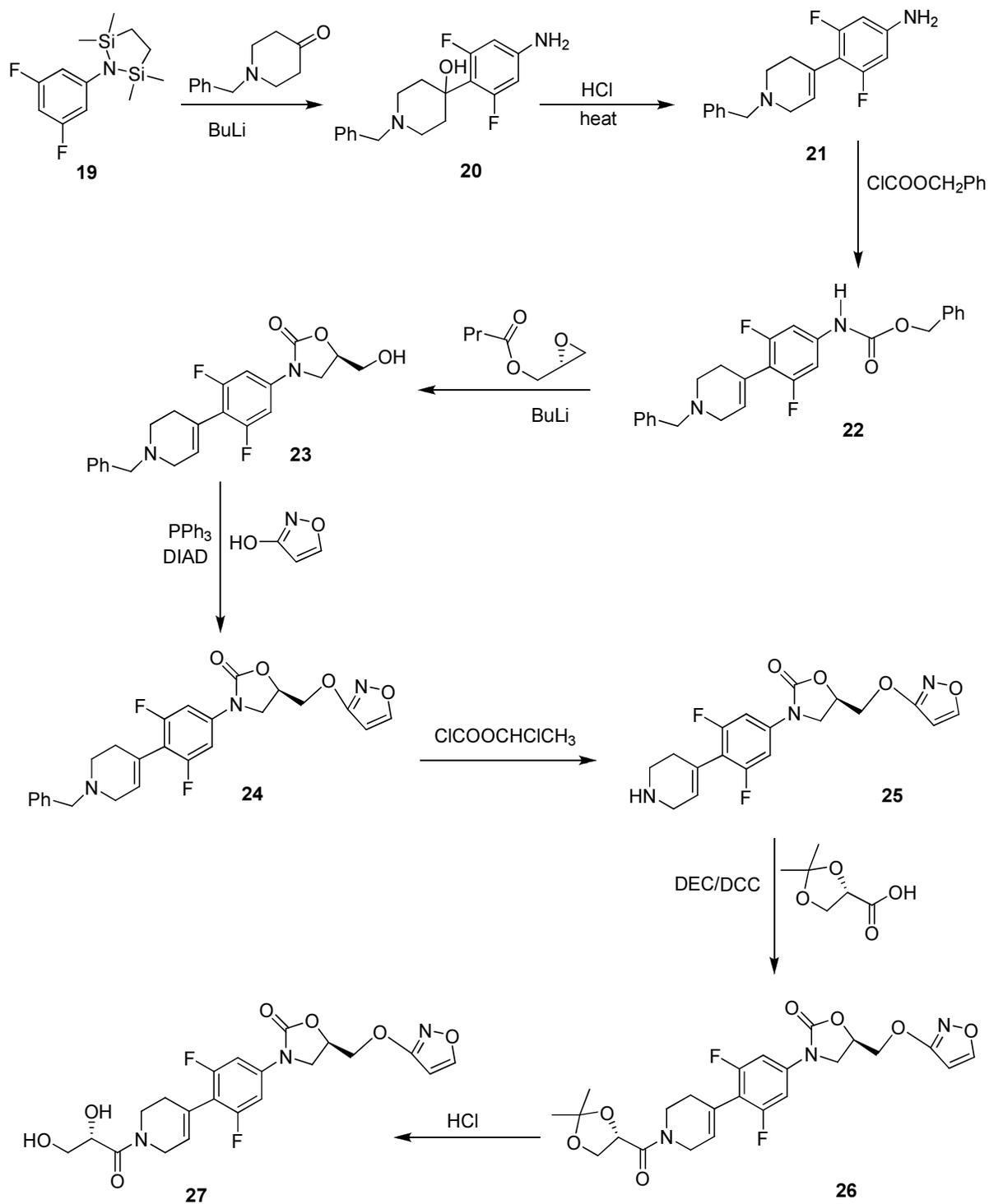


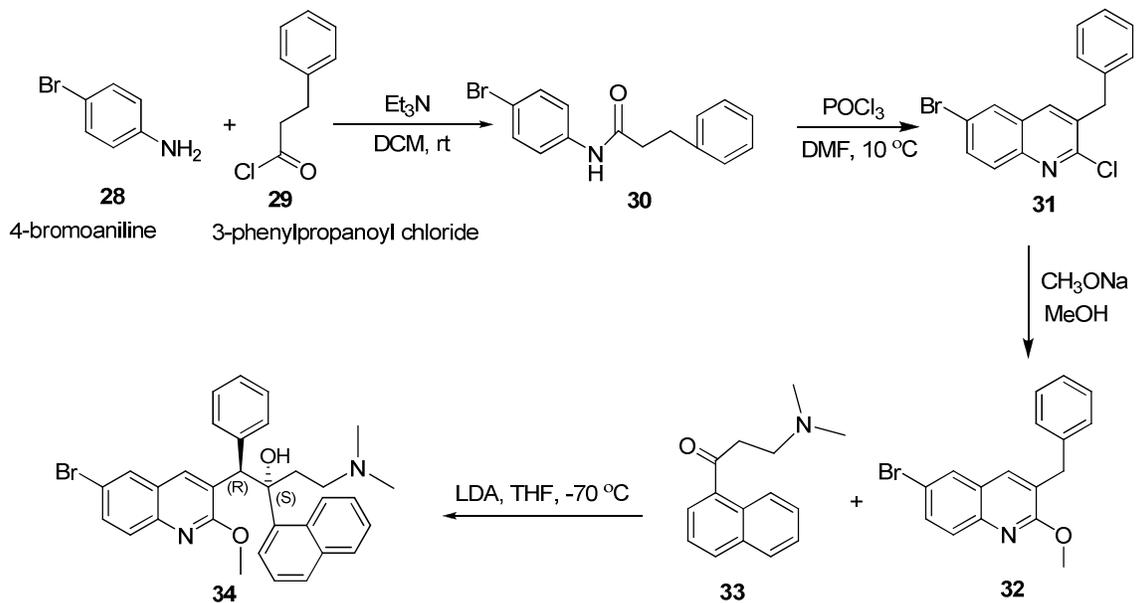
Scheme 2: Synthesis of Moxifloxacin



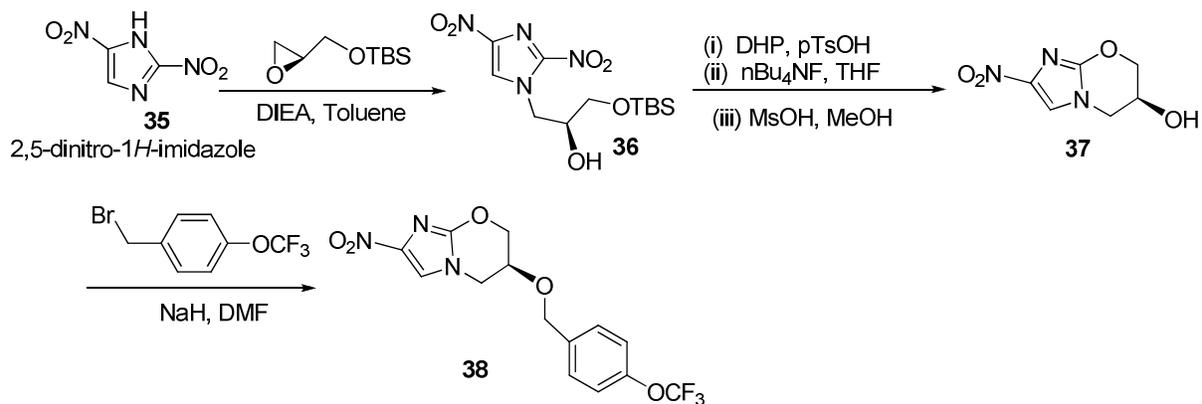
Scheme 3: Synthesis of linezolid



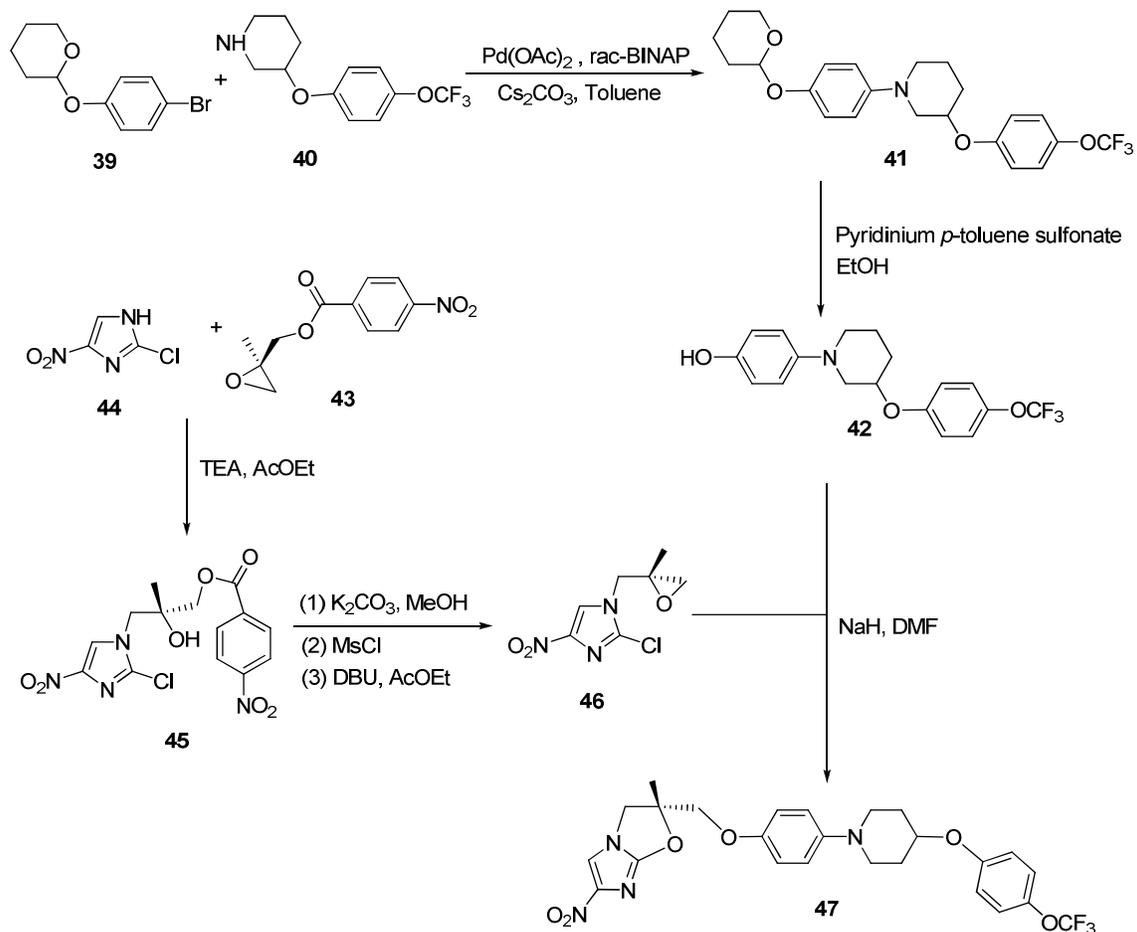
**Scheme 4:** Synthesis of AZD-5847 (Posizolid)



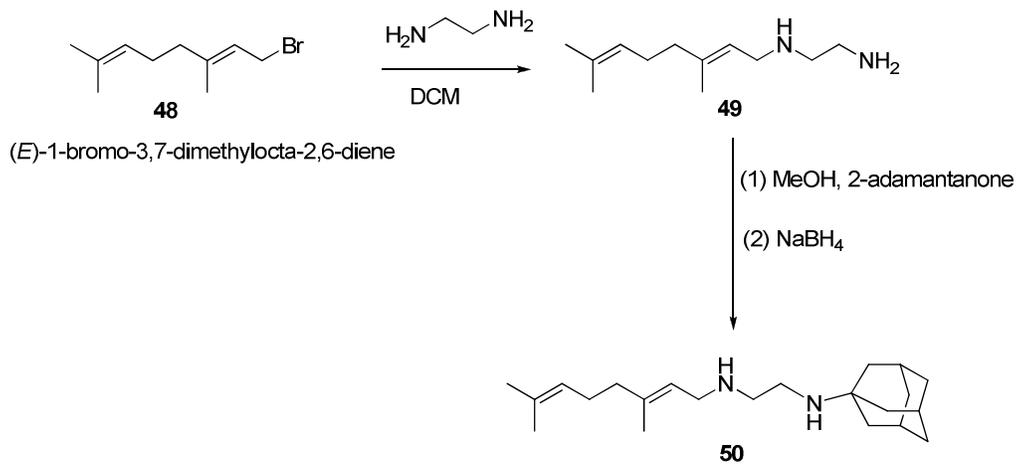
Scheme 5: Synthesis of TMC-207



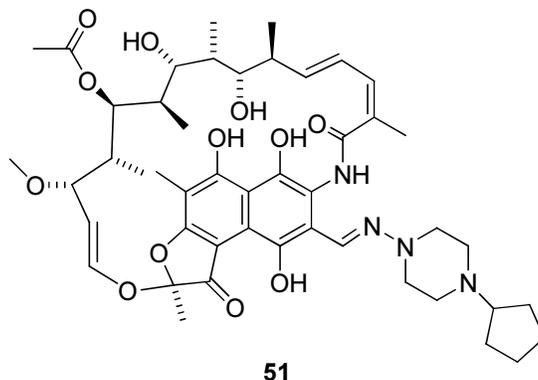
Scheme 6: Synthesis of PA-824



Scheme 7: Synthesis of OPC-67683



Scheme 8: Synthesis of SQ-109



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