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Role of Nucleoside and Macrolides Analogs in the Treatment of Infectious Disease Tuberculosis: A Short Review

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Abstract: Tuberculosis remains the leading cause of mortality worldwide. The second line of anti-TB drug Nucleoside analogs has been introduced in several compounds to improve their anti-TB activity against mycobacterium as well as their multidrug-resistant strains. Several Nucleoside analogs have shown very good activity. A series of Nucleoside analogs have been evaluated for their activity against *M. tuberculosis*. Some Nucleoside analogs exhibited significant activity when compared with first-line drugs and could be a good starting point to develop new lead compounds in the fight against multi-drug resistant tuberculosis.

Keywords: Nucleoside analogs, infectious disease, tuberculosis.

Introduction

Tuberculosis (TB) remains at the beginning of the 21st century the world's leading infectious disease with a global prevalence of more than a billion people. One-third of the world's population is currently infected; more than 5000 people die of TB every day. A great number of people are carriers of the latent form that creates a dangerous source of illness in the future. The pandemic of AIDS has had a major impact on the worldwide TB problem. Another factor contributing to the rise in TB and responsible for the increased death rate is the emergence of new strains of *M. tuberculosis* (*Mtb*) resistant to some of the current anti-TB drugs, so-called multidrug-resistant TB (MDR-TB). According to alarming WHO data, TB is spread everywhere, with most cases in South-East Asia. Mycobacteria are designated as the *transition forms* existing between bacteria and fungi. Mycobacterium refers to a genus of acid-fast organisms. These microorganisms are slender, nonmotile, Gram-positive rods, and fail to produce either spores or capsules. The various species invariably comprise of *M. africanum, M. avium intracellular, M. boria, M. chelonei, M. fortuitum, M. gastri, M. gordonae, M. kanasaii, M. trivale, M. smegmatis*, and *M. xenopi*. TB is an infection caused by the mycobacteria *M. tuberculosis*, which most often affects the lungs, and is characterized by symptoms such as acute inflammation, tissue necrosis, and frequently by the development of open sores. In a few cases, the pathogen penetrates the lymph or blood, and the infection can spread to other body tissues. Modern therapy for tuberculosis is very effective, although it can be long and difficult. The pathogen quickly develops resistance to therapy using a single drug [1-5]. Moreover, many strains also developed resistance to bi- and even multi-drug therapy, and therefore anti-TB drugs, as a rule, are used in the form of a combination of two or three drugs. Drugs used for TB therapy are very different in terms of activity and toxicity, and they are divided into two groups. Drugs in the first group include those medicinal drugs with a high level of efficacy and relatively low toxicity. Isoniazid, ethambutol, pyrazinamide as well as the antibiotics rifampicin and streptomycin are included in this group. The majority of patients using these drugs can be successfully healed. Sometimes it becomes necessary to use a drug of the second group because of microbial resistance and/or depending on the patient. Included in this group of drugs are ethionamide, antibiotics (cycloserine, capreomycin, kanamycin) as well as a very structurally simple drug called *p*-aminosalicylic acid. These drugs are somewhat more toxic than drugs in the first group, and they have certain limitations. Chemotherapy of TB should include the use of two or more effective drugs for preventing an increase in the number of resistant mutants. Treatment should last long enough to prevent relapses of slow-growing intracellular organisms [1-5].

Treatments

Excellent results for patients with non-drug-resistant TB can be obtained with a 6-month course of treatment; for the first 2 months, isoniazid, rifampin, ethambutol, and pyrazinamide are given, followed by isoniazid and rifampin for the remaining 4 months. Administration of rifampin in combination with isoniazid for 9 months also is an effective therapy for all forms of disease caused by strains of *M. tuberculosis* susceptible to both agents. Because of microbial resistance, it may be necessary to resort to "second-line" drugs in addition; thus, treatment may be initiated with 5 to 6 drugs. This category of agents includes *moxifloxacin* or *gatifloxacin*, *ethionamide*, *aminosalicylic acid*, *cycloserine*, *amikacin*, *kanamycin*, *capreomycin*, and *linezolid*. In HIV-infected patients receiving protease inhibitors and/or nonnucleoside reverse transcriptase inhibitors, drug interactions with the rifamycins (rifampin, *rifapentine*, *rifabutin*) are an important concern. Antimicrobial drugs with activity against MAC include rifabutin, *clarithromycin*,

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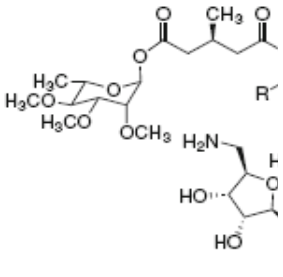
azithromycin, streptomycin, and fluoroquinolones. Clarithromycin and azithromycin are more effective than rifabutin for prophylaxis of *M. avium* complex infection in patients with AIDS. Clarithromycin or azithromycin, in combination with ethambutol (to prevent the development of resistance), is an effective treatment for *M. avium* complex infection in HIV-infected individuals. Capreomycin (CAPASTAT) is an antimycobacterial cyclic peptide elaborated by *Streptococcus capreolus.* It consists of 4 active components-capreomycins IA, IB, IIA, and IIB. The agent used clinically contains primarily IA and IB. Bacterial resistance to capreomycin develops when it is given alone; such microorganisms show cross-resistance with kanamycin and *neomycin*. Capreomycin is used only in conjunction with other appropriate antitubercular drugs in the treatment of pulmonary tuberculosis when bactericidal agents cannot be tolerated or when causative organisms have become resistant. Capreomycin must be given intramuscularly. The recommended daily dose is 15 to 30 mg/kg per day or up to 1 g for 60 to 120 days, followed by 1 g two to three times a week. The adverse reactions associated with the use of capreomycin are hearing loss, tinnitus, transient proteinuria, cylindruria, and nitrogen retention. Severe renal failure is rare. Eosinophilia is common. Leukocytosis, leukopenia, rashes, and fever have also been observed. Injections of the drug may be painful [6-10].

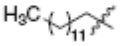
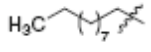
Problems

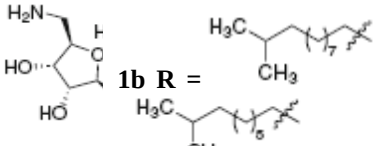
Bacterial Resistance to Drugs One of the more important problems in the chemotherapy of tuberculosis is bacterial resistance. The primary reason for the development of drug resistance is poor patient adherence. To prevent noncompliance and the attendant development of drug-resistant tuberculosis, directly observed therapy is advisable for most patients, in which a healthcare provider observes the patient ingest the medications 2 to 5 times weekly. Where drug resistance is suspected but sensitivities are not yet known (as in patients who have undergone several courses of treatment), therapy should be instituted with 5 or 6 drugs, including 2 or 3 that the patient has not received in the past. Such a regimen might include isoniazid, rifampin, pyrazinamide, ethambutol, streptomycin, and ethionamide. Some physicians include isoniazid in the therapeutic regimen, even if microorganisms are resistant, because of some evidence that disease with isoniazid-resistant mycobacteria does not "progress" during such therapy. Others prefer to discontinue isoniazid to lessen the possibility of toxicity. Therapy should be continued for at least 24 months [6-10].

Nucleoside analogs

Nucleoside analogs are the drugs typically used in the therapy of infectious diseases including *M. tuberculosis (Mtb*) and cancer [11]. The need for drugs that have activity against resistance *Mtb* strains and then makes the nucleoside analogs particularly attractive because they have unique mechanisms of action from currently used anti-TB drugs [11]. Among the nucleoside analogs currently under investigation, the capuramycin and caprazamycin antibiotics have the most potent activity [12,13]. Caprazamycin (**1a–g**) and capuramycin (**2a**) are isolated from the *Streptomyces griseus* 447-S3 and Streptomyces sp. MK730-62F2 and show activity against drug-resistant *Mtb* strains. Capuramycin analog SQ-641 (**2b**) has shown moderate activity against *Mtb*. The uridine unit and the protic amide are essential for bactericidal activity (Fig. 1) [14,15]. At the R_2 position, lipophilic groups, including medium-size alkyl chains, phenethyl, and phenyl-type substitutions, retained moderate activity but benzyl-type substitution showed decreased activity. When different lipophilic groups were placed at the R_1 position, installation of a decanoate substituent showed the largest increase in whole cell activity compared to shorter alkanoate chains, likely due to the increased lipophilicity, which increases intracellular uptake into *Mtb*. CPZEN-45 (**2f**), a caprazamycin analog, the uridine, and the aminoribose are crucial for antibacterial activity [13,16]. Insertion of ester substituents at R_1 with R_2 alkyl chains showed that tridecane (**2c**) and octadecane (**2d**) esters showed equipotent activity, whereas a 21-carbon chain with unsaturation at C(18) showed reduced potency. The effect of the amide substituent R_2 (**2e**) was explored, which showed that the potency usually increased up to a 21-carbon alkyl chain and showed reduced potency with even longer alkyl substituents. Aniline-amide substituents with n-butyl (CPZEN-45, **2f**), n-hexyl (**2g**), and hexyloxy (**2h**) showed potent activity against *Mtb*. Highly lipophilic drugs are, not good agents for lead optimization courses; thus significant work is still vital to discover superior agents from CPZEN-45 (**2f**) as a lead drug. Translocase I (mraY) is a vital enzyme involved in the biosynthesis of peptidoglycans, which makes it an attractive target due to its unique presence in bacteria. Caprazamycin (**1a-g**) and capuramycin (**2a**) inhibit Translocase I with an IC_{50} of 18 nM and 90 nM, respectively [17-19]. The lead compounds of the series are SQ-641 (**2b**), which has a MIC of 0.67-1.35 mM against drug-susceptible *Mtb* and 0.081-2.71 mM against MDR-TB, and CPZEN-45 (**2e**), which has a MIC of 2.26 and 9.07 mM against drug-susceptible and MDR-TB, respectively. SQ-641 shows promising efficacy in the murine model of TB infection and exhibits synergistic effects with EMB, STR, and SQ-109 [11]. CPZEN-45 also exhibits no significant toxicity and a new mechanism of action making these nucleosides attractive candidates for TB drug development.



1a R =  **1e R =** 



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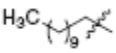
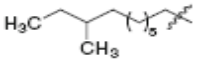
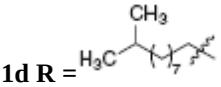
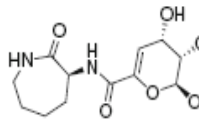
1c R =  **1g R=**

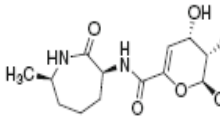




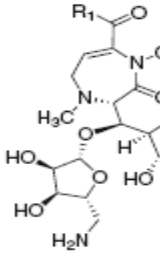
1d R =



2a Capuramycin



2b SQ-641



2c-2h

2c R_1 =COR_2, R_2 = $OC_{13}H_{27}$

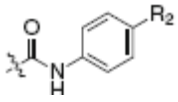
2g R_2=*n*-Hex

2d R_1=COR_2, R_2=$OC_{18}H_{37}$

2h R_2=OC_6H_{13}

2e R_1=COR_2, R_2=NH alkyl

R_1=



2f R_2=*n*-Bu; CPZEN-45

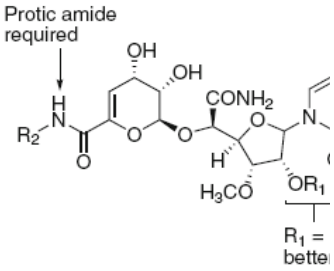


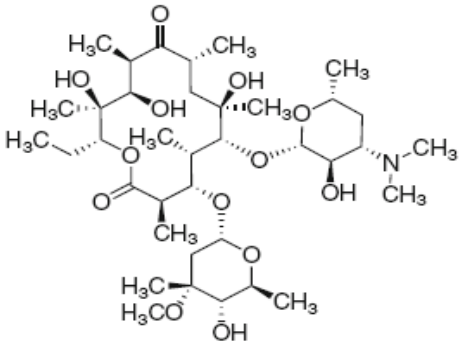
Fig. 1: Structure of Caprazamycins (**1a-g**) and Capuramycins, SQ-641 and CPZEN-45 (**2a-h**) and structural activity for required capuramycins

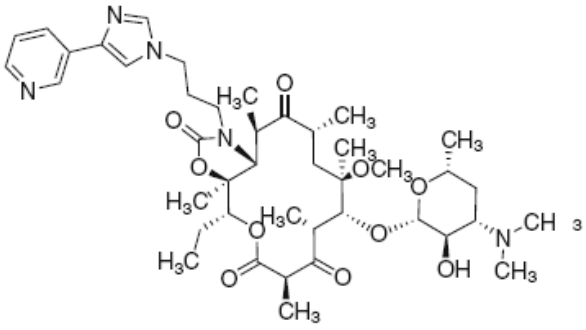
Macrolides: Macrolide antibiotics contain a many-membered lactone ring (14-membered rings for erythromycin and clarithromycin and a 15-membered ring for azithromycin) to which are attached one or more deoxy sugars. Clarithromycin differs from erythromycin only by methylation of the hydroxyl group at the 6 positions, and azithromycin differs by the addition of a methyl-substituted nitrogen atom into the lactone ring. These structural modifications improve acid stability and tissue penetration and broaden the spectrum of activity. *Erythromycin* was discovered in 1952 by McGuire and coworkers in the metabolic products of a strain of *Streptomyces erythreus*. *Clarithromycin* and azithromycin are semisynthetic derivatives of erythromycin [1-3].

In the early 1950s, the first-generation prototypical macrolide, erythromycin (EM, **3a**), was discovered. It is a natural antibiotic isolated from *Saccharopolyspora erythrea* [20,21]. Erythromycin consists of a 14-membered lactone ring with two attached sugar groups: L-cladinose at the C (3) position and desosamine at the C(5) position. EM shows antibacterial activity against Gram-positive bacteria, but no activity has been observed against *Mtb* [20,21].

To increase potency against *Mtb*, a series of EM analogs was synthesized with modifications at the 2, 3, 6, 9, 11, and 12 positions of the 14-membered lactone ring, as well as at the 40 positions of cladinose and the 200 positions of desosamine [23,24] (Fig. 2).

Specific modifications on the lactone ring such as 6-substitution, 11, 12-carbamate, 11, 12-carbazate, and 9-oxime substitutions enhance potency [23]. Substitution of fluorine at position 2 in ketolides appears to improve both potency and selectivity (i.e., cytotoxicity vs activity against *Mtb*). The C(6) substituent is critical for the activity of the ketolides [22], as it affords acid stability by preventing internal hemiketalization with the 3-keto group [20]. In general, ketolides are less potent than the corresponding cladinose-containing compounds for all substituents on the 6-position [21]. Among 9-oxime substituted ketolides and macrolides, there is a correlation between the lipophilicity of the substituent on the 9-position (defined as calculated log P) and the potency [22], with some C (9) oximes showing submicromolar MIC against *Mtb*. The substituent at 11, and 12 positions appears to significantly affect potency. A variety of aryl-substituted 11, 12-carbamate and carbazate macrolides, and ketolides demonstrated low or submicromolar MICs [23]. Also, the aryl substituent may be involved in determining cytotoxicity. The substituted 11, 12-carbazate compounds demonstrated significant dose-dependent inhibition of *Mtb* growth in mice, with a 10–20-fold reduction of colony-forming units (CFUs) in lung tissue [22].





Erythromycin (EM) **3a**
Telithromycin **3b**

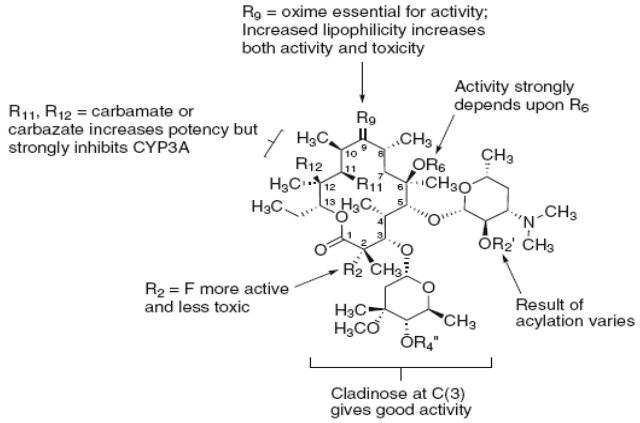


Fig. 2: Erythromycin (EM), **3a**, and telithromycin, **3b**, and General SAR for macrolides

To further enhance lipophilicity, the 20 and 400-positions on desosamine and cladinose rings, respectively, have been modified via esterification, which generally improved potency and sometimes decreased CYP3A4 inhibition (more commonly in the cladinose-containing macrolides), although the substituent on the 9-position is generally more important than modifications on 20 and 400 positions [21].

Macrolides bind reversibly to the 50S subunit of 70S bacterial ribosomes, which inhibits protein synthesis [21,23]. Although macrolides are effective for other bacterial infections, including some mycobacteria, they have not demonstrated significant efficacy against *Mtb*. Ribosome methylation is the most widespread mechanism involved with macrolide resistance in *Mtb*, and the gene ermMT plays an essential role [10-14]. Therefore, the current development goal for macrolides has been primarily to overcome bacterial resistances resulting from methylation of the rRNA and drug efflux [23,24]. Furthermore, since macrolides are well-known inhibitors of CYP3A4, a cytochrome P450 enzyme, developing compounds with decreased inhibition against CYP3A4 is critical. In addition to low efficacy against *Mtb*, the pharmacokinetics of EM are somewhat unsatisfactory, as it is unstable to gastric acid and displays a short serum half-life (~1.4 h) [20]. The second-generation macrolides such as clarithromycin and roxithromycin improved both properties [21], but clarithromycin showed only weak activity against *Mtb* either in vitro or in vivo (200 mg/kg dose for low-dose aerosol infection mouse models), suggesting that second-generation macrolides cannot be expected to offer significant antimicrobial clinical benefits for TB [22]. Further improvements focused on the replacement of the L-cladinose substituent, as it is associated with both drug efflux (one mechanism for the development of macrolide resistance) and metabolic instability of the macrolides. This third generation of macrolides replaced the cladinose ring with a ketone moiety (ketolides), leading to more metabolically stable drugs [20-24]. However, it appears that C (3) cladinose is important for the antitubercular potency of macrolides, which remain more potent than either ketolides or other substituents such as 3-OH and 3-carbamoyloxy groups. Telithromycin (**3b**), the first clinically approved ketolide, has been developed for use against respiratory pathogens but is not active against *Mtb* [20-24]. Currently, there have been some improvements in vitro activity with macrolides against *Mtb*, but to date, no promising drug candidate has emerged. Preclinical work in this area is ongoing.

Despite being a global public health problem, TB has remained a neglected disease. In the era of MDR-TB and XDR-TB, there is an urgent need for new anti-TB drugs that are more effective and have less toxicity [25]. There is also a need for newer and innovative anti-TB drug delivery systems. This review attempted to summarize evidence regarding the efficacy and potential of Nucleoside analogs and macrolide analogs as new anti-TB drug molecules in the treatment of active TB disease with diverse molecular structures and chemical properties. In conclusion, Nucleoside analogs were active against the *Mtb*. It suggests that this class of compounds may be selectively targeted to *Mtb* growth and could be a good starting point to find new lead compounds.

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