Nature nurtures the design of new semi-synthetic macrolide antibiotics

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Erythromycin and its analogs are used to treat respiratory tract and other infections. The broad use of these antibiotics during the last 5 decades has led to resistance that can range from 20% to over 70% in certain parts of the world. Efforts to find macrolides that were active against macrolide-resistant strains led to the development of erythromycin analogs with alkyl-aryl side chains that mimicked the sugar side chain of 16-membered macrolides, such as tylosin. Further modifications were made to improve the potency of these molecules by removal of the cladinose sugar to obtain a smaller molecule, a modification that was learned from an older macrolide, pikromycin. A keto group was introduced after removal of the cladinose sugar to make the new ketolide subclass. Only one ketolide, telithromycin, received marketing authorization but because of severe adverse events, it is no longer widely used. Failure to identify the structure-relationship responsible for this clinical toxicity led to discontinuation of many ketolides that were in development. One that did complete clinical development, cethromycin, did not meet clinical efficacy criteria and therefore did not receive marketing approval. Work on developing new macrolides was re-initiated after showing that inhibition of nicotinic acetylcholine receptors by the imidazolyl-pyridine moiety on the side chain of telithromycin was likely responsible for the severe adverse events. Solithromycin is a fourth-generation macrolide that has a fluorine at the 2-position, and an alkyl-aryl side chain that is different from telithromycin. Solithromycin interacts at three sites on the bacterial ribosome, has activity against strains resistant to older macrolides (including telithromycin), and is mostly bactericidal. Pharmaceutical scientists involved in the development of macrolide antibiotics have learned from the teachings of Professor Satoshi Omura and progress in this field was not possible without his endeavors.

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INTRODUCTION

The term ‘macrolide’ antibiotic to physicians and microbiologists means erythromycin and its analogs, such as clarithromycin, azithromycin and roxithromycin. Clarithromycin and roxithromycin possess a 14-membered macrocyclic ring, whereas azithromycin is 15-membered. The 14-membered ring in erythromycin was expanded semi-synthetically by an introduction of a nitrogen to make the 15-membered macrolide, azithromycin.1 The large family of 16-membered macrolide antibiotics, with tylosin being the leader, is mostly used in veterinary practice. There are many hundreds of macrolide antibiotics that belong to these classes that have been described in detail by Professor Satoshi Omura in the book, Macrolide Antibiotics: Chemistry, Biology, and Practice.2 This book is essential reading for those interested in macrolide antibiotics, as it covers not only chemistry but also pharmacokinetics and structure-activity relationships that have been learned and re-learned over the decades. Additional macrocyclic natural products, such as fidaxomicin, are sometimes referred to as macrolides but are not considered in this review as they are not related to erythromycin and also have a different mechanism of action.3

Erythromycin and its analogs have been widely used in medicine to treat respiratory, genital and skin infections. The properties of the macrolide class that support their use include: (i) good oral bioavailability, (ii) high concentrations in tissues and fluids such as the lung and pulmonary epithelial lining fluid, (iii) meaningful intracellular concentration, (iv) limited spectrum of activity that does not eradicate anaerobic Gram-negative microflora, especially in the intestine where these organisms play a protective role and (v) a profile of being generally safe and well-tolerated. The macrolides also have strong anti-inflammatory properties that provide symptomatic relief from the pain from inflammatory cytokines that are released at the sites of infection.4 All of these characteristics have led to macrolides being among the most successful of antibiotic classes in the outpatient setting as well as in the hospital.

ERYTHROMYCIN AND SEMI-SYNTHETIC ANALOGS

Erythromycin, a fermentation product from the bacteria Saccharopolyspora erythraea, was isolated in 1949 from a soil sample collected in the Philippines that was part of an intense screening program to find new antibiotics by Eli Lilly and Co.5 Although orally bioavailable, the macrocyclic ring is not stable in acid. At gastric pH, there is conversion to anhydro-erythromycin, loss in antibacterial activity, and nausea and vomiting.6 The instability of the macrolide core ring to acidic environments is an advantage for the soil microbe that...
with decreased or no activity. Therefore, more stable side chains of the macrolides because metabolism of the side chains lead to metabolites with lower activity relationships of the 16-membered macrolides. This class of antibiotics reached 48% in the US and over 70% in Asia. However, from the pharmaceutical point of view, improvements in acid stability was a necessity. Efforts to stabilize the ring led to the discovery of acid-stable, second-generation macrolide antibiotics such as roxithromycin, clarithromycin and azithromycin (Figure 1). The discovery of acid-stable, second-generation macrolides and a keto group replaced the cladinose, making the analogs more potent. A macrolide antibiotic with improved activity remained elusive.

MODIFICATIONS BASED ON PIKROMYCIN AND TYLOSIN

Pikromycin, a 14-membered macrolide without a cladinose sugar (Figure 3), was the first macrolide antibiotic identified but due to low activity, it was not developed and erythromycin became the core for new macrolide development. Learning from tylosin and pikromycin chemistry, chemists at the pharmaceutical company, Aventis, designed telithromycin (Figure 4). In telithromycin, a side chain at the 11, 12-position simulated the sugar side chain of the 16-membered macrolides and a keto group replaced the cladinose, making the molecule smaller like pikromycin. As with the compounds made at Abbott Laboratories, telithromycin had activity against macrolide-resistant strains, but also had 4-8-fold greater potency than the older macrolides. Co-crystal structures confirmed that like the older macrolides, telithromycin bound to the peptide tunnel on the 23S RNA of the 50S ribosome, and interacted with Domain V binding to A2058 and A2059. In addition, it also interacted distantly at Domain II via the side chain. Telithromycin’s activity against macrolide-resistant bacteria is reportedly due to the fact that telithromycin interacts with the bacterial ribosome at two distinct sites.

TELITHROMYCIN AND SEVERE ADVERSE EVENTS

Telithromycin was approved for broad use by regulatory agencies to treat simple and complicated upper and lower respiratory tract infections. It is marketed under the brand name Ketek, derived from the macrolide subclass ketolide, which recognized the keto group in

Figure 1 Chemical structures of erythromycin, clarithromycin, azithromycin and roxithromycin.
Advanced Life Sciences.19 Advanced Life Sciences completed Phase clinical trials at Abbott, but then licensed to the start-up company, position or on the cladinose ring, was synthesized and tested in early 2000. Unlike others, which were modified at the C6 position, unlike others, which were modifications at the C11–C12 position or on the cladinose ring, was synthesized and tested in early clinical trials at Abbott, but then licensed to the start-up company, Advanced Life Sciences.19 Advanced Life Sciences completed Phase 3 clinical trials, but failed to demonstrate efficacy in more severe respiratory infections and cethromycin was demonstrated to be non-inferior to clarithromycin at the lowest approved dose of 250 mg of clarithromycin in simple pneumonia.20 The dose used for cethromycin provided free blood levels that were low as over 95% of cethromycin is bound to plasma protein.20 Also, low blood levels were noted with repeated administration which was likely due to the compound’s induction of CYP3A4, inducing its own metabolism and gastrointestinal tract effects, precluding doses sufficient to achieve clinical efficacy.20 The drug did not obtain marketing approval. Enanta Pharmaceuticals and Shionogi developed a modithromycin, but clinical development was halted.21 Hindered by the Ketek adverse event profile (thus far unexplained, but considered to possibly be related to the ketolide structure), Abbott, Merck, Johnson and Johnson, Pfizer, Ksan and other pharmaceutical companies terminated their ketolide programs.22

OTHER KETOLIDES

Several companies developed competitive programs to synthesize ketolide analogs like telithromycin in the late 1990’s and the early 2000’s. Cethromycin, a ketolide that had a side chain at the C6 position, unlike others, which were modifications at the C11–C12 position or on the cladinose ring, was synthesized and tested in early clinical trials at Abbott, but then licensed to the start-up company, Advanced Life Sciences.19 Advanced Life Sciences completed Phase 3 clinical trials, but failed to demonstrate efficacy in more severe respiratory infections and cethromycin was demonstrated to be non-inferior to clarithromycin at the lowest approved dose of 250 mg of clarithromycin in simple pneumonia.20 The dose used for cethromycin provided free blood levels that were low as over 95% of cethromycin is bound to plasma protein.20 Also, low blood levels were noted with repeated administration which was likely due to the compound’s induction of CYP3A4, inducing its own metabolism and gastrointestinal tract effects, precluding doses sufficient to achieve clinical efficacy.20 The drug did not obtain marketing approval. Enanta Pharmaceuticals and Shionogi developed a modithromycin, but clinical development was halted.21 Hindered by the Ketek adverse event profile (thus far unexplained, but considered to possibly be related to the ketolide structure), Abbott, Merck, Johnson and Johnson, Pfizer, Ksan and other pharmaceutical companies terminated their ketolide programs.22

SOLITHROMYCIN—THE FIRST FLUOROKETOLIDE

In addition to the companies noted above, Optimer Pharmaceuticals, a small biopharmaceutical company in San Diego, had likewise generated a library of macrolides and ketolides using their expertise in macrolide and carbohydrate chemistries. From the library, licensed to Cempra Pharmaceuticals, Inc. in 2006, Cempra selected solithromycin for clinical development (Figure 5).23 Conclusive experiments performed by Dr Daniel Bertrand and his staff demonstrated that telithromycin blocked the activity of key nicotinic acetylcholine receptors (nAChRs) in the eye, liver, neuromuscular junction and brain.24,25 The structure responsible for these adverse events was hypothesized to be the imidazolyl-pyridine moiety at the end of the side chain, novel to telithromycin. Interestingly, drugs designed to target central nervous system receptors frequently incorporate this moiety. Compounds targeting the central nervous system are potent receptors.24 Thus, solithromycin is not expected to have telithromycin-associated side effects. In addition to the unique side chain, solithromycin also has a fluorine at the 2 position of the macrocyclic ring. This fluorine prevents the ketolide group from enolizing as seen with telithromycin.25 The fluorine also interacts with the bacterial ribosome at the peptide tunnel and confers solithromycin activity against macrolide-resistant strains (including telithromycin-resistant strains). Thus, solithromycin has three sites of interaction on the 23S RNA of bacterial ribosomes, making it the 4th generation of

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Figure 6 Semi-synthetic route for solithromycin.\textsuperscript{27,32}
macrolides. Unlike cethromycin, it does not induce CYP3A4,\textsuperscript{27} is well absorbed (oral bioavailability is 63% and 78% for 400 mg and 800 mg, respectively),\textsuperscript{28} and is well-distributed to tissues and body fluids such as the epithelial lining fluid.\textsuperscript{27} Solithromycin has also been developed as an intravenous formulation. An oral suspension formulation is being tested in pediatric trials.\textsuperscript{29}

Following pre-clinical development of solithromycin, two global phase 3 trials for the treatment of moderate to moderately severe community-acquired bacterial pneumonia were successfully completed.\textsuperscript{30,31} At the time that this paper was written, New Drug Applications and a Marketing Authorization Application have been submitted to the regulatory agencies in the US and EU, respectively, for marketing authorizations.

**SEMI-SYNTHESIS OF SOLITHROMYCIN**

Solithromycin is a complex molecule having thirteen chiral centers. The manufacture of solithromycin is conducted in the following 9 steps: protection/deprotection of the 2’-OH of desosamine, activation of C-11 and C-12, installation of a carbamate at C-11 and C-12, removal of the cladinose, oxidation at C-3, fluorination at C-2, formation of the aryl-1,2,3-triazole moiety and formation of the desired polymorph.\textsuperscript{27,32} The scheme for the semi-synthetic route is shown in Figure 6. The manufacture of solithromycin begins with commercially available clarithromycin. Clarithromycin was chosen as the starting material as it is a convenient way to establish the majority of the chiral centers. However, two new chiral centers are made in the synthesis of solithromycin. In step 3, the 11, 12-carbamate is formed with a high degree of stereochemical control and this transformation is well known in the literature.\textsuperscript{33–36} Creation of a second chiral center occurs during the fluorination at C-2 of the ketolide intermediate. Fluorination with N-Fluorobenzenesulfonylimide and a suitable base produces the fluoro-intermediate with the S configuration. The R isomer is not observed as a significant side product, indicating that the fluorine adds from one face of the macrolide ring. Attempts to make the R isomer proved unsuccessful by a number of different approaches, further demonstrating that the fluorination is stereospecific.

The aryl-1,2,3-triazole moiety is another unique feature of solithromycin. Regio-stereoisomer control of the substitution is achieved by employing the copper-catalyzed azide–alkyne cycloaddition methodology.\textsuperscript{37,38} Under copper catalysis, the 1,4-regioisomer is selectively produced from the azide intermediate and 3-ethynylaniline.

Although solithromycin is a challenging molecule to manufacture, the process chemistry from clarithromycin to solithromycin has been demonstrated on the multi-metric ton scale with batch sizes ranging from 250 to 400 kg depending on the step. At full commercial production, the overall yield from clarithromycin to solithromycin is expected to range from 35 to 40%.

A total synthesis of solithromycin that took 16 steps starting from seven chiral building blocks was recently reported.\textsuperscript{39} These chiral building blocks are in turn built using chiral auxiliaries and chiral catalysts in 23 steps, including those required to make synthetic desosamine. Therefore, 40 chemical transformations (including a step to set the polymorph) are required for the total synthesis of solithromycin via the Meyer chemistry. The ability to manufacture metric tons of solithromycin by this route is yet to be determined.

**RECENT ADVANCES**

Wockhardt Ltd. in India has described a new ketolide that also has activity against macrolide-resistant strains (Figure 7).\textsuperscript{40} Their phase 1 results (both single and multiple ascending dose studies) have been announced.\textsuperscript{41,42} This compound, known as WCK 4873 or nafithromycin, has the following unique features: (1) 11, 12-lactone versus the 11, 12-carbamate of telithromycin and solithromycin; (2) the side chain backbone has an amino group and methyl group substituents; (3) the side chain is attached at the 2 position of the pyridine moiety compared with attachment at the 3 position of the pyridine in telithromycin.

Semi-synthetic approaches starting with fermentation derived starting materials have resulted in the synthesis of thousands of analogs by pharmaceutical companies in over a half century since the discovery of erythromycin.\textsuperscript{6,43,44} However, there are some parts of the molecule, such as the substitution at many carbons of the macrocyclic ring, that have not been accessible to chemical modifications. To extend the universe of modifications around the macrocyclic core ring, two main approaches have been taken; biosynthetic and total synthesis. The biosynthetic approach to new molecules is dependent on the expression of genes from *S. erythreus* in a heterologous host, such as *Escherichia coli*.\textsuperscript{45} Microbes are clever and efficient chemists. New methods to exploit these ‘microbial chemists’ is the heterologous expression of genes involved in the biosynthesis of macrolides. The genes involved in the macrocyclic or polyketide core are 6-deoxerythronolide B and deoxy sugars as well as the genes involved in self-resistance. To alter macrocycle biosynthesis and obtain ‘unnatural’ erythromycin compounds, the genes have been heterologously expressed in *E. coli*. This is a herculean task as there are at least 20 genes involved with the polyketide synthesis. It also requires appropriate substrates. Nonetheless, these hurdles have been overcome with expression of erythromycin and a few macrocyclic analogs.\textsuperscript{45} One very important aspect to a biosynthetic approach to new macrolides is the control of the many chiral centers present in the macrocyclic ring. Here, microbial biosynthesis may have an edge over total synthesis.

The total synthesis of macrolide analogs has been reported in the literature for more than 35 years.\textsuperscript{46–50} These approaches have exploited new chemistries to make analogs not available from fermentation derived starting materials. In particular, the Meyer group derived starting materials such as erythromycin developed from fermentation that allows for the synthesis of macrolides and azalides that cannot be prepared from fermentation. A number of chiral building blocks are constructed and then assembled to form macrocycles and azalides with unique ring substitutions brought in with the chiral building blocks.\textsuperscript{39} Such novel macrolides may lead to an enhanced spectrum of activity against Gram-positive organisms and also greater activity against Gram-negative bacteria. However, the processes must be scalable and inexpensive if they are to compete with semi-synthesis or biosynthetic processes.

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