Streptogramins, Oxazolidinones, and Other Inhibitors of Bacterial Protein Synthesis

Tariq A. Mukhtar, and Gerard D. Wright

Chem. Rev., **2005**, 105 (2), 529-542• DOI: 10.1021/cr030110z • Publication Date (Web): 08 January 2005

Streptogramins, Oxazolidinones, and Other Inhibitors of Bacterial Protein Synthesis

Tariq A. Mukhtar and Gerard D. Wright*

Antimicrobial Research Centre, Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5

Received May 17, 2004

Contents

Tariq Mukhtar was born in Toronto in 1975. He obtained his B.Sc. (Hons) degree in Biochemistry from McMaster University in 1998, where he subsequently started postgraduate studies. He is currently a Ph.D. student in Biochemistry and Biomedical Sciences studying the molecular mechanisms of resistance to type B streptogramins and is supervised by G. D. Wright.

Gerry Wright is Professor and Chair of the Department of Biochemistry and Biomedical Sciences at McMaster University in Hamilton, Ontario. He received his Ph.D. degree in Chemistry in 1990 for work on antifungal targets and spent 2 years as a Natural Sciences and Engineering Research Council of Canada postdoctoral fellow in Professor Christopher Walsh's laboratory at the Harvard Medical School researching the molecular mechanisms of vancomycin resistance. He joined the Department of Biochemistry at McMaster in 1993 and received a Medical Research Council of Canada Scholarship. Gerry's research program includes antibiotic-resistance mechanisms, antibiotic biosynthesis, and discovering new targets for antibacterial and antifungal agents. He presently holds a Canada Research Chair in Antibiotic Biochemistry.

1. Introduction

The process of protein translation and, in particular, the macromolecular ribozyme that is the ribosome were among the first recognized molecular targets for antibiotics. A great number of these antibiotics have since found clinical use or therapeutic promise over the past 50 years (Table 1). Translation and the ribosome remain outstanding drug targets with numerous efforts directed toward further mining the potential of this ribozyme and associated activities in drug design. The recently available high-resolution structures of virtually all components of translation and the intact ribosome now make structure-based drug design across the components of the whole process a reality.[1–4] The chemical diversity of compounds that can productively interfere with ribosome action is astonishing and includes cationic aminoglycosides and neutral carbohydrates, macrolides, peptides, and diverse small molecules (Figure 1). These act by exploiting numerous binding sites

* To whom correspondence should be addressed. Phone: (905) 525-9140, ext. 22454. Fax: (905) 522-9033. E-mail: wrightge@mcmaster.ca.

10.1021/cr030110z CCC: $53.50
Published on Web 01/08/2005

Table 1. Antibiotics that Target Bacterial Translation

antibiotic	molecular target	binding site, subunit
aminoglycosides	16S rRNA	A, 30S
tetracyclines	16S rRNA	A, 30S
macrolides	23S rRNA	P, 50S
streptogramins	23S rRNA	P, 50S
oxazolidinones	23 RNA	P, 50S
lincosamides	23S rRNA	P, 50S
chloramphenicol	23S rRNA	P, 50S
edeine	16S rRNA	P/E, 30S
thiostrepton	23S rRNA	P, 50S
everninomycins	23S rRNA	P, 50S
pleuromutilins	23S rRNA	P, 50S
fusidic acid	EF-G	
mupirocin	Ile t-RNA synthetase	

on the ribosome and ancillary proteins necessary for translation fidelity.

The availability of 3D structures of the various protein and rRNA components required for translation represents the beginning of a new era in antibiotic biochemistry.[5] Co-structures of ribosome-targeted antibiotics with the intact ribosomal subunits are now available for a growing number of antibiotics, permitting for the first time examination of structure–function analysis of this complex structure.[1] Already this work has resulted in a new understanding of antibiotic–ribosome interaction that has served to rationalize decades of painstaking biochemical research on antibiotic mode of action and resistance. Furthermore, the availability of these remarkable structures, even at the relatively low resolution presently on hand, has permitted the first forays into structure-based drug design that no doubt will launch a new generation of ribosome-directed antibiotics.

The importance of the ribosome as a drug target can readily be seen in other reviews in this special issue of *Chemical Reviews*. This review will address the structure, mode of action, and resistance to the streptogramin and oxazolidinone antibiotics, two distinct classes of antibiotics that have recently been brought to market for treatment of bacterial infections. In addition, a brief discussion of some new and old classes of antibiotics that block translation will be presented.

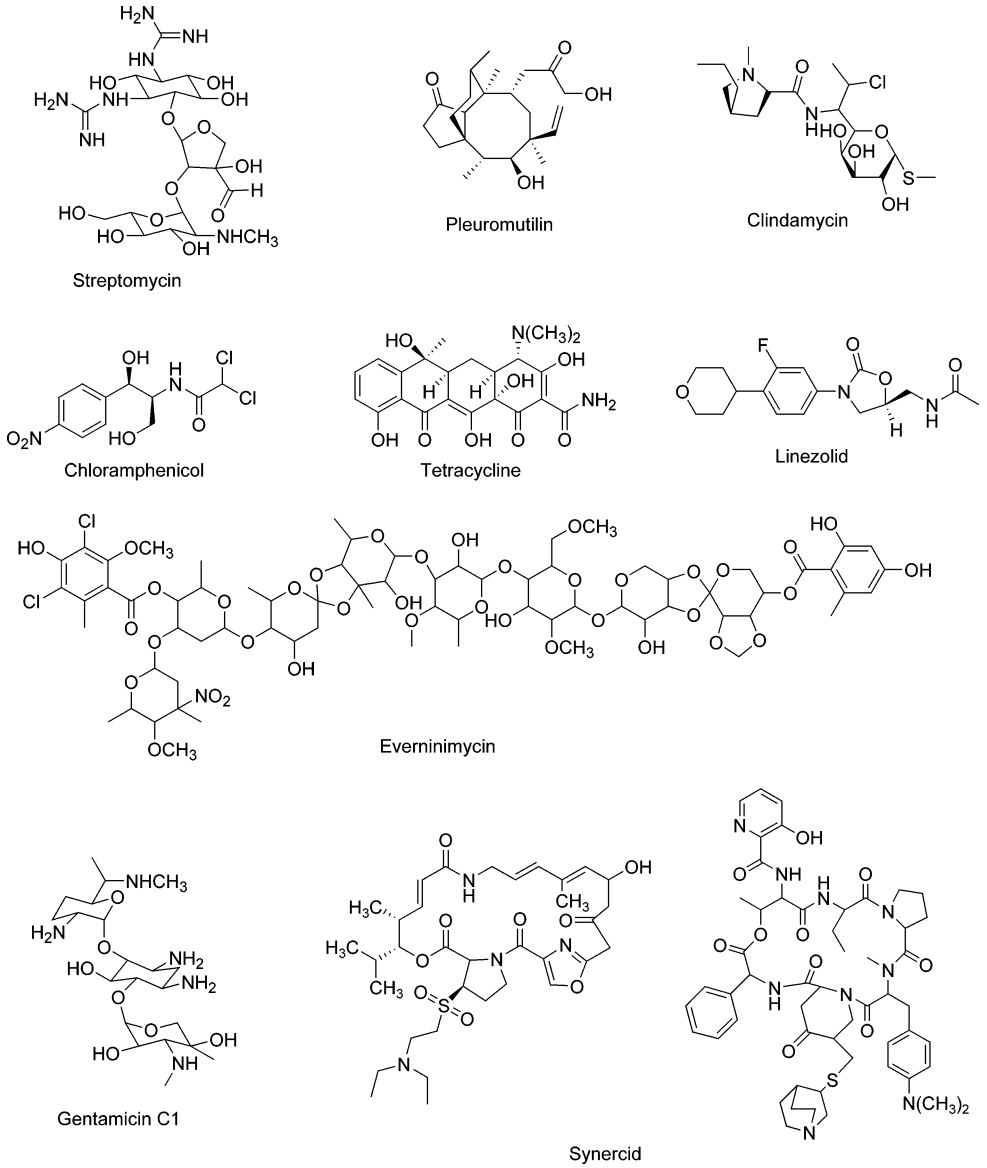


Figure 1. Structural diversity in inhibitors of bacterial translation.

2. Molecular Basis of Protein Translation

Bacterial protein synthesis is an iterative process consisting of initiation, peptide elongation, and termination events (Figure 2). This process is carried out by a number of cytoplasmic factors associated with ribosomes, which are large ribonucleoprotein assemblies made up of two unequal subunits (30S and 50S) that associate at the onset of translation initiation events. The ribosomal architecture is best viewed in context of the three tRNA binding sites that span both subunits: the amino−acyl tRNA binding/decoding A site, the peptidyl−tRNA binding P site, and the E site, where the uncharged tRNA exits. The active site of the ribosome, the peptidyl-transferase complex (PTC), lies at the interface of the A and P sites on the 50S subunit, and the growing peptides exit the ribosome via a 100 Å hydrophobic tunnel that opens at the back of the PTC.[6]

Initiation of translation begins with the formation of a ternary complex consisting of the 30S subunit, mRNA, and the initiating tRNA charged with formyl methionine (fMet).[7] The fMet-tRNA binds to the P site and is the only tRNA to do so as all subsequent amino acyl-tRNAs (aa-tRNAs) must enter through the decoding A site (Figure 2, Step 1). The initiation step is dependent on three initiation factors: IF-1, IF-2, and IF-3. IF-1 is approximately 70 amino acids in length, possessing a large S1 RNA binding domain, and binds in the A site.[8] IF-2 promotes GTP-dependent binding of the fMet-tRNA to the 30S subunit, whereas IF-3 is believed to act as a fidelity factor during the assembly of this ternary initiation complex and as a means of preventing association of the two subunits prior to initiation.[9] Upon formation of the ternary initiator complex the 50S subunit associates with the 30S subunit followed by release of the initiation factors (Figure 2, Step 2).

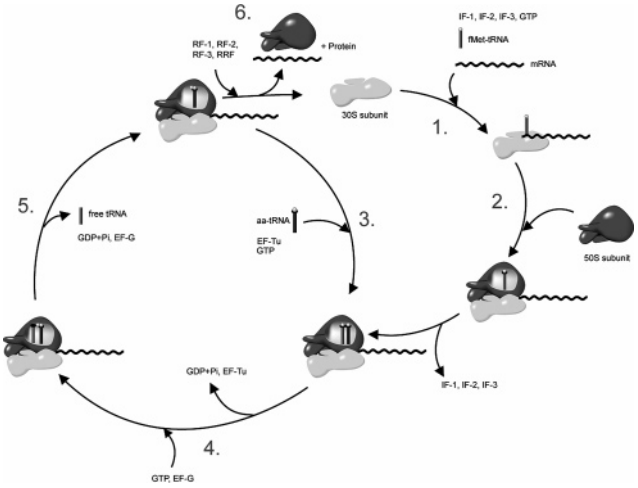


Figure 2. Overview of bacterial translation. **Step 1. Initiation.** Association of initiation factors (IF-1, IF-2, IF-3), mRNA, and fMet tRNA (GTP-dependent binding to P site) with 30S ribosomal subunit. **Step 2.** Association of 50S ribosomal subunit to the ternary complex, completing initiation complex. **Step 3. Elongation**. Ef-Tu·GTP delivers aa-tRNA into the A site followed by GTP hydrolysis and accommodation into the A site. **Step 4.** Peptidyl transfer occurs, transferring the amino acid from the tRNA in the P site to the aa-tRNA in the A site. EF-G and GTP aid in the translocation of the tRNA from the A and P sites to the P and E sites. **Step 5.** The free tRNA in the E site exits the ribosome along with the release of GDP+Pi and EF-G. This is an iterative process in which the A site is now unoccupied and prepared to receive an incoming aa-tRNA. **Step 6. Termination**. Termination occurs upon encountering a stop codon. Release factors (RF-1, RF-2, RF-3, RRF) dissociate the complex, releasing the polypeptide, mRNA, and ribosomal subunits, preparing them for recycling.

The next step in protein synthesis is elongation. This consists of a series of codon−anticodon decoding, peptide synthesis, and translocation events that have been the focus of intense research over the past decade. An elongation factor, EF-Tu bound with GTP, is responsible for delivering the aa-tRNA to the A site of the ribosome, where the decoding takes place (Figure 2, Step 3). Decoding is an elegant molecular process in which the ribosome ensures the accuracy of the incoming aa-tRNA into the A site.[10] Although the aa-tRNA contains the anticodon for the respective codon on the mRNA, this does not solely account for the fidelity of protein synthesis. In fact, it has been demonstrated that the energy difference between a cognate codon−anticodon interaction and a near cognate interaction is not large enough to account for the high accuracy with which the ribosomes carry out their function.[11,12] The additional accuracy is contributed by a process of dynamic conformational changes in the ribosome decoding center that detect orientation and geometry, resulting in incorporation of correct amino acids onto the nascent chain.[13,14]

The decoding process consists of a series of molecular checkpoints that ensure the correct incorporation of amino acids.[10] Ensuring that the correct amino acid is incorporated occurs in two stages: prior to GTP hydrolysis and after GTP hydrolysis. Decoding begins with the reversible binding of the anticodon of an EF-Tu·aa-tRNA complex to the codon at the A site of the ribosome. If the interaction is noncognate in nature, the complex will be released without hydrolysis of GTP.[15] Although the dissociation constants for cognate and near cognate codon−anticodon interactions do differ, the initial selection process does not readily distinguish between the two on the basis of this difference. In principle, any EF-Tu·aa-tRNA complex resulting in near-cognate interactions can bind the A site. Discrimination between cognate and near-cognate codon−anticodon pairing then occurs at the second stage of proofreading, in which geometry and overall fit of the aa-tRNA plays a significant role in its stability and ultimately the rate at which it is accommodated into the A site.[14,16] Accommodation thus becomes a necessary factor for a specific amino acid to be incorporated in the growing peptide. Upon accommodation into the A site, conformational changes in EF-Tu and the ribosome induce GTP hydrolysis, and EF-Tu release[16,17] and peptidyl transferase reaction between the peptidyl-tRNA and aa-tRNA can take place (Figure 2, Step 4).

A fundamental process in the elongation step is translocation. Translocation is the ratchet-like movement of the ribosome along the mRNA by one codon, resulting in the tRNA shifting from the A site to the P site to the E site. The rate of this particular step

is greatly increased by EF-G, which aids in translocation in a GTP-dependent manner. The use of tRNA mimics has recently shown this process to be a complex series of interactions and events in which the 3′ end of the aa-tRNA in the A site undergoes a 180° rotation around a local 2-fold rotation axis in the PTC, and shift of the rest of the tRNA molecule, to enter the P site. This event occurs concurrently with peptidyl transfer.[18] Various residues within the PTC cavity have also been suggested to guide and rotate the tRNA as it passes from A site to P site and subsequently guide the nascent peptide through the exit tunnel.[18]

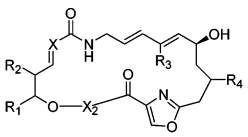
Peptidyl transfer occurs in the PTC, which is comprised only of RNA, and thus the ribosome is clearly a ribozyme. The details of the chemical mechanism of acyl transfer remain controversial, and in particular, the contributions of acid−base catalysis vs substrate proximity have been reviewed.[5,19]

The final step of protein synthesis, termination, occurs when the ribosome encounters a stop codon in the mRNA at the A site (Figure 2, Step 6). Various cytoplasmic factors, known as release factors, release the polypeptide and prepare the ribosome for recycling (RF1, RF2, RF3). RRF in conjunction with EF-G is responsible for separating the subunits and the associated components.

3. Streptogramin Antibiotics

3.1. Structures and Biosynthesis

Streptogramin antibiotics are natural products produced by various members of the Streptomyces genus. This family of antibiotics consists of two subgroups, type A and type B, which are simultaneously produced by the same bacterial species in a ratio of roughly 70:30.[20,21] Group A streptogramins are cyclic polyunsaturated macrolactones that are comprised of a hybrid peptide/polyketide structure and are cyclized through an internal ester bond between the carboxyl of the C-terminal amino acid (generally Pro) and an internal hydroxyl group (Figure 3). Structural variations in type A streptogramins can arise from desaturation of the Pro residue and by its substitution for Ala or Cys.[22] Examples of group A streptogramins are pristinamycin II_A (same as virginiamycin M_1), madumycin II, and the semisynthetic derivative dalfopristin (Figure 3).



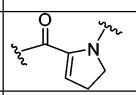
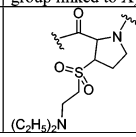
Antibiotic	R1	R2	R3	R4	X2	X
Pristinamycin II_A (Virginiamycin M_1)	isopropyl	methyl	methyl	O		CH
Madumycin II	isopropyl	methyl	methyl	OH	Alanine	CH
Griseoviridin	methyl	H	H	OH	Cysteine (SH group linked to X)	C
Dalfopristin	isopropyl	methyl	methyl	O		CH

Figure 3. Structures of type A streptogramin antibiotics.

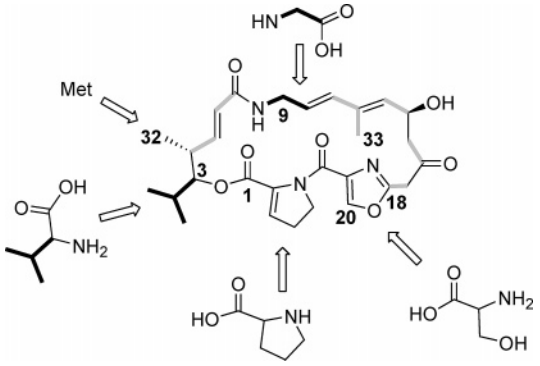
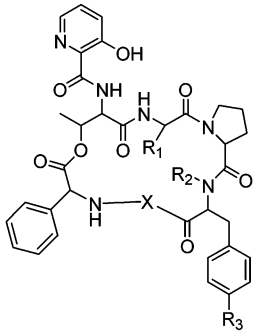


Figure 4. Biosynthetic origins of various components of group A streptogramin antibiotics.

The biosynthetic origins of the components of the group A streptogramin virginiamycin M_1 have been investigated through traditional precursor fermentation experiments (Figure 4).[23] The C-terminal dehydroproline predictably arises from Pro.[23] A two-enzyme system comprised of an FMN-dependent monooxygenase and an FMN reductase that catalyzes Pro oxidation has been purified and characterized from the pristinamycin II_A producer *Streptomyces pristinaespiralis*.[24,25] The oxazole ring is derived from Ser,[23,26] which cyclizes in a fashion reminiscent of other natural product antibiotics such as microcin (reviewed in ref 27). The rest of the molecule is largely comprised of acetate units derived from a polyketide synthesis scaffold with the exception of positions 9 and 10 (and likely the amide nitrogen), which are derived from Gly, and the isopropyl group that originates in Val (via crotonyl CoA). Methyl groups 32 and 33 (Figure 4) originate from different sources, respectively, Met and the methyl group of acetate, derived from an uncharacterized decarboxylation mechanism.[23]

Group B streptogramins are cyclic hepta- or hexadepsipeptides, e.g., pristinamycin IA, virginiamycin S, the semisynthetic quinupristin (Figure 5). The nomenclature in this field is highly redundant with several molecules reported in the literature having an identical structure but different names; for example, pristinamycin IA ≡ streptogramin B ≡ vernamycin Bα ≡ mikamycin IA ≡ ostreogrycin B ≡ PA114B1.[28] The general composition of group B streptogramins is 3-hydroxypicolinic acid-L-Thr-D-aminobutyric acid (or D-Ala)-L-Pro-L-Phe (or 4-*N*,*N*-(dimethylamino)-L-Phe)-X−L-phenylglycine. Residue X is most commonly L-4-oxo or 4-hydroxypipecolic acid but can also be Asp or Pro. The invariant N-terminal Thr residue is N-acetylated with 3-hydroxypicolinic acid and forms a cyclizing ester linkage with the C-terminal carboxyl group of the peptide via its secondary hydroxyl group.

The biosynthesis of the unusual amino acids that comprise type B streptogramins has not been extensively studied. Labeled precursor feeding experiments have shown that phenylalanine is the source of phenylglycine[29,30] and both 4-oxopipecolic acid and 3-hydroxypicolinic acid are derived from Lys[30,31]



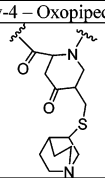
Antibiotic	R1	R2	R3	X
Pristinamycin IA	CH_2CH_3	CH_3	$N(CH_3)_2$	4 – Oxopipecolic Acid
Virginiamycin S1	CH_2CH_3	CH_3	$N(CH_3)_2$	4 – Oxopipecolic Acid
Virginiamycin S2	CH_2CH_3	H	H	4 – Hydroxypipecolic Acid
Vernamycin B δ	CH_3	CH_3	$NHCH_3$	4 – Oxopipecolic Acid
Vernamycin C	CH_2CH_3	CH_3	$N(CH_3)_2$	Aspartic Acid
Patricin A	CH_2CH_3	CH_3	H	Proline
Patricin B	CH_2CH_3	CH_3	H	Pipecolic Acid
Ostreogrycin B3	CH_2CH_3	CH_3	$N(CH_3)_2$	3-hydroxy-4 – Oxopipecolic Acid
Quinupristin	CH_2CH_3	CH_3	$N(CH_3)_2$	

Figure 5. Structures of type B streptogramin antibiotics.

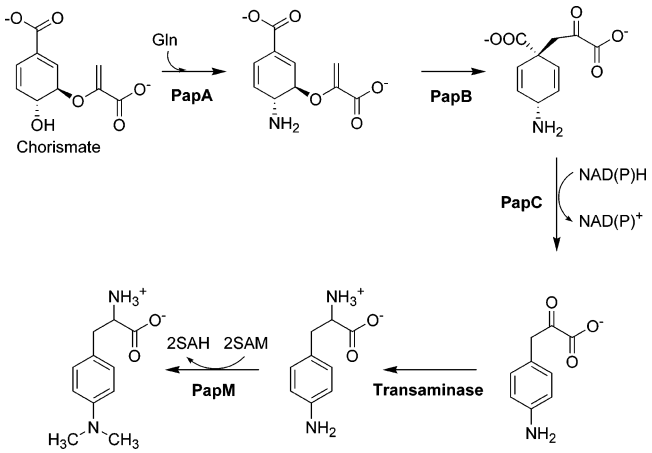


Figure 6. Biosynthesis of 4-*N,N*-(dimethylamino)-L-Phe required for type B streptogramin antibiotics.

during the biosynthesis of virginiamycin S_1 by *Streptomyces virginiae*. A four-gene system for the production of 4-*N,N*-(dimethylamino)-L-Phe has been cloned and partially characterized from the pristinamycin producer *Streptomyces pristinaespiralis*.[32] Three of the genes are reminiscent of bacterial *p*-aminobenzoic acid formation with *papA* acting as a chorismate aminotransferase, *papB* as a mutase, and *papC* as a predicted dehydrogenase that catalyzes formation of 4-aminphenylpyruvic acid (Figure 6). An unidentified transaminase then converts the ketone to 4-aminoPhe. The final steps in the biosynthesis of 4-*N,N*-(dimethylamino)-L-Phe are two successive methylations of the 4-amino group catalyzed by the enzyme PapM, which has been partially characterized and confirmed to be *S*-adenosylmethionine (SAM) dependent (Figure 6).[32]

The other components of type B streptogramins for which some biochemical information is known are the Lys-derived 3-hydroxypicolinic acid and 4-oxopipecolic acid. Sequencing of DNA downstream from known regulatory genes of virginiamycin S biosynthesis in *S. virginiae* identified four genes, *visA*−*D*, predicted to be involved in amino acid biosynthesis.[33] Both *visA* and *visC* are predicted to encode enzymes that generate 1-piperidine 2-carboxylic acid. VisA has been purified and shown to be a pyridoxal−phosphate-dependent Lys 2-aminotransferase capable of generating 1-piperidine 2-carboxylate.[33] Inactivation of the *visA* gene in *S. virginiae* completely blocked biosynthesis of virginiamycin S_1, and addition of 3-hydroxypicolinic acid but not pipecolic acid to the culture medium rescued this null phenotype, suggesting that VisA is required for formation of the former (Figure 7).[33] Paradoxically, the intracellular levels of 1-piperidine 2-carboxylic acid in the *visA* null mutant were comparable to the wild-type organism. The *visC* and *visD* genes show homology to 1-piperidine 2-carboxylic acid, generating Lys cyclodeaminase and P-450 oxidase, respectively. These genes and their products have not been well studied, but they are predicted to be involved in the synthesis of 4-oxopipecolic acid (Figure 7).[34]

The presence of nonprotein amino acids in type B streptogramins predicts the requirement of nonribosomal peptide synthetases (NRPSs) for antibiotic assembly (see review by Sieber and Marahiel in this issue). The NRPS-encoding genes responsible for biosynthesis of pristinamycin I from *S. prinstinaespiralis* have been purified[35] and cloned.[36,37] Three NRPS genes, *snbA*, *snbC*, and *snbDE*, are organized in a 1,2, and 3 module arrangement (Figure 8). The first gene encodes SnbA, which activates 3-hydroxypicolinic acid;[36] this gene has been cloned and designated *visB* in *S. virginiae*.[34] An epimerization domain in the aminobutyric acid module of *snbC* is consistent with the D-stereochemistry of this amino acid in the final product. Similarly, the presence of

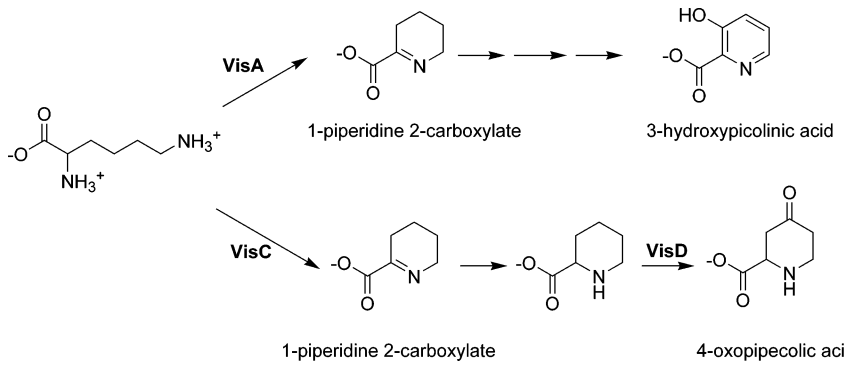


Figure 7. Biosynthesis of 3-hydroxypicolinic acid and 4-oxopipecolic acid components of type B streptogramin antibiotics.

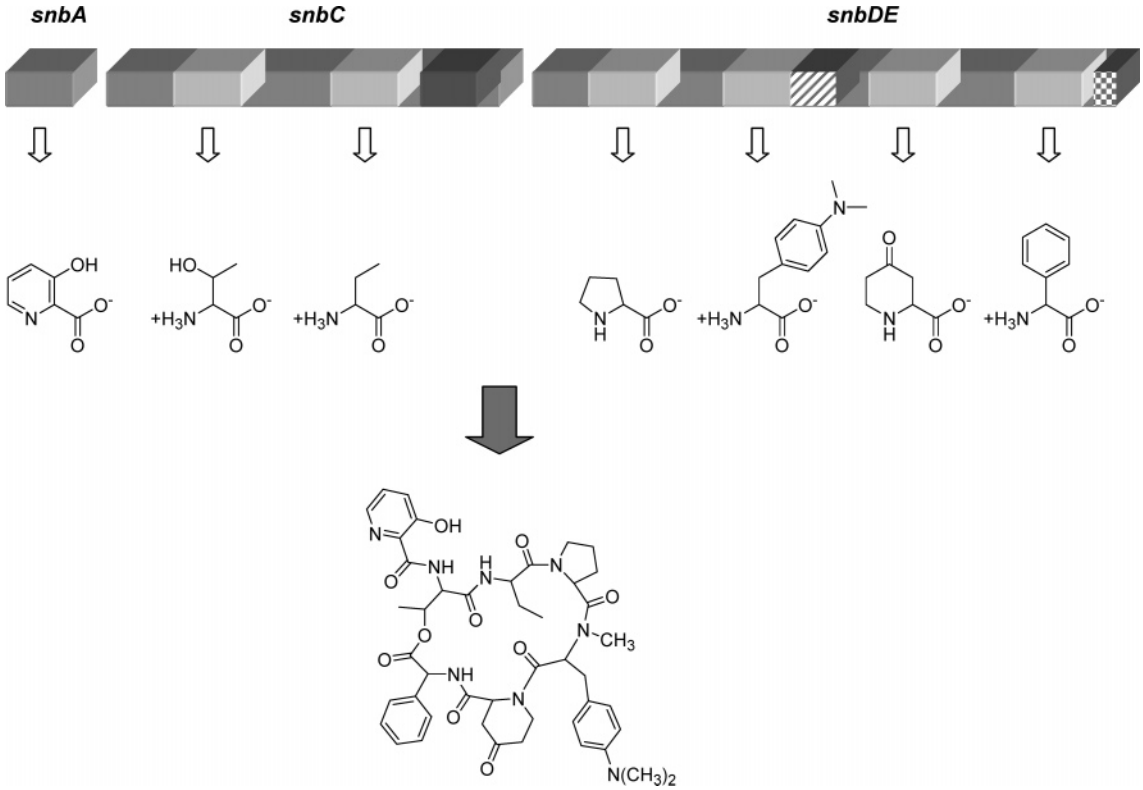


Figure 8. Organization of the nonribosomal assembly line for the biosynthesis of pristinamycin I.

a methyltransferase domain flanking the 4-*N*,*N*-(dimethylamino)-L-Phe activation domain of SnbDE is consistent with N-methylation of the amide bond (Figure 8).

The biosynthesis of streptogramins is highly regulated by γ-butyrolactone autoregulators that affect gene expression at nanomolar levels.[38] These diffusible small molecules bind to highly specific transcription factors, e.g., BarX of *S. virginiae*.[39] These quorum-dependent autoregulators, analogous to the homoserine lactones of some Gram-negative bacteria,[40] have been implicated in regulation of secondary metabolism and cellular differentiation in a number of actinomycetes.[41,42]

3.2. Synergy and Mode of Action

Both the A and B streptogramins bind the P site of the 50S ribosome, a property that is shared with such structurally diverse antibiotics as the macrolides, lincosamides, and thiopeptides (Table 1). Streptogramins are unique in that the two components (A and B) are separately bacteriostatic, yet when working in combination, they act synergistically on inhibition of bacterial growth and can become bactericidal.[43] Group A streptogramins bind to the PTC only in the absence of aminoacyl-tRNAs and block substrate attachment to the donor and acceptor sites, preventing the early events in elongation. In addition, the binding of type A streptogramins causes a conformational change in the 50S ribosome that increases the activity of the type B streptogramins by 100-fold.[43] Type B streptogramins, on the other hand, prevent the extension of protein chains, cause the release of incomplete peptides, and can bind to the ribosomes at any step of protein synthesis.[43]

The structure of virginiamycin M bound to the *Haloarcula marismortui* 50S subunit supports the inhibition of the PTC activity as the antibiotic bridges both the A and P sites.[44] The structure of the 50S ribosomal subunit from *Deinococcus radiodurans* in complex with both dalfopristin and quinupristin has also been reported (Figure 9).[45] The structure con-

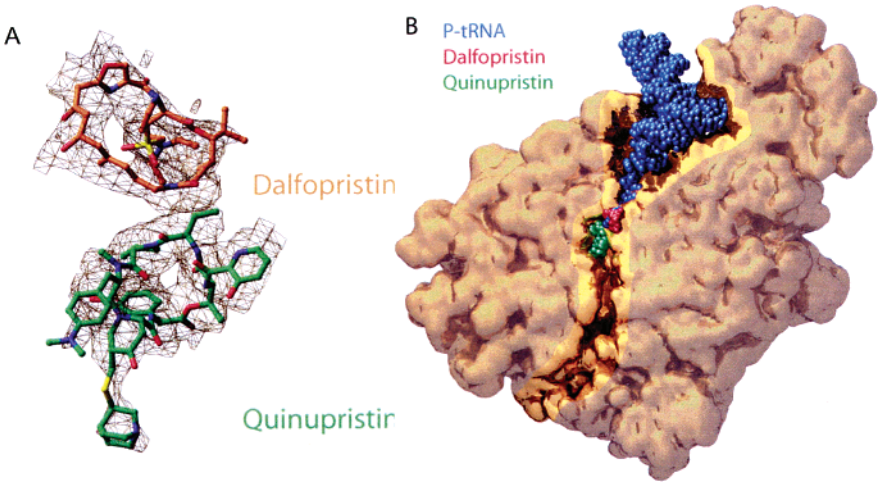


Figure 9. Structure of dalfopristin and quinupristin bound to the large subunit of the bacterial ribosome. (A) Electron density of the dalfopristin and quinupristin bound to the ribosome. (B) Space-filling model showing the positioning of dalfopristin, quinupristin, and the peptidyl t-RNA. The peptide exit tunnel is shown to demonstrate the impact of quinupristin binding. (Reprinted with permission from ref 45. Copyright 2004 BioMed Central.)

firmed many of the biochemical studies performed with streptogramins and the ribosome and also sheds new light on the basis of synergy for these antibiotics. The structure demonstrated that quinupristin binds at the entrance to the exit tunnel through which the nascent peptide travels. The antibiotic−ribosome interaction appears to be dominated by hydrophobic interactions and also via hydrogen bonds to residues A2062 and C2586 (Figure 5). Interestingly, the quinuclidinylthio moiety that imparts good solubility and pharmacological properties to quinupristin occupies an empty space in the subunit. This is consistent with the fact that various modifications in this position do not interfere with antibiotic binding.

Like virginiamycin M, dalfopristin is positioned in a pocket in the PTC.[45] It also appears to be bound by a network of hydrophobic interactions in addition to hydrogen bonds with residues G2505 and G2061. These studies indicate that dalfopristin interferes with the correct positioning of the substrates for the A and P sites. Therefore, once the P site is occupied by the substrate, then binding of dalfopristin can be hindered. This is consistent with studies demonstrating that translating ribosomes are not susceptible to dalfopristin.[46,47]

The synergistic nature of these antibiotics is assisted by hydrophobic interactions between the streptogramins. The structure indicates that both antibiotics contact A2062 through both hydrophobic interactions and hydrogen bonds. This is also consistent with biochemical data, suggesting that A2062 undergoes conformational changes upon binding of streptogramins.[48,49] Perhaps the most interesting suggestion of this study was the description of conformational changes in the PTC upon dalfopristin binding. These changes are shown to be in residue U2585. This residue appears to be rotated by 180° in the streptogramin-bound state as compared to the native, making hydrogen bonds with residues C2606 and G2588. Harms et al. performed simulations that indicated that if there are no long-range conformational changes upon binding of dalfopristin, then U2585 in the native and rotated conformations have nearly identical energy states. The binding of dalfopristin therefore provides sufficient energy to move the residue through the energy barrier to the rotated conformation, thereby making new contacts via hydrogen bonds. The spontaneous reversal is predicted to be slow even after removal of dalfopristin due to similarity in energy state to the native form. This observation is consistent with studies on the post-antibiotic effect of the drug (retention of antibiotic activity even after circulating levels of antibiotic have dropped below the minimal inhibitory concentration).

3.3. Clinical Utility

Despite the fact that the streptogramins were first discovered in the 1950s, these antibiotics only found marginal use in Europe in the following decades as antibacterial agents in the clinic. They did however, and still do, find use as feed additives in agriculture. The rise in antibiotic resistance in the 1980s and 1990s, largely as a result of the emergence of vancomycin-resistant enterococci (VRE), spurred renewed interest in antibiotic discovery in pharmaceutical companies. Medicinal chemists at Rhône-Poulenc worked to improve the drug-like properties of pristinamycin, resulting in the synthesis and characterization of the semisynthetic streptogramins dalfopristin (type A) and quinupristin (type B). Their combination in a 7:3 ratio resulted in the antibiotic Synercid, which received FDA approval in 1999 for the treatment of bacteremia caused by vancomycin-resistant *Enterococcus faecium* and skin and skin structure infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*.

A number of studies have been performed that examine the in-vitro and in-vivo antibacterial activity of quinupristin and dalfopristin. Bouanchaud reviewed the susceptibility of the quinupristin/dalfopristin combination, illustrating its effectiveness against a wide range of organisms.[50] Synercid's spectrum of activity includes most Gram-positive pathogens such as species of Staphylococci, Streptococci, and Enterococci, including multi-antibiotic-

resistant strains. *Enterococcus faecalis*, however, is intrinsically resistant to the effects of Synercid, likely due to the presence of Lsa, a predicted dalfopristin−clindamycin efflux pump.[51] Furthermore, important aerobic Gram negatives including *Moraxella catarrhalis*, *Legionella* spp., *Mycoplasma* spp., *Neisseria* spp., and to a lesser degree *Haemophilus influenzae* are also susceptible to Synercid. Its antibacterial activity has also been confirmed against a number of anaerobic organisms such as *Bacteroides* spp., *Lactobacillus* spp., *Clostridium perfringens*, and *Clostridium difficile*. The wide spectrum of antibacterial activity for quinupristin/dalfopristin therefore provides a therapeutic alternative for life-threatening bacterial infections.

3.4. Streptogramin Resistance

Despite the relatively recent development and clinical entry of Synercid, multiple mechanisms of resistance to streptogramins are known. This may be a reflection of the significant agricultural use of this class of antibiotic over several decades. There are three major acquired streptogramin-resistance mechanisms: active efflux, target modification, and antibiotic inactivation.

Efflux of type B streptogramin antibiotics has been found to be associated with ATP-binding transport pumps that are specific for the 14- and 15-membered macrolide antibiotics as well as streptogramin B, for example, MsrA, from *S. aureus* RN4220.[52] Efflux of type A streptogramins has also been associated with several proteins including Lsa, which is intrinsic to *E. faecalis*,[51] and Vga[53] and VgaB,[54] which have been found on plasmids in *S. aureus*.

Another mechanism of streptogramin resistance is target modification. Covalent modification of the 23S rRNA by an rRNA methylase encoded by the *erm* gene is a highly prevalent mechanism of streptogramin B resistance. Erm-mediated ribosomal mono- and dimethylation of the N^6 of A2058 (*E. coli* numbering) of the 23S RNA confers resistance not only to type B streptogramins but also to macrolides such as erythromycin and to lincosamides such as clindamycin.[55] This gives rise to the so-called MLS_B-resistance phenotype (M = macrolide, L = lincosamide, S_B = type B streptogramin) that is predominantly found in Gram-positive bacteria. There are a number of different *erm* genes that have been identified in a variety of organisms, and resistance is likely a result of a conformational change occurring in the ribosome.[56] The type A streptogramins are not affected by Erm-mediated resistance, and as a result, synergy between the two types of streptogramins is maintained.

An important aspect of this type of resistance is the regulation of the respective *erm* genes. Expression of the MLS resistance in staphylococci may be inducible or constitutive. The inducible nature of the resistance determinant depends on regions adjacent to the gene itself. Specifically, inducible *erm* gene transcripts contain an upstream regulatory element that contain four inverted repeats.[55] These give rise to two stem loop structures that in the absence of erythromycin sequester both the ribosome binding site and initiation codon for the *erm* gene. When erythromycin is present, the antibiotic binds the ribosome, resulting in stalling of translation. This stalling event likely kinetically displaces the stem loop structures and allows translation of the methylase. Therefore, gene regulation is maintained by a feedback mechanism; when all the ribosomes are methylated, erythromycin cannot bind, in turn stalling does not occur, and the mRNA adopt the stem loop structures again.[56]

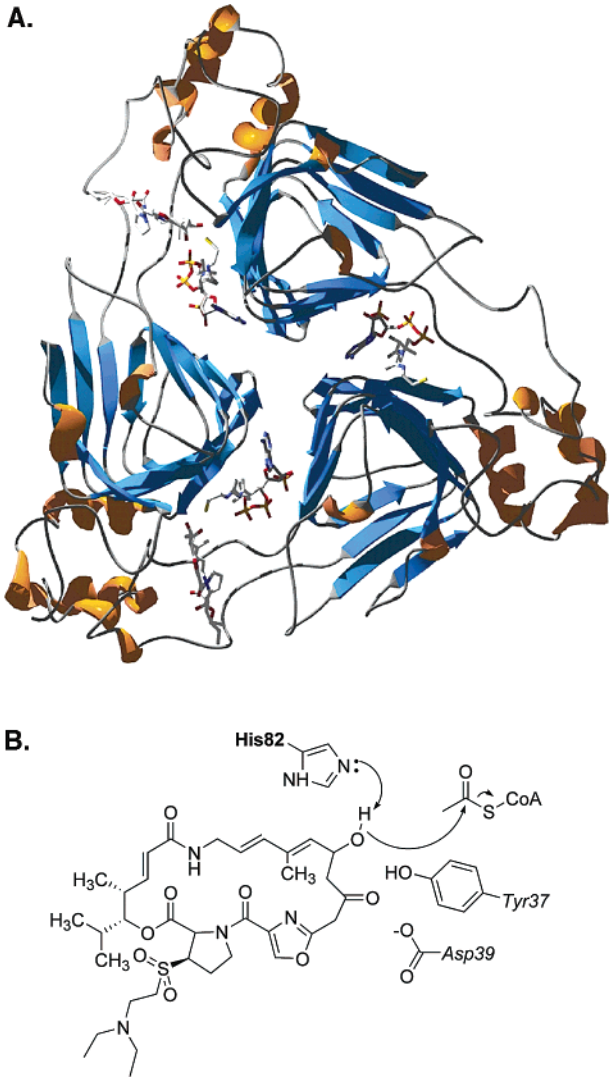


Figure 10. VatD as a representative inactivator of type A streptogramin antibiotics. (A) 3D structure of VatD cocrystallized with Virginiamycin M_1 and CoA.[58] (B) Predicted molecular mechanism of acetyltransfer of VatD.

Recently, Tait-kamradt et al. reported clinical isolates of *S. pneumoniae* conferring the MS_B phenotype that did not harbor *ermB* or mutations in the 23S rRNA.[57] These isolates were found to have modified L4 ribosomal proteins with either a three amino acid substitution (G69TG to T69PS) or in one case a six amino acid insertion in this region.

The final type of acquired resistance to streptogramins include a cadre of antibiotic inactivation enzymes. These consist of acetyltransferases, which modify type A streptogramins, and lyases, which inactivate type B streptogramins. The acetyltransferases transfer an acetyl group from acetyl−CoA on

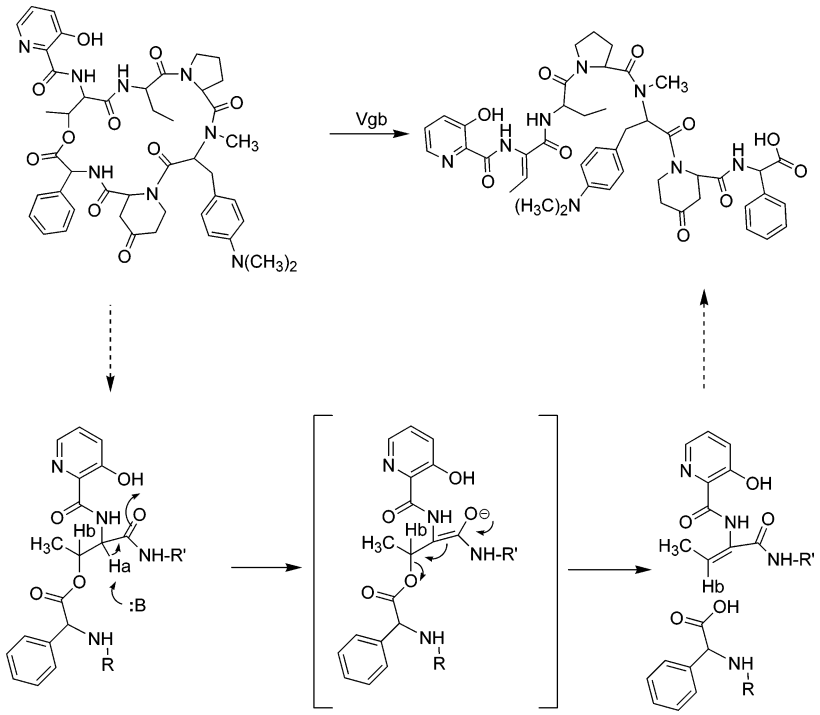


Figure 11. Predicted molecular mechanism of Vgb-catalyzed inactivation of type B streptogramin antibiotics.

to the secondary hydroxyl of the type A streptogramin. This hydroxyl makes multiple contacts with G2505 (*E. coli* numbering) of the 23S rRNA in the PTC as evidenced by the 3D structure of the antibiotic bound to the ribosome,[45] and therefore, acetylation of this hydroxyl results in a steric block of drug−target interaction. On the other hand, the lyases cause the cleavage of the ester linkage on type B streptogramins, resulting in linearization of the peptide and loss of the bioactive conformation necessary for ribosome binding.

A number of streptogramin A acetyltransferases have been identified in many pathogenic organisms. The crystal structure for VatD, an acetyltransferase from *E. faecium*, has been solved in the apo form,[58,59] bound to substrate acetyl−CoA,[58,59] product CoA,[58] and the streptogramins virginiamycin M_1[58] and dalfopristin[59] (Figure 10A). The enzyme is a homotrimer with each subunit folded into three domains. These domains consist of a large coiled LβH, an extended loop domain, and C-terminal domain. Recent mutagenesis studies have supported a predicted mechanism where His82 acts as the general base and is a major determinant of catalytic rate enhancement by VatD (Figure 10B).[59]

The other streptogramin inactivation mechanism associated with pathogenic bacteria is conferred by Vgb lyase, which inactivates type B antibiotics. Early studies on type B streptogramin-inactivating enzymes suggested that this enzyme was a lactonase in which hydrolysis was responsible for linearizing the cyclic peptide.[60−63] However, in 1996 Bateman et al. reported type B streptogramin-inactivating activity in crude cell-free extracts of *Streptomyces lividans*. The extract inactivated the antibiotic etamycin through an elimination mechanism as opposed to hydrolysis.[20] Recent studies on Vgb lyase, using purified recombinant enzyme from *S. aureus*, and orthologues encoded on the chromosomes of *Bordetella pertussis* and *Streptomyces coelicolor* have demonstrated that this enzyme also inactivates type B streptogramins through an elimination mechanism as opposed to hydrolysis (Figure 11).

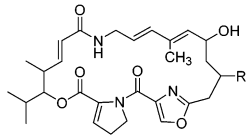
In 1998, Suzuki et al. reported an enzyme from the streptogramin-producing organism *S. virginiae* that was capable of inactivating type A streptogramins by a previously unidentified mechanism.[64] This inactivation occurred through reduction of the 16-carbonyl group of virginiamycin M, resulting in 16R-dihydroVM. Although this inactivation mechanism has not yet been reported in the clinics, the possibility for its appearance is an event that both researchers and health practitioners should be aware of.

3.5. New Streptogramins

Synercid is not orally available and is administered by intravenous routes. Efforts have therefore been made to generate new orally active streptogramins. For example, RPR 106972 is a 2:1 (molar) mixture of pristinamycin I_B (RPR 112808) and pristinamycin II_B (RPR 106950) (Figure 12).[65] Pristinamycin I_B differs from a closely related molecule pristinamycin I_A at the *N*-methyl-4-(dimethylamino)phenylalanine residue. In this particular case pristinamycin I_B contains a *N*-methyl-4-(methylamino)phenylalanine at this position. In addition, pristinamycin II_B differs from pristinamycin II_A in the dehydroproline residue in which it is replaced with proline.

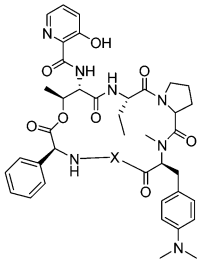
Rhône Poulenc Rorer (subsequently Aventis Pharma) also developed additional type B streptogramins through chemical modification of the 4-oxopipecolic acid moiety and type A streptogramins through modification of the dehydroproline residue. In particular, a new oral streptogramin designated XRP 2868, a combination of RPR132552A and RPR 202868

A.



	R
(16R)-16-dimethylamino-16-desoxopristinamycin II_A	$N(CH_3)_2$
(16R)-16-desoxo-16-fluoropristinamycin II_B	F
(16R)-16-desoxo-16-(trifluoromethoxy)pristinamycin II_B	OCF_3
(16R)-16-desoxo-16-(ethylnyl)pristinamycin II_A	C≡CH

B.



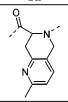
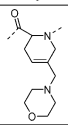
	X
2″-methylpyrido[2,3-5γ,5δ]pristinamycin I_E	
5γ(S),5δ(R)-[5γa,5δb]-1,4-hexahydrothiazepino pristinamycin I_E	
5δ-(morpholinomethyl)-5 δ5γ-dehydropristinamycin I_E	

Figure 12. New semisynthetic streptogramin antibiotics: (A) type A streptogramins; (B) type B streptogramins.

(Figure 12), has been shown to be very effective against a number of Gram-positive and Gram-negative organisms and particularly effective against pneumococcal and *Haemophilus* strains.[66] A number of patents have been issued to Aventis Pharma for the development of novel streptogramin derivatives which have shown to have activity against Gram-positive organisms, particularly streptococci, enterococci, and staphylococci.[67] Some examples of these structures have been summarized in Figure 12.

The growing problem of streptogramin resistance as well as a desire to improve pharmacological properties will drive the development of new agents in the future. The availability of 3D strucutres of the ribosome with bound antibiotics and of resistance enzymes will greatly facilitate these efforts.

4. Oxazolidinone Antibiotics

4.1. Discovery and Structure–Activity Relationships

The oxazolidinones are the only new chemical class of antibiotic that have been discovered and successfully implemented in the clinic over the past 40 years. These bacteriostatic molecules find no congeners in natural product compounds and were first synthesized by chemists at E.I. du Pont de Nemours & Co. in the mid-1980s (e.g., DuP-721, Figure 13).[68,69] While these compounds showed good antibacterial activity against Gram-positive bacteria such as Staphylococci, Streptococci, and Enterococci,[68] the compound class was not pursued as a result of toxicity issues in animals studies.[70] Scientists at Pharmacia subsequently found that a structure–toxicity relationship could be established for the oxazolidinones and initiated a campaign to develop these compounds as novel antibacterial agents (historically reviewed in ref 70).

Initial structure–activity relationship studies revealed a core structure requirement for the 5-S configuration at position 5 of the oxizolidinone ring, an acylaminomethyl group linked to C5 and N-aryl ring substitution (Figure 13). This work was expanded to explore functionalization of the aryl ring that resulted in improved activity or expanded antibacterial spectrum. For example, substitution with azole groups added increased activity toward the Gram-negative pathogens *H. influenzae* and *Moraxella catarrhalis*,[71] and compounds incorporating thiomorpholine resulted in activity against mycobacteria.[72] Substitution by piperazine,[73] morpholine,[74] as well as fluorination[75] was also explored. The details of this SAR program have recently been reviewed.[70]

The outcome of this drug discovery program is the antibiotic linezolid (Figure 13), which received FDA approval in 2000 and is marketed under the trade name Zyvox.[76] Linezolid is active both in oral and injectable forms and is indicated for the treatment of a variety of infections caused by Gram-positive bacteria.

4.2. Mode of Action

Oxazolidinones bind to the P site of the 50S subunit with micromolar affinity[77,78] and inhibit bacterial translation.[79] NMR studies indicate that the solution structures and the ribosome-bound structures of the antibiotics are very similar.[78] Despite binding to the P region of the ribosome in a region that overlaps with chloramphenicol and lincosamide binding sites, oxazolidinones have no effect on the PTC activity.[77] Instead, inhibition of translation appears to be at the level of translation initiation.[80] In particular, binding of oxazolidinones at the P site interferes with binding of the initiator fMet-tRNA to this site during the formation of the initiating complex (Figure 2).[81] A recent study indicated that oxazolidinones also promote frame shifting and stop codon read through in an *E. coli* system.[82]

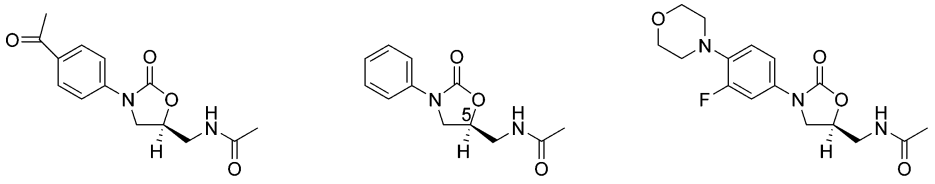


Figure 13. Structures of oxazolidinone antibiotics.

Mapping of 23 rRNA mutations that result in oxazolidinone resistance (see below) have further localized the binding site to the P site, and kinetic studies of a number of inhibitors of translation with oxazolidinone-resistance ribosomes reveal some cross-resistance with chloramphenicol but not the A-site-specific antibiotic sparsomycin.[83] Careful work by Pompliano's group at Bristol-Myers Squibb suggests that oxazolidinones induce a conformational shift in the peptidyl transferase region that is specific to the structure of the individual antibiotic.[83] Therefore, one could imagine a suite of similar compounds with different resistance patterns; high-resolution crystal structures of various oxazolidinones in complex with the ribosome will greatly facilitate interpretation of the available data and its use in structure-guided drug design.

Taken together, the available research points to a mechanism of action where oxazolidinones bind to the P site of the 50S subunit, in particular adjacent to the 23S rRNA, preventing fMet-tRNA from binding in a fashion that permits the formation of the first peptide bond that begins mRNA translation. Additional effects on fidelity (e.g., frame shifting) may also contribute to the mechanism of action.

4.3. Resistance to Oxazolidinones

Widespread resistance to linezolid in the clinic is thus far rare. In a survey of over 40 000 isolates of Gram-positive cocci between 1998 and 2000, only eight strains were resistant (MIC $\geq$ 8 μg/mL). These included species of the genera *Enterococcus*, *Staphylococcus*, and *Streptococcus* and two strains of vancomycin-resistant enterococci. Prolonged use in patients has selected for resistance in methicillin-resistant *Staphylococcus aureus*[84,85] and enterococci[86] and has been shown to emerge in enterococci[87] and others during in-vitro serial dilution or mutagenesis studies.[88,89] Importantly, resistant bacteria in patients who have not had prior treatment with linezolid has been reported as well.[90,91]

Resistance occurs by site mutations in the domain V region of the 23S rRNA, consistent with the mode of action (see above). Until recently, all clinically derived linezolid resistance was associated with a G $\rightarrow$ U mutation at position 2576 of the 23S rRNA. These strains retain resistance to linezolid even in the presence of the ribosomal methylation enzyme Erm(C).[92] However, Meka et al. recently reported a T2500A mutation in *S. aureus* in addition to loss of a copy of the 23 RRNA gene in some isolates.[93] Most bacterial species carry multiple copies of the rDNA genes, and in *S. aureus* there are 5−6 23S rDNA genes. Heterozygous mutations in these genes result in a gradient of linezolid susceptibility. For example, strains with two of six possible G2576T mutations gave linezolid MIC of 8 μg/mL, while mutation of five of six gave MIC of 32 μg/mL compared to the wild-type, linezolid-susceptible, bacterium with MIC of 2 μg/mL.[85] Reversion of this mechanism has been reported in a strain of *S. aureus* with four of five copies bearing the G2576T mutation following repeated serial passage in antibiotic-free medium.[94]

Other ribosomal mutations have been reported during in vitro selection experiments,[82,87−89] and the addition of mutations in DNA repair mechanisms increases the frequency of linezolid-resistant mutations[95] Sander et al. used *Mycobacterium smegmatis*, which only carries one copy of the 23S rDNA, to explore the mechanisms of linezolid resistance identifiable after serial passage experiments. As expected, 23S rRNA mutations were identified but also some nonribosomal mutants emerged in the screen, possibly the result of altered uptake or efflux.[96] Resistance to all antimicrobial agents is predictable; however, thus far widespread resistance remains rare for the oxazolidinones likely for two reasons. First, the fact that these agents are not natural products, and therefore, the microbial community has not 'seen' this chemical scaffold in the past. Second, ribosomal mutations must be hardwired into the chromosome and the presence of multiple 23S rDNA genes makes homozygocity, which is associated with high-level resistance, rare. This argues well for the longevity of this class of agent and presents an opportunity to initiate program development in new members of the class to be ready if (or rather when) resistance becomes more of an issue.

4.4. Second-Generation Agents

Since the launch of linezolid there has been great interest in additional SAR studies directed at the oxazolidinone class of antibiotics to identify new agents with improved properties such as bacterial spectrum and to counter emerging resistance. For example, combinatorial synthesis of *S*-oxide, fluoroacetamido analogues of linezolid, such as compounds **1** and **2** in Figure 14, provide increased activity against *H. influenzae* and *M. catarrhalis*.[97] Similarly, indolinyl derivatives such as compounds of general structure **3** also showed improved activity against these Gram-negative pathogens. A series of pyrrol derivatives (**4**) was shown to have activity against various mycobacterial species including multidrug-resistant strains.[98]

In a creative approach two groups recently reported a series of compounds linking oxazolidinone and fluoroquinolone (ciprofloxacin) moieties, e.g., compound **7** (Figure 14).[99−101] The best of these analogues exhibited a broad spectrum of antibacterial activity, e.g., including bacterial targets not covered by linezolid such as *E. coli*, and activity against linezolid- and fluoroquinolone-resistant bacteria. This clever combination approach has the potential to be further optimized to achieve improved drug-like properties.

5. Other Antibacterial Inhibitors of Bacterial Translation

The structural diversity of compounds shown in Figure 1 and Table 1 graphically demonstrate that inhibitors of bacterial translation encompass a very broad chemical space that has great potential to be exploited in the development of new antimicrobial agents. Furthermore, as the lesson of the clinical development of the streptogramins demonstrates, reexamination of 'old' agents also has great potential

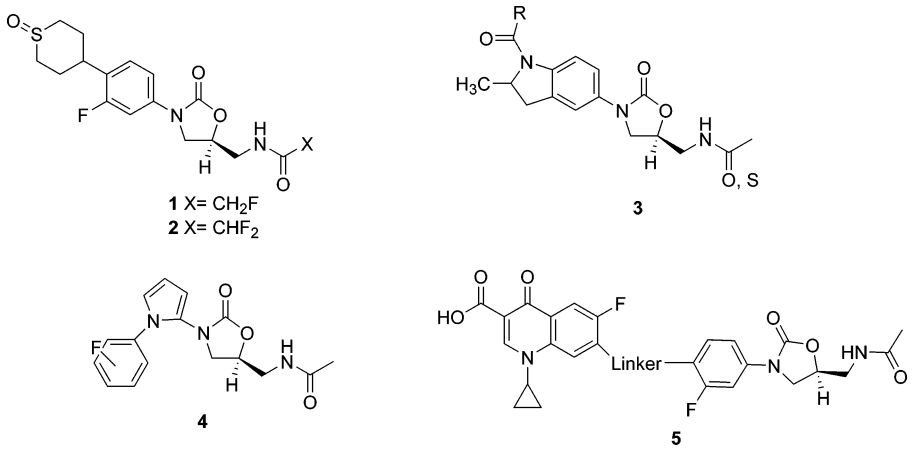


Figure 14. Sample of some newer oxazolidinone antibiot504ics.

in the development of clinically important drugs. In this section a brief discussion of some old and new inhibitors of translation will be presented.

5.1. Chloramphenicol

The antibiotic chloramphenicol (Figure 1) was discovered in 1947 from extracts of *Streptomyces venezuelae*.[102] It has excellent broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria and was widely used clinically following its discovery. This antibiotic however is no longer in significant use as a result of potential fatal, irreversible aplastic anemia which has been associated with it.[103] The basis for this rare side effect is unknown but is likely genetic. Therefore, a sensitive screen of genotype could reinvigorate the use of this agent in a safe fashion.

Chloramphenicol binds to the P site of the ribosome in an area of the PTC that largely overlaps with dalfopristin.[45,104] A second binding site at the entrance of the peptide exit tunnel has also been suggested.[44,105] The crystal structure of chloramphenicol bound to the ribosome reveals the importance of intermediary Mg^{2+} ions in antibiotic binding to the PTC,[104] a fact that has the potential to be exploited in the development of new analogues. One of the key interactions is between the primary alcohol at C3 and a Mg^{2+} ion. The most prevalent mechanism of resistance to chloramphenicol is via a series of acetyltransferases (many of which are structurally similar to the Vat proteins described above) that modify this important OH group.[106]

5.2. Lincosamides

The lincosamide antibiotics also bind the ribosome P site in a region that partially overlaps the chloramphenicol/dalfopristin and erythromycin binding sites.[104] The first member of the class, lincomycin, was discovered as a product of *Streptomyces lincolnensis* in 1962,[107] and the semisynthetic derivative, clindamycin (Figure 1), continues to find some clinical use, especially in the treatment of infections caused by anaerobic bacteria.[108] Resistance to clindamycin occurs primarily via the Erm-mediated methylation of the 23S rRNA as described above as part of the MLS_B phentotype (L = lincosamide).

5.3. Pleuromutilins

The pleuromutilins (Figure 1) are fungal natural products discovered over 50 years ago.[109] They have found use in agriculture and veterinary medicine but as a result of poor pharmacological properties have not been used to treat infections in humans (see references in ref 110). The pleuromutilins bind to the PTC as assessed by chemical footprinting studies.[111] While at present these antibiotics are unsuitable for clinical use, recent medicinal chemistry efforts to improve stability to human cytochrome P-450s, which results in rapid degradation of the compounds, has been reported.[110]

6. Conclusions

Bacterial translation is reemerging as a front-line target for the modern discovery of antibiotics as evidenced by the fact that of the very few new antibacterial agents to receive regulatory approval in the past 5 years, three of these are translation inhibitors: Synercid, linezolid, and the ketolide, telithromycin. Furthermore, the availability of high-resolution crystal structures of the bacterial ribosome and the ability to solve co-structures with inhibitors of translation now provides the opportunity to launch structure-based initiatives to develop new agents that block translation. At the same time, highly important biochemical and molecular biological tools such as a strain of *E. coli* with all of the rRNA genes inactivated[112] will greatly facilitate characterization of mode of action, dose dependency, and resistance. The potential of using the ribosome as a platform for new drug discovery is in fact being pursued by a number of drug discovery companies. Investigation of the biochemistry, structure, and clinical application of inhibitors of bacterial translation is therefore poised to enter a new 'golden era', mirroring the discovery phase during the 1950s. The challenge of resistance however will remain significant in these efforts. Overcoming existing and eventual resistance will require the ongoing and concerted efforts of medicinal chemists working together with microbiologists, pharmacologists, structural biologists, and biochemists to generate the next generation of translation inhibitors.

7. Acknowledgments

We thank Arif Mukhtar for assistance in preparing Figure 2. Research in our lab on inhibitors of bacterial translation and associated resistance has been supported by the Canadian Institutes for Health Research. T. Mukhtar is support by an Ontario Graduate Student Scholarship, and G. Wright is supported by a Canada Research Chair in Antibiotic Biochemistry.

8. References

(1) Knowles, D. J.; Foloppe, N.; Matassova, N. B.; Murchie, A. I. *Curr. Opin. Pharmacol.* **2002**, *2*, 501.
(2) Bower, J.; Drysdale, M.; Hebdon, R.; Jordan, A.; Lentzen, G.; Matassova, N.; Murchie, A.; Powles, J.; Roughley, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2455.
(3) Barluenga, S.; Simonsen, K. B.; Littlefield, E. S.; Ayida, B. K.; Vourloumis, D.; Winters, G. C.; Takahashi, M.; Shandrick, S.; Zhao, Q.; Han, Q.; Hermann, T. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 713.
(4) Foloppe, N.; Chen, I. J.; Davis, B.; Hold, A.; Morley, D.; Howes, R. *Bioorg. Med. Chem.* **2004**, *12*, 935.
(5) Wilson, D. N.; Nierhaus, K. H. *Angew. Chem., Int Ed. Engl.* **2003**, *42*, 3464.
(6) Nissen, P.; Hansen, J.; Ban, N.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 920.
(7) Gualerzi, C. O.; Brnadi, L.; Caserta, E.; La Teana, A.; Spurio, R.; Tomsic, J.; Pon, C. L. In *The Ribosome*; Garrett, R. A., Douthwaite, S. R., Lijas, A., Metheson, A. T., Moore, P. B., Noller, H. F., Eds.; ASM Press: Washington, DC, 2000.
(8) Carter, A. P.; Clemons, W. M., Jr.; Brodersen, D. E.; Morgan-Warren, R. J.; Hartsch, T.; Wimberly, B. T.; Ramakrishnan, V. *Science* **2001**, *291*, 498.
(9) Pioletti, M.; Schlunzen, F.; Harms, J.; Zarivach, R.; Gluhmann, M.; Avila, H.; Bashan, A.; Bartels, H.; Auerbach, T.; Jacobi, C.; Hartsch, T.; Yonath, A.; Franceschi, F. *EMBO J.* **2001**, *20*, 1829.
(10) Ogle, J. M.; Carter, A. P.; Ramakrishnan, V. *Trends Biochem. Sci.* **2003**, *28*, 259.
(11) Rodnina, M. V.; Pape, T.; Fricke, R.; Kuhn, L.; Wintermeyer, W. *J. Biol. Chem.* **1996**, *271*, 646.
(12) Pape, T.; Wintermeyer, W.; Rodnina, M. V. *EMBO J.* **1998**, *17*, 7490.
(13) O'Connor, M.; Dahlberg, A. E. *J. Mol. Biol.* **1995**, *254*, 838.
(14) Stark, H.; Rodnina, M. V.; Rinke-Appel, J.; Brimacombe, R.; Wintermeyer, W.; van Heel, M. *Nature* **1997**, *389*, 403.
(15) Pape, T.; Wintermeyer, W.; Rodnina, M. *EMBO J.* **1999**, *18*, 3800.
(16) Rodnina, M. V.; Daviter, T.; Gromadski, K.; Wintermeyer, W. *Biochimie* **2002**, *84*, 745.
(17) Valle, M.; Sengupta, J.; Swami, N. K.; Grassucci, R. A.; Burkhardt, N.; Nierhaus, K. H.; Agrawal, R. K.; Frank, J. *EMBO J.* **2002**, *21*, 3557.
(18) Agmon, I.; Auerbach, T.; Baram, D.; Bartels, H.; Bashan, A.; Berisio, R.; Fucini, P.; Hansen, H. A.; Harms, J.; Kessler, M.; Peretz, M.; Schluenzen, F.; Yonath, A.; Zarivach, R. *Eur. J. Biochem.* **2003**, *270*, 2543.
(19) Green, R.; Lorsch, J. R. *Cell* **2002**, *110*, 665.
(20) Bateman, K. P.; Yang, K.; Thibault, P.; White, R. L.; Vining, L. C. *J. Am. Chem. Soc.* **1996**, *118*, 5335.
(21) Pechere, J. C. *Drugs* **1996**, *51*, 13.
(22) Johnston, N. J.; Mukhtar, T. A.; Wright, G. D. *Curr. Drug Targets* **2002**, *3*, 335.
(23) Kingston, D. G. I.; Kolpak, M. X.; LeFevre, J. W.; Borup-Grochtmann, I. *J. Am. Chem. Soc.* **1983**, *105*, 5106.
(24) Thibaut, D.; Ratet, N.; Bisch, D.; Faucher, D.; Debussche, L.; Blanche, F. *J. Bacteriol.* **1995**, *177*, 5199.
(25) Blanc, V.; Lagneaux, D.; Didier, P.; Gil, P.; Lacroix, P.; Crouzet, J. *J. Bacteriol.* **1995**, *177*, 5206.
(26) Purvis, M. B.; Kingston, D. G. I.; Fujii, N.; Floss, H. G. *J. Chem. Soc., Chem. Commun.* **1987**, 302.
(27) Roy, R. S.; Gehring, A. M.; Milne, J. C.; Belshaw, P. J.; Walsh, C. T. *Nat. Prod. Rep.* **1999**, *16*, 249.
(28) Cocito, C. *Microbiol. Rev.* **1979**, *43*, 145.
(29) Reed, J. W.; Kingston, D. G. *J. Nat. Prod.* **1986**, *49*, 626.
(30) Molinero, A. A.; Kingston, D. G.; Reed, G. H. *J. Nat. Prod.* **1989**, *52*, 99.
(31) Reed, J. W.; Purvis, M. B.; Kingston, D. G.; Biot, A.; Grosselé, F. *J. Org. Chem.* **1989**, *54*, 1161.
(32) Blanc, V.; Gil, P.; Bamas-Jacques, N.; Lorenzon, S.; Zagorec, M.; Schleuniger, J.; Bisch, D.; Blanche, F.; Debussche, L.; Crouzet, J.; Thibaut, D. *Mol. Microbiol.* **1997**, *23*, 191.
(33) Namwat, W.; Kinoshita, H.; Nihira, T. *J. Bacteriol.* **2002**, *184*, 4811.
(34) Namwat, W.; Kamioka, Y.; Kinoshita, H.; Yamada, Y.; Nihira, T. *Gene* **2002**, *286*, 283.
(35) Thibaut, D.; Bisch, D.; Ratet, N.; Maton, L.; Couder, M.; Debussche, L.; Blanche, F. *J. Bacteriol.* **1997**, *179*, 697.
(36) de Crécy-Lagard, V.; Blanc, V.; Gil, P.; Naudin, L.; Lorenzon, S.; Famechon, A.; Bamas-Jacques, N.; Crouzet, J.; Thibaut, D. *J. Bacteriol.* **1997**, *179*, 705.
(37) de Crécy-Lagard, V.; Saurin, W.; Thibaut, D.; Gil, P.; Naudin, L.; Crouzet, J.; Blanc, V. *Antimicrob. Agents Chemother.* **1997**, *41*, 1904.
(38) Yamada, Y.; Sugamura, K.; Kondo, K.; Yanagimoto, M.; Okada, H. *J. Antibiot. (Tokyo)* **1987**, *40*, 496.
(39) Kawachi, R.; Akashi, T.; Kamitani, Y.; Sy, A.; Wangchai-soonthorn, U.; Nihira, T.; Yamada, Y. *Mol. Microbiol.* **2000**, *36*, 302.
(40) Miller, M. B.; Bassler, B. L. *Annu. Rev. Microbiol.* **2001**, *55*, 165.
(41) Horinouchi, S. *Front. Biosci.* **2002**, *7*, d2045.
(42) Choi, S. U.; Lee, C. K.; Hwang, Y. I.; Kinosita, H.; Nihira, T. *Arch. Microbiol.* **2003**, *180*, 303.
(43) Vannuffel, P.; Cocito, C. *Drugs* **1996**, *51*, 20.
(44) Hansen, J. L.; Moore, P. B.; Steitz, T. A. *J. Mol. Biol.* **2003**, *330*, 1061.
(45) Harms, J. M.; Schluenzen, F.; Fucini, P.; Bartels, H.; Yonath, A. E. *BMC Biol.* **2004**, *2*, 4.
(46) Vannuffel, P.; Di Giambattista, M.; Cocito, C. *Nucleic Acids Res* **1994**, *22*, 4449.
(47) Porse, B. T.; Kirillov, S. V.; Awayez, M. J.; Garrett, R. A. *RNA* **1999**, *5*, 585.
(48) Chinali, G.; Di Giambattista, M.; Cocito, C. *Biochemistry* **1987**, *26*, 1592.
(49) Cocito, C.; Di Giambattista, M.; Nyssen, E.; Vannuffel, P. *J. Antimicrob. Chemother.* **1997**, *39 Suppl A*, 7.
(50) Bouanchaud, D. H. *J. Antimicrob. Chemother.* **1997**, *39* (Suppl. A), 15.
(51) Singh, K. V.; Weinstock, G. M.; Murray, B. E. *Antimicrob. Agents Chemother.* **2002**, *46*, 1845.
(52) Ross, J. I.; Eady, E. A.; Cove, J. H.; Cunliffe, W. J.; Baumberg, S.; Wootton, J. C. *Mol. Microbiol.* **1990**, *4*, 1207.
(53) Alignet, J.; Loncle, V.; el Sohl, N. *Gene* **1992**, *117*, 45.
(54) Alignet, J.; El Solh, N. *Gene* **1997**, *202*, 133.
(55) Weisblum, B. *Antimicrob. Agents Chemother.* **1995**, *39*, 577.
(56) Leclercq, R.; Courvalin, P. *Antimicrob. Agents Chemother.* **1991**, *35*, 1267.
(57) Tait-Kamradt, A.; Davies, T.; Appelbaum, P. C.; Depardieu, F.; Courvalin, P.; Petitpas, J.; Wondrack, L.; Walker, A.; Jacobs, M. R.; Sutcliffe, J. *Antimicrob. Agents Chemother.* **2000**, *44*, 3395.
(58) Sugantino, M.; Roderick, S. L. *Biochemistry* **2002**, *41*, 2209.
(59) Kehoe, L. E.; Snidwongse, J.; Courvalin, P.; Rafferty, J. B.; Murray, I. A. *J. Biol. Chem.* **2003**, *278*, 29963.
(60) Hou, C. T.; Perlman, D.; Schallock, M. R. *J. Antibiot.* **1970**, *23*, 35.
(61) Kim, C. H.; Otake, N.; Yonehara, H. *J. Antibiot.* **1974**, *27*, 903.
(62) Le Goffic, F.; Capmau, M. L.; Abbe, J.; Cerceau, C.; Dublanchet, A.; Duval, J. *Ann. Microbiol. (Paris)* **1977**, *128B*, 471.
(63) Allignet, J.; Loncle, V.; Mazodier, P.; el Solh, N. *Plasmid* **1988**, *20*, 271.
(64) Suzuki, N.; Lee, C. K.; Nihira, T.; Yamada, Y. *Antimicrob. Agents Chemother.* **1998**, *42*, 2985.
(65) Spangler, S. K.; Jacobs, M. R.; Appelbaum, P. C. *Antimicrob. Agents Chemother.* **1996**, *40*, 481.
(66) Pankuch, G. A.; Kelly, L. M.; Lin, G.; Bryskier, A.; Couturier, C.; Jacobs, M. R.; Appelbaum, P. C. *Antimicrob. Agents Chemother.* **2003**, *47*, 3270.
(67) Bonfiglio, G.; Furneri, P. M. *Expert Opin. Investig. Drugs* **2001**, *10*, 185.
(68) Slee, A. M.; Wuonola, M. A.; McRipley, R. J.; Zajac, I.; Zawada, M. J.; Bartholomew, P. T.; Gregory, W. A.; Forbes, M. *Antimicrob. Agents Chemother.* **1987**, *31*, 1791.
(69) Gregory, W. A.; Brittelli, D. R.; Wang, C. L.; Wuonola, M. A.; McRipley, R. J.; Eustice, D. C.; Eberly, V. S.; Bartholomew, P. T.; Slee, A. M.; Forbes, M. *J. Med. Chem.* **1989**, *32*, 1673.
(70) Barbachyn, M. R.; Ford, C. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 2010.
(71) Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953.
(72) Barbachyn, M. R.; Hutchinson, D. K.; Brickner, S. J.; Cynamon, M. H.; Kilburn, J. O.; Klemens, S. P.; Glickman, S. E.; Grega, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 680.
(73) Tucker, J. A.; Allwine, D. A.; Grega, K. C.; Barbachyn, M. R.; Klock, J. L.; Adamski, J. L.; Brickner, S. J.; Hutchinson, D. K.; Ford, C. W.; Zurenko, G. E.; Conradi, R. A.; Burton, P. S.; Jensen, R. M. *J. Med. Chem.* **1998**, *41*, 3727.

(74) Brickner, S. J.; Hutchinson, D. K.; Barbachyn, M. R.; Manninen, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Grega, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 673.
(75) Barbachyn, M. R.; Toops, D. S.; Grega, K. C.; Hendges, S. K.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, J. D.; Stapert, D.; Yagi, B. H.; Buysse, J. M.; Demyan, W. F.; Kilburn, J. O.; Glickman, S. E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1009.
(76) Barrett, J. F. *Curr. Opin. Investig. Drugs* **2000**, *1*, 181.
(77) Lin, A. H.; Murray, R. W.; Vidmar, T. J.; Marotti, K. R. *Antimicrob. Agents Chemother.* **1997**, *41*, 2127.
(78) Zhou, C. C.; Swaney, S. M.; Shinabarger, D. L.; Stockman, B. J. *Antimicrob. Agents Chemother.* **2002**, *46*, 625.
(79) Shinabarger, D. L.; Marotti, K. R.; Murray, R. W.; Lin, A. H.; Melchior, E. P.; Swaney, S. M.; Dunyak, D. S.; Demyan, W. F.; Buysse, J. M. *Antimicrob. Agents Chemother.* **1997**, *41*, 2132.
(80) Swaney, S. M.; Aoki, H.; Ganoza, M. C.; Shinabarger, D. L. *Antimicrob. Agents Chemother.* **1998**, *42*, 3251.
(81) Patel, U.; Yan, Y. P.; Hobbs, F. W., Jr.; Kaczmarczyk, J.; Slee, A. M.; Pompliano, D. L.; Kurilla, M. G.; Bobkova, E. V. *J. Biol. Chem.* **2001**, *276*, 37199.
(82) Thompson, J.; O'Connor, M.; Mills, J. A.; Dahlberg, A. E. *J. Mol. Biol.* **2002**, *322*, 273.
(83) Bobkova, E. V.; Yan, Y. P.; Jordan, D. B.; Kurilla, M. G.; Pompliano, D. L. *J. Biol. Chem*. **2003**, *278*, 9802.
(84) Tsiodras, S.; Gold, H. S.; Sakoulas, G.; Eliopoulos, G. M.; Wennersten, C.; Venkataraman, L.; Moellering, R. C.; Ferraro, M. J. *Lancet* **2001**, *358*, 207.
(85) Wilson, P.; Andrews, J. A.; Charlesworth, R.; Walesby, R.; Singer, M.; Farrell, D. J.; Robbins, M. *J. Antimicrob. Chemother.* **2003**, *51*, 186.
(86) Johnson, A. P.; Tysall, L.; Stockdale, M. V.; Woodford, N.; Kaufmann, M. E.; Warner, M.; Livermore, D. M.; Asboth, F.; Allerberger, F. J. *Eur. J. Clin. Microbiol. Infect. Dis.* **2002**, *21*, 751.
(87) Prystowsky, J.; Siddiqui, F.; Chosay, J.; Shinabarger, D. L.; Millichap, J.; Peterson, L. R.; Noskin, G. A. *Antimicrob. Agents Chemother.* **2001**, *45*, 2154.
(88) Kloss, P.; Xiong, L.; Shinabarger, D. L.; Mankin, A. S. *J. Mol. Biol.* **1999**, *294*, 93.
(89) Xiong, L.; Kloss, P.; Douthwaite, S.; Andersen, N. M.; Swaney, S.; Shinabarger, D. L.; Mankin, A. S. *J. Bacteriol*. **2000**, *182*, 5325.
(90) Jones, R. N.; Della-Latta, P.; Lee, L. V.; Biedenbach, D. J. *Diagn. Microbiol. Infect. Dis.* **2002**, *42*, 137.
(91) Rahim, S.; Pillai, S. K.; Gold, H. S.; Venkataraman, L.; Inglima, K.; Press, R. A. *Clin. Infect. Dis.* **2003**, *36*, E146.
(92) Sakoulas, G.; Gold, H. S.; Venkataraman, L.; Moellering, R. C., Jr.; Ferraro, M. J.; Eliopoulos, G. M. *J. Antimicrob. Chemother.* **2003**, *51*, 1039.
(93) Meka, V. G.; Pillai, S. K.; Sakoulas, G.; Wennersten, C.; Venkataraman, L.; DeGirolami, P. C.; Eliopoulos, G. M.; Moellering, R. C., Jr.; Gold, H. S. *J. Infect. Dis.* **2004**, *190*, 311.
(94) Meka, V. G.; Gold, H. S.; Cooke, A.; Venkataraman, L.; Eliopoulos, G. M.; Moellering, R. C., Jr.; Jenkins, S. G. *J. Antimicrob. Chemother.* **2004**.
(95) Willems, R. J.; Top, J.; Smith, D. J.; Roper, D. I.; North, S. E.; Woodford, N. *Antimicrob. Agents Chemother.* **2003**, *47*, 3061.
(96) Sander, P.; Belova, L.; Kidan, Y. G.; Pfister, P.; Mankin, A. S.; Bottger, E. C. *Mol. Microbiol.* **2002**, *46*, 1295.
(97) Singh, U.; Raju, B.; Lam, S.; Zhou, J.; Gadwood, R. C.; Ford, C. W.; Zurenko, G. E.; Schaadt, R. D.; Morin, S. E.; Adams, W. J.; Friis, J. M.; Courtney, M.; Palandra, J.; Hackbarth, C. J.; Lopez, S.; Wu, C.; Mortell, K. H.; Trias, J.; Yuan, Z.; Patel, D. V.; Gordeev, M. F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4209.
(98) Sbardella, G.; Mai, A.; Artico, M.; Loddo, R.; Setzu, M. G.; La Colla, P. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 1537.
(99) Gordeev, M. F.; Hackbarth, C.; Barbachyn, M. R.; Banitt, L. S.; Gage, J. R.; Luehr, G. W.; Gomez, M.; Trias, J.; Morin, S. E.; Zurenko, G. E.; Parker, C. N.; Evans, J. M.; White, R. J.; Patel, D. V. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4213.
(100) Hubschwerlen, C.; Specklin, J. L.; Sigwalt, C.; Schroeder, S.; Locher, H. H. *Bioorg. Med. Chem.* **2003**, *11*, 2313.
(101) Hubschwerlen, C.; Specklin, J. L.; Baeschlin, D. K.; Borer, Y.; Haefeli, S.; Sigwalt, C.; Schroeder, S.; Locher, H. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4229.
(102) Ehrlich, J.; Bartz, Q. R.; Smith, R. M.; Joslyn, D. A.; Burkholder, P. R. *Science* **1947**, *106*, 417.
(103) Yunis, A. A. *Am. J. Med.* **1989**, *87*, 44N.
(104) Schlunzen, F.; Zarivach, R.; Harms, J.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. *Nature* **2001**, *413*, 814.
(105) Long, K. S.; Porse, B. T. *Nucleic Acids Res.* **2003**, *31*, 7208.
(106) Murray, I. A.; Shaw, W. V. *Antimicrob. Agents Chemother.* **1997**, *41*, 1.
(107) Mason, D. J.; Deietz, A.; DeBoer, C. In *Antimicrobial Agents and Chemotherapy-1962*; Sylvester, J. C., Ed.; American Society for Microbiology: Ann Arbor, MI, 1963.
(108) Kasten, M. J. *Mayo Clin. Proc.* **1999**, *74*, 825.
(109) Kavanigh, F.; Hervey, A.; Robbins, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*.
(110) Springer, D. M.; Sorenson, M. E.; Huang, S.; Connolly, T. P.; Bronson, J. J.; Matson, J. A.; Hanson, R. L.; Brzozowski, D. B.; LaPorte, T. L.; Patel, R. N. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1751.
(111) Poulsen, S. M.; Karlsson, M.; Johansson, L. B.; Vester, B. *Mol. Microbiol.* **2001**, *41*, 1091.
(112) Asai, T.; Zaporojets, D.; Squires, C.; Squires, C. L. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1971.

CR030110Z