Chapter 14
Protein Synthesis Inhibitors from Smaller Antibiotic Classes

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Abstract Inhibition of microbial protein synthesis is one of the most commonly used and important mechanisms of action for antibiotics. In addition to the four larger antibiotic classes (aminoglycosides, tetracyclines, oxazolidinones and macrolides) that operate by this mechanism, several smaller antibiotic classes also employ it. This review covers key members and important developments in the lincosaminide, streptogramin, phenicol, pleuromutilin, fusidane, pseudomonic acid, and thiopeptide classes of fermentation-derived antibiotics. Retapamulin is the most recently approved antibiotic from this group while some other members of these seven classes are currently in the antibiotic development pipeline. In addition, many other smaller classes of previously discovered antibiotics remain under-explored and under-utilized, which, if further examined, could represent additional opportunities and starting materials for creating new scaffolds for novel antibiotics.

14.1 Introduction

The steadily rising levels of microbial resistance to antibiotics have been thoroughly publicized for over two decades and accompanied by many calls for action (Boucher et al. 2009; Bush et al. 2011; Fong and Drlica 2008; Freire-Moran et al. 2011; IDSA 2010; Pulcini et al. 2012). In one response to such calls, the current antibiotic pipeline of new agents at some stage of clinical development is slowly being filled with representatives from many different antibiotic classes (Bush and Pucci 2011; Butler and Cooper 2011; Coates et al. 2011; Donadio et al. 2010; Kirst 2010a, 2012). Inhibition of protein biosynthesis in bacteria is one of the most

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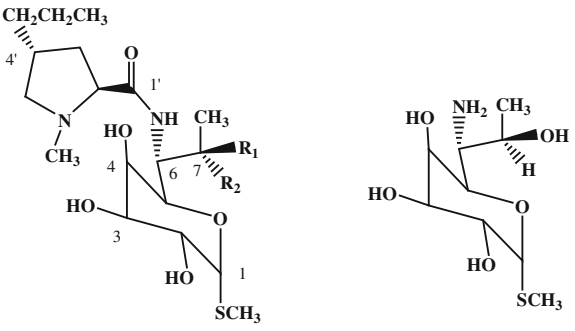
F. Marinelli and O. Genilloud (eds.), *Antimicrobials*,
DOI: 10.1007/978-3-642-39968-8_14,

utilized mechanisms of action (MOA) for eliciting antibacterial activity (Lange et al. 2007). The ribosome is the target site of action for microbial protein biosynthesis which different antibiotics disrupt in many diverse ways (Blanchard et al. 2010; Hermann 2005; McCoy et al. 2011; Yonath 2005). The importance of this MOA is confirmed by four other chapters of this book (see Kirst and Marinelli, Kirst, Genilloud and Vincente, Zappia et al. this volume) that are each devoted to one of the large antibiotic classes of protein synthesis inhibitors, namely, aminoglycosides, tetracyclines, macrolides, and oxazolidinones. Complementing those four reviews, this chapter summarizes several smaller classes of other antibiotics that also exert their antimicrobial activity by inhibition of protein synthesis.

14.2 Lincosaminides

Lincomycin is an older antibiotic produced from fermentation of *Streptomyces lincolnensis* (Mason et al. 1963). It is a relatively small molecule whose structure is composed of an uncommon amino acid attached via an amide bond to a unique amino-octose named lincosamine (Fig. 14.1) (Mitscher 2010). Based on the latter name, this antibiotic class is known as lincosaminides (or lincosamides). Clindamycin is a more potent semi-synthetic derivative of lincomycin in which chlorine has replaced the 7-hydroxyl group (Fig. 14.1) (Lewis 1974). Clindamycin has generally superseded lincomycin in clinical use due to features such as higher potency and better oral absorption. Clindamycin is used to treat infections caused by Gram-positive bacteria, many anaerobes, and certain other pathogens (Guay 2007; Sivapalasingam and Steigbigel 2010). It became particularly useful upon clinical introduction due to its greater activity against the Gram-negative anaerobe, *Bacteroides fragilis*. However, it was further noted that the opportunistic anaerobe *Clostridium difficile* could proliferate and cause a dangerous condition known as pseudomembranous colitis if the beneficial gastrointestinal bacteria became too



Lincomycin: R_1 = OH, R_2 = H
Clindamycin: R_1 = H, R_2 = Cl

Lincosamine

Fig. 14.1 Structures of lincomycin, clindamycin, and lincosamine

severely depleted or overrun due to antibiotic activity against them in the gastrointestinal tract (Bartlett 2008). Another important clinical use of clindamycin is to treat infections caused by Gram-positive bacteria including community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) (Dryden 2010; Guay 2007). Current levels of clindamycin resistance appear to be relatively low, but as for all antibiotics, resistance is becoming more prevalent (Frei et al. 2010; Kluka 2011; Marcinak and Frank 2006). Hospital-acquired MRSA strains are more resistant (Dryden 2010).

Clindamycin binding occurs at the peptide exit tunnel and extends to the peptidyl transferase center (PTC) of the ribosome (McCoy et al. 2011; Tenson et al. 2003). As discussed in the preceding chapter for macrolides (see Kirst, this volume), the binding site for lincosaminides in the exit tunnel region overlaps the binding sites for macrolide and streptogramin B antibiotics. As a consequence, either *erm* gene-governed N-methylation of an adenine residue or nucleotide point mutations at this site can reduce antibiotic binding for members of all three of these structurally-unrelated classes. This mutual reduction in binding results in cross-resistance between the macrolide, lincosaminide, and streptogramin B (MLS_B resistance) antibiotics (Leclercq and Courvalin 1991; Roberts 2011; Weisblum 1995). At the PTC, clindamycin inhibits transpeptidation where its binding site overlaps and competes with chloramphenicol and tiamulin. X-ray studies have been published illustrating structures for co-crystals of clindamycin bound in the large ribosomal subunits from *Deinococcus radiodurans*, *Haloarcula marismortu*, and *Escherichia coli* (Dunkle et al. 2010; Schlunzen et al. 2001; Tu et al. 2005). Analysis of these complexes identified differences in binding between the three of them, although the latter two were more consistent with each other at several positions. These differences emphasized the need for caution in assuming that ribosomes from different species are so conserved that they always bind antibiotics in the same arrangements (Kannan and Mankin 2011; Wilson 2011).

Other mechanisms of resistance to lincosaminides are efflux systems and enzymatic inactivation, but they are less common than target site modifications. Efflux systems transport unaltered antibiotic out of the cell to counteract its uptake, maintain low intracellular concentrations, and keep it from the ribosome. Lincosaminide efflux is controlled by *lmr*, *car*, and *lsa* genes (Roberts 1999, 2008, 2011). Enzymatic inactivation occurs by O-adenylylation that is coded by *lnu* (formerly *lin*) genes (Morar et al. 2009). Adenylylation occurs at the 4-hydroxyl group in *S. aureus* and the 3-hydroxyl group in *Enterococcus faecium* for clindamycin, but at the 3-hydroxyl group of lincomycin in both bacteria (Mitscher 2010).

Pirlimycin is an old member of this class that is used for veterinary applications such as treatment of bovine mastitis (Saini et al. 2011). Mirincamycin is an old pair of *cis/trans* isomers that has more recently been further tested for anti-malarial activity (Khemawoot et al. 2011). However, only a few efforts to synthesize new derivatives in this class have been recently reported (Kirst 2010b).

14.3 Streptogramins

The streptogramins are an old antibiotic class originally obtained from fermentation of *Streptomyces graminofaciens* (Charney et al. 1953). They have been re-isolated on several later occasions and given different names at those times, such as pristinamycins from *Streptomyces pristinaespiralis*, virginiamycins from *Streptomyces virginiae*, and others (Johnston et al. 2002). The class is composed of two separate and structurally unrelated series (Fig. 14.2). Compounds in the streptogramin A series have a 23-membered mixed polyketide and amino acid macrocyclic core whereas compounds in the streptogramin B series are macrocyclic depsipeptides (Kirst 2010b; Mitscher 2010). Each series by itself possesses insufficient potency and is only bacteriostatic, but a combination of the A and B series has a dual target and exhibits synergistic bactericidal activity (Canu and Leclercq 2001).

The first product, Pyostacine®, was a fixed combination containing a 7:3 ratio of streptogramin A:B (Bacque et al. 2005). Extensive SAR studies were conducted over many years especially to improve water solubility and efficacy upon injection. These efforts resulted in the creation and development of a 7:3 combination by weight of dalfopristin:quinopristin named Synercid® (Fig. 14.2). It is used for

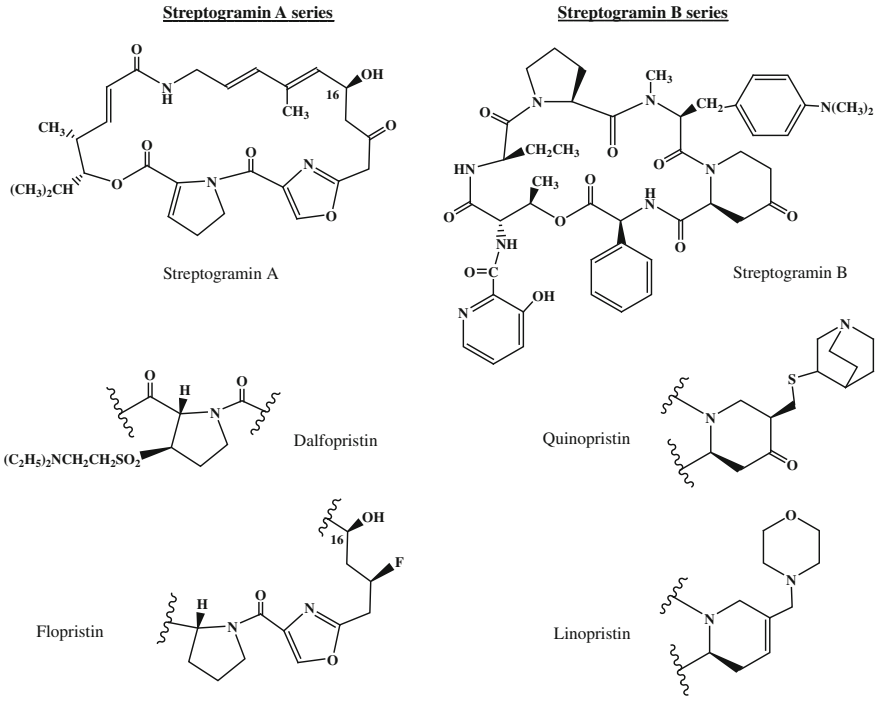


Fig. 14.2 Structures of streptogramins and derivatives

parenteral treatment of multidrug-resistant Gram-positive bacterial infections, including vancomycin-resistant *E. faecium* (Manfredi 2005; Nailor and Sobel 2009). Upon approval in the U.S. in 1999, it became the first approved new antibiotic to have activity against vancomycin-resistant Gram-positive bacteria, but newer antibiotics have now generally replaced it (Anstead and Owens 2004; Dang et al. 2007). Further SAR studies that were focused on oral administration have subsequently yielded another combination containing 70 % flopristin and 30 % linopristin (Fig. 14.2) (Bacque et al. 2005). This latter orally bioavailable combination coded NXL-103 (formerly XRP2868) has been undergoing Phase II clinical trials (Devasahayam et al. 2010; Politano and Sawyer 2010).

As previously mentioned, streptogramin B is the third component in the MLS_B resistance phenotype that occurs due to overlapping binding sites of MLS_B antibiotics around the peptide exit tunnel of the ribosome (Canu and Leclercq 2001; Leclercq and Courvalin 1991; Tenson et al. 2003; Weisblum 1995). The A series binds to a site at the PTC that changes ribosome conformation in a manner which strengthens binding of the B series. As a result, binding of both streptogramin components produces a synergistic irreversible bactericidal effect due to greater stabilization of binding relative to each component alone (Hermann 2005; Yonath 2005). X-ray crystallographic analyses have been reported for complexes between ribosomes from *H. marismortui* and either streptogramin B (Tu et al. 2005) or streptogramin A (Hansen et al. 2003) and for ribosomes from *D. radiodurans* complexed with both Synercid components (Harms et al. 2004).

In addition to ribosomal modifications that reduce antibiotic binding, clinically important resistance to streptogramins arises from enzymatic inactivation of the antibiotics (Mlynarczyk et al. 2010). Inactivation of the B series by *vgb* gene-coded lyases produces a linear peptide in which the macrocycle is cleaved at the ester linkage via a beta-elimination reaction involving dehydration of the threonine subunit to a 2-amino-2-butenoic acid residue (Korczynska et al. 2007). The A series is inactivated by *vat* genes coding for acetylation of the 16-hydroxyl group. Efflux utilizes ATP-binding transporters coded by *msr* genes for the B series and *vga* genes for the A series (Roberts 1999).

14.4 Phenicols

Chloramphenicol is another old antibiotic that was initially obtained as a fermentation product from *Streptomyces venezuelae* (Ehrlich et al. 1947). However, its relatively small and uncomplicated structure lends it to efficient manufacturing by total synthesis, which long ago superseded fermentation as the product source (Yang et al. 2009). The only active antimicrobial isomer of the four possibilities is the one that possesses *2R, 3R* stereochemistry (Fig. 14.3) (Loncaric and Wulff 2001). Clinical use is limited by toxicity, especially a relatively low incidence of aplastic anemia but which is unpredictable, untreatable, and generally fatal (Maviglia et al. 2009). However, chloramphenicol is valuable for treating typhoid

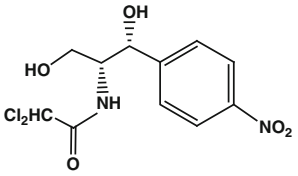


Fig. 14.3 Structure of chloramphenicol

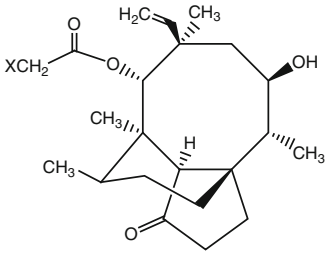
fever or other serious infections for which less toxic alternatives are not available. Due to the absence of many new Gram-negative antibiotics in development, chloramphenicol is one of several older antibiotics being reconsidered for possible uses (Falagas et al. 2008; Maviglia et al. 2009). Florphenicol and thiamphenicol are analogs that are used to treat respiratory infections in veterinary medicine (Schwarz and Kehrenberg 2006).

The MOA for chloramphenicol is inhibition of ribosomal protein synthesis at the PTC by interfering with binding of tRNA at the A site (McCoy et al. 2011). X-ray studies of chloramphenicol bound to large ribosomal subunits from three different species identified a different binding site in *H. marismortui* compared to the binding site in *D. radiodurans* (Dunkle et al. 2010; Hansen et al. 2003; Schlunzen et al. 2001). The results observed from *E. coli* were more consistent with those from *H. marismortui*. However, both binding sites may be relevant targets for chloramphenicol since each site overlaps sites used by other antibiotics to which cross-resistance has been observed (McCoy et al. 2011; Yonath 2005).

The most significant mechanism of clinical resistance is inactivation by chloramphenicol acetyltransferase (CAT) that acetylates the primary hydroxyl group (Schwarz et al. 2004). CAT does not confer resistance to thiamphenicol or florphenicol. Efflux of chloramphenicol only is coded by the *cml* gene, while efflux via the *flo, cfr,* and *fex* genes produce resistance to all members of this class (Doublet et al. 2005; White et al. 2000).

14.5 Pleuromutilins

The parent compound of this class is pleuromutilin, a tricyclic diterpenoid antibiotic (Fig. 14.4) that was isolated from culture broths of *Pleurotis* species (now reclassified as the genus *Clitopilus*) (Hartley et al. 2009; Kavanagh et al. 1951). Although clinical applications of pleuromutilin and a semisynthetic derivative, azamulin, were explored, neither compound became a successful product. However, due to the unique structure and MOA, SAR studies of this class were continued. Those efforts produced two useful semi-synthetic derivatives, first with tiamulin and later with valnemulin (Fig. 14.4). Both derivatives became important commercial products in the veterinary field for treatment and control of infectious diseases in swine and poultry (Hu and Zou 2009; Novak and Shlaes 2010; Tang et al. 2012).



Pleuromutilin: X = OH
Tiamulin: X = $SCH_2CH_2N(CH_2CH_3)_2$
Valnemulin: X = $S\text{-}C(CH_3)_2\text{-}CH_2\text{-}NH\text{-}CO\text{-}CH(NH_2)\text{-}CH(CH_3)_2$

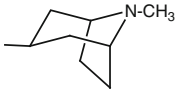
Retapamulin: X = S 

Fig. 14.4 Structures of pleuromutilin and derivatives

Early efforts to develop commercial products for human medicine in the pleuromutilin class were generally unsuccessful due to problems such as unsatisfactory pharmacokinetics, short half-life, and too rapid metabolism. However, a later semi-synthetic derivative, retapamulin (Fig. 14.4), has recently received U.S. regulatory approval for the topical treatment of certain human skin infections (Jacobs 2010; Weinberg and Tyring 2010). This approval is especially noteworthy for being the second natural product-originated antibiotic to be recently approved for human use (after daptomycin, see Baltz, this volume) and the first representative coming from a class that has been previously unrepresented in human medicine (pleuromutilins). Additional semi-synthetic derivatives are being evaluated in clinical trials, which may result in additional compounds from this class becoming useful antibiotics in human medicine (Butler and Cooper 2011; Donadio et al. 2010; Kirst 2012).

In spite of six decades between the discovery of pleuromutilin and the approval of retapamulin, interest in this class has remained high due to the unique structure and good spectrum of activity against Gram-positive bacteria. Very little cross-resistance has been reported thus far between the mutilins and other antibiotic classes, and in vitro studies have shown a low potential for development of resistance (Hu and Zou 2009; Tang et al. 2012). Its MOA has been well-established as an inhibitor of protein synthesis (Poulsen et al. 2001). Pleuromutilin binds at the PTC where it inhibits the formation of peptide bonds. Its binding site overlaps those of clindamycin, chloramphenicol, and the disaccharide substituent of carbomycin (McCoy et al. 2011). X-ray crystallographic studies of tiamulin complexed with the large ribosomal subunits from both *H. marismortui* and *D. radiodurans* have identified the antibiotic binding site (Gurel et al. 2009; Schlunzen et al. 2004). In one report, resistance to tiamulin in *E. coli* was caused by a point mutation in ribosomal protein L3 that hindered antibiotic binding (Bøsling et al. 2003).

14.6 Fusidanes

Fusidic acid is an old steroidal antibiotic that was obtained from fermentation of *Fusidium coccineum* (Godtfredsen et al. 1962). Its structure consists of a tetracyclic triterpenoid scaffold whose perimeter is arrayed with a variety of substituents, including a doubly unsaturated carboxylic acid moiety attached to C-17 in the D-ring (Fig. 14.5) (Godtfredsen et al. 1965; Cooper 1966). Fusidic acid is the only developed member of this small group of antibiotics known as fusidanes, which includes helvolic acid, helvolinic acid, and cephalosporin P1. Fusidic acid is structurally unrelated to other antibiotics that are used in human or veterinary medicine. It is currently used principally as a topical antibiotic against Gram-positive bacteria, especially staphylococci.

Given the dearth of new antibiotics, fusidic acid is one of several older antibiotics that have been targeted for potentially increased use and importance (Maviglia et al. 2009; Pulcini et al. 2012). It has been noted for its good Gram-positive activity including MRSA, oral bioavailability, minimal toxicity, and low development of cross-resistance with other antibiotics (Schofer and Simonsen 2010). But despite these several favorable features, it has never been previously registered for the U.S. market. However, a novel loading dose regimen has been developed to improve clinical effects and to hinder resistance development (Cempra 2011). Fusidic acid has completed a Phase II clinical trial for ABSSSI and a Phase I trial for prosthetic joint infections to support registration in the U.S. (Cempra 2012; Corey et al. 2011; Sutcliffe 2011).

Fusidic acid possesses a unique antimicrobial MOA in which protein synthesis is inhibited through binding to a protein called elongation factor G (EF-G). EF-G is one component of the complex that performs the successive steps in the ribosomal elongation cycle in which the amide bonds of a growing protein are sequentially formed with the incoming amino acids (Agrawal et al. 2000). The system is driven by hydrolysis of GTP using the GTP-ase which is EF-G. Binding of fusidic acid to EF-G stabilizes the complex following GTP hydrolysis and hinders dissociation of EF-G from the ribosome, which interrupts the continuity of

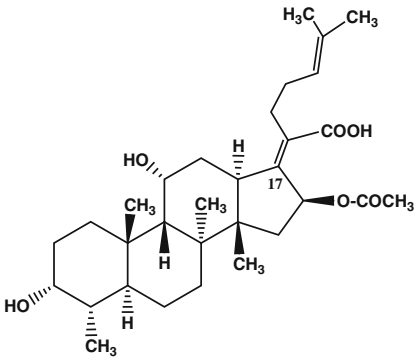


Fig. 14.5 Structure of fusidic acid

the elongation cycle and stalls further protein synthesis. A crystal structure of the complex has been published (Gao et al. 2009). Bacterial resistance to fusidic acid has been recently summarized (Farrell et al. 2011). The most common mechanism involves ways to reduce binding affinity and to protect EF-G from binding with fusidic acid, either by alterations of EF-G (*fusA*) or protein L6 (*fusE*) or by formation of protective proteins (*fusB, fusC, fusD*). Less common mechanisms are enzymatic inactivation and reduced uptake. Combinations with other antibiotics and limited duration of use have been used to help reduce development of resistance.

14.7 Pseudomonic Acids

The pseudomonic acids constitute a small antibiotic class produced from fermentation of the bacterium, *Pseudomonas fluorescens* (Fuller et al. 1971). Mupirocin is a mixture of pseudomonic acids in which pseudomonic acid A is the predominant component. Thus, mupirocin is often used synonymously with pseudomonic acid A (Hothersall et al. 2007). Its structure is a highly oxygenated polyketide-derived carboxylic acid (Fig. 14.6) (Chain and Mellows 1977). It is used as a topical antibiotic to treat a variety of skin and related infections caused by several Gram-positive bacteria and for decolonization of *S. aureus* from nasal passages (Coates et al. 2009). It exhibits bactericidal activity against Gram-positive bacteria that include MRSA and beta-lactamase producing strains.

Mupirocin is an inhibitor of protein synthesis that exerts its activity by a unique MOA (Thomas et al. 2010). It binds to bacterial isoleucyl-tRNA synthetase, which prevents binding between ATP and isoleucine and thus prevents the synthesis of isoleucyl-tRNA. Consequently, isoleucyl-tRNA is unavailable when needed for incorporation into growing proteins in the ribosome (Vondenhoff and Van Aerschot 2011). As anticipated for a unique MOA, cross-resistance to other antibiotics has not been demonstrated (Lee 2007). However, as eventually happens for all antibiotics, development of resistance to mupirocin has been slowly occurring (Thomas et al. 2010). Such resistance to mupirocin has been observed due to either alterations in the native isoleucyl-tRNA synthetase gene (*ileS*) that reduce binding of mupirocin or incorporation of another synthetase gene (*mupA*) (Patel et al. 2009).

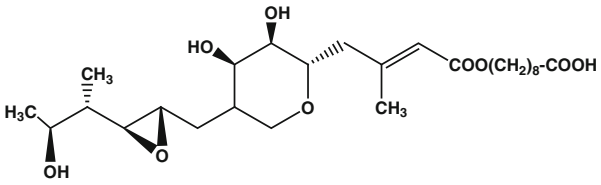


Fig. 14.6 Structure of pseudomonic acid A

Inhibition of aminoacyl-tRNA synthetases has been proposed as a novel MOA to use in the search for new antibiotics, but thus far the impact of this approach has been limited (Pohlmann and Brotz-Oesterhelt 2004).

14.8 Thiopeptides

The thiopeptide class is so-named due to its high sulfur content from the numerous sulfur-containing heterocycles within their highly modified peptidyl-based structures (Bagley et al. 2005; Hughes and Moody 2007). This class has been steadily expanding as new members continue to be isolated and identified. Many thiopeptides are high molecular weight compounds possessing very complex structures, although some smaller members are also known. One of the oldest and best-known members of this class is thiostrepton, which was initially isolated from the fermentation of *Streptomyces azureus* (Pagano et al. 1956). Its complex structure was not fully elucidated until 1983 (Fig. 14.7a) (Anderson et al. 1970; Hensens and Albers-Schonberg 1983). Total synthesis of thiostrepton was not achieved until 2004 (Nicolaou et al. 2004).

Although many thiopeptides exhibit good antimicrobial activity, especially against Gram-positive bacteria including MRSA, they have not yet been successfully developed for treating serious infections in human medicine. Low water solubility of these large and often lipophilic molecules creates problems for parenteral administration and their size hinders oral bioavailability. One advantage is their relatively low toxicity to mammalian systems since there is minimal overlap or similarity between their mammalian and bacterial targets. Some efforts have been made to overcome the problems by creation of semi-synthetic derivatives (Kirst 2012). However, because of the size and complexity of most thiopeptide structures, chemical derivatization of such compounds has thus far been generally slow and limited to modifying those functional groups located around the periphery of the molecule that can be readily accessed and selectively manipulated. Two semi-synthetic derivatives of GE2270A are a recent example of efforts to find important clinical candidates within this class (Fig. 14.7b) (LaMarche et al. 2011; Leeds et al. 2011). Some veterinary applications for another thiopeptide, thiopeptin, have been investigated, such as treatment of liver abscesses and lactic acidosis in ruminents (Lechtenberg et al. 1998; Muir et al. 1981).

Many thiopeptides interfere with some component(s) of bacterial protein synthesis, although they often differ on the distinct steps being affected (Lentzen et al. 2003; Mikolajka et al. 2011). Thiostrepton inhibits protein synthesis by binding to the complex of 23S rRNA and the L11 protein (Harms et al. 2008). This location is associated with the binding sites of translation factors (EF-Tu, EF-G) and GTPase-promoted hydrolysis of GTP. Both thiostrepton and micrococcin inhibit the same target, EF-G dependent translation, but their small structural differences lead to opposite effects; thiostrepton decreases turnover of EF-G while micrococcin increases turnover (Lentzen et al. 2003; Mikolajka et al. 2011). This site(s) does

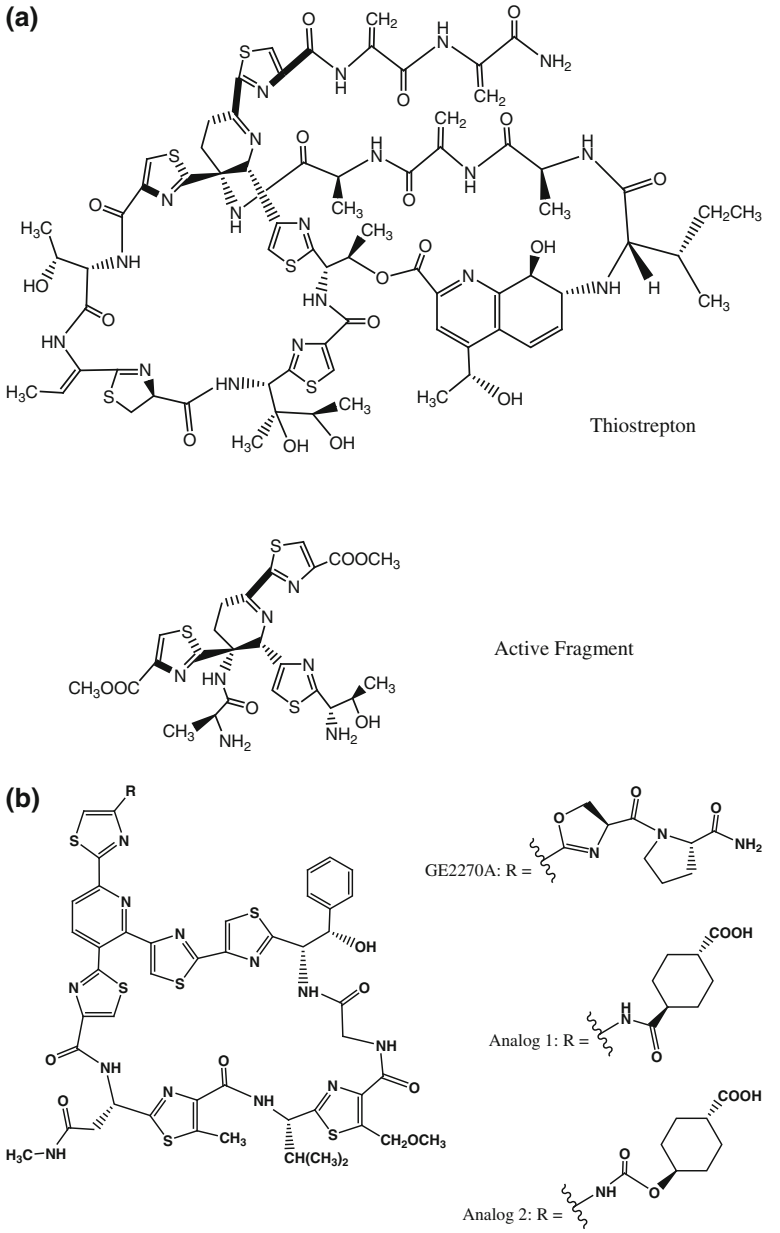


Fig. 14.7 **a** Structures of thiostrepton and an active fragment. **b** Structures of GE2270A and derivatives

not overlap the binding site of other antibiotics, so cross-resistance is not a problem. Other thiopeptides show both similarities and differences in their MOA compared to thiostrepton. For example, GE2270A inhibits bacterial protein synthesis by a mechanism involving binding with elongation factor Tu (EF-Tu) (Leeds et al. 2011).

Some recent effort has been successful in reducing the size of these compounds while still retaining some antimicrobial activity. During a total synthesis program, a fragment from the structure of thiostrepton (Fig. 14.7a) was discovered to possess moderate anti-MRSA activity (Nicolaou et al. 2005). This result demonstrates that a substantial portion of the large structure is unnecessary for moderate antimicrobial activity and could be replaced. Another study constructed a synthetic library of compounds based upon fragments that were identified from analyzing a model of thiostrepton-ribosome binding (Bower et al. 2003). The thiostrepton-ribosome binding site was also examined by proximity-induced covalent capture (Baumann et al. 2008). It will prove interesting to see if these or other strategies can identify smaller pharmacophores that SAR studies can use as starting points to create improved analogs having stronger antibiotic features.

Synthetic challenges with this class offer opportunities for biosynthetic engineering to provide a complementary strategy for producing new analogs. The intriguing origins of the many uncommon components assembled in thiopeptide structures have been attributed to extensive post-translational modifications of peptides initially formed by biosynthesis on the ribosome (Arndt et al. 2009; Li et al. 2011). Further studies are being conducted by several research groups to learn further details about the biosynthetic pathways and then to manipulate and modify them to produce new entities and to eventually achieve combinatorial biosynthesis in this class (Li and Kelly 2010, Li et al. 2011).

14.9 Miscellaneous

In addition to the seven smaller classes reviewed in this chapter, many other antibiotics are known that employ some aspect of inhibition of microbial protein synthesis as their MOA. Such a list includes some agents that have never experienced much if any significant clinical development, such as sparsomycin, pactamycin, anisomycin, viomycin, puromycin, kirromycin, negamycin, TAN-1057, and edeine (Hermann 2005; Lange et al. 2007; McCoy et al. 2011; Yonath 2005). Some of these agents use a unique MOA not shared by other antibiotics and represent new uncharted territory.

Another example is capreomycin, an old but still useful second-line anti-tuberculosis drug used in cocktails of multiple agents in order to hinder development of resistance and to combat multi-drug resistant (MDR) strains of *M. tuberculosis* (Ma et al. 2007). One member of the orthosomycin class, everninomicin D (Ziracin®), experienced some clinical development before it was eventually dropped due to toxicity (Lee 2007). While more details on MOA are

still needed, orthosomycins inhibit protein synthesis through a unique mechanism of binding at a site relatively far removed from the PTC, resulting in no cross-resistance with other antibiotics (Mikolajka et al. 2011). However, there appears to be no follow-up after everninomicin D was dropped from development.

Completely synthetic compounds that target critical steps in microbial protein biosynthesis are also being investigated. GSK2251052 (AN3365) is a novel boron-containing antibiotic that was discovered at Anacor Pharmaceuticals and licensed to GSK (Fig. 14.8). It functions as an inhibitor of leucyl-tRNA synthetase and is one of the few new antibiotics that exhibit activity against Gram-negative bacteria and anaerobes (Citron and Goldstein 2011). It is currently in Phase II clinical trials for treatment of hospital-acquired Gram-negative infections (Bush and Pucci 2011; Butler and Cooper 2011; Coates et al. 2011; Sutcliffe 2011).

Another inhibitor of protein synthesis is GSK1322322, a synthetic analog of the original natural product lead structure of actinonin (Chen et al. 2000). GSK1322322 has a hydrazino-pyrimidine structure (Fig. 14.8) and functions as an inhibitor of peptide deformylase (PDF), a ubiquitous and critical bacterial enzyme long considered as a potential new target for antibiotics (Sharma et al. 2009, see East, this volume). PDF cleaves the N-formyl group from the N-terminus of the

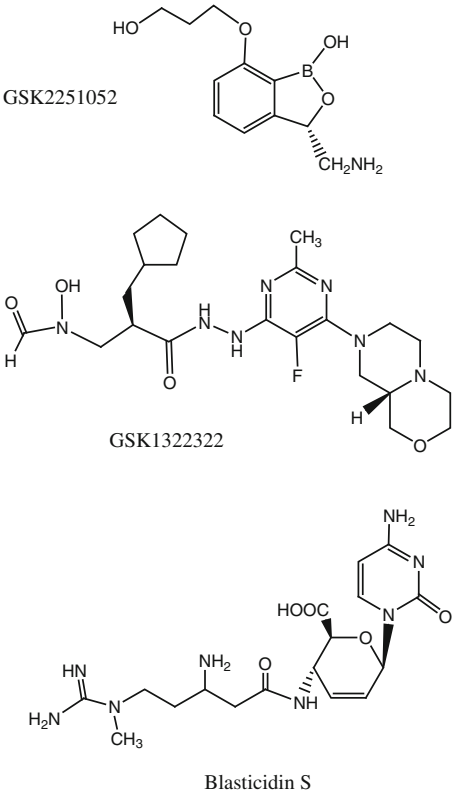


Fig. 14.8 Structures of GSK2251052, GSK1322322, and blasticidin S

protein synthesis initiator f-met on the peptide chain in preparation for the final steps to release and activate the desired peptide product. Failure to properly cleave the N-formyl moiety at the correct time during protein biosynthesis disrupts the ribosomal process. Although several previous PDF inhibitors have been studied and dropped from further development, GSK1322322 is in Phase II clinical trial (Bush and Pucci 2011; Butler and Cooper 2011; Coates et al. 2011; Kirst 2012; Sutcliffe 2011; see East, this volume).

Rib-X Pharmaceuticals presented their RX-04 program in fourteen posters at the 2011 ICAAC (Rib-X 2012). A starting point was provided by blasticidin S, an old nucleoside antibiotic (Fig. 14.8) which is obtained from fermentation of *Streptomyces griseochromogenes* and is a known inhibitor of protein synthesis (Kumasaka et al. 2007; Takeuchi et al. 1958). An initial structure for a PTC—blasticidin S complex has been described (Hansen et al. 2003). In the current effort, X-ray crystallography and computational analyses of ribosome-blasticidin S co-crystals were used to identify potential new scaffold fragments for SAR elaborations and to suggest new directions for synthesizing novel entities that would exhibit stronger ribosomal binding and greater antimicrobial activity (Kanyo et al. 2011). From these studies, three separate new platforms were presented as leads for future synthetic efforts and SAR studies in order to create new broad spectrum antibiotic structures (Marra et al. 2011).

14.10 Conclusions

Recent events described in this review clearly illustrate that creative ideas for new antibiotics are still being generated even within such a highly investigated field as protein synthesis inhibitors. In addition to new antibiotics coming from the four large classes of protein synthesis inhibitors, several smaller classes are also starting to contribute new agents into the antibiotic development pipeline. One member of the pleuromutilin class, retapamulin, recently received regulatory approval. Other members from these (and other) smaller classes should follow in due course.

In addition to the seven classes in this chapter, a vast wealth of other previously isolated fermentation-derived antibiotics remains under-explored and under-developed. This large collection of known natural products is a rich lode that is nowhere yet fully mined for either chemistry or microbiology and it warrants more serious attention. It is reasonable to expect that many of these compounds inhibit protein synthesis, but they have never been (fully) tested to learn their detailed MOA. New targets may be discovered that are currently unused or unknown within that large and complex MOA of protein synthesis inhibitors. Thus, new opportunities await researchers to again use antibiotics to discover new microbiology. The fermentative origin of compounds along with advances in microbial genetics opens greater opportunities for controlled manipulations of biosynthetic

pathways and combinatorial biosynthesis to complement chemical synthesis in creating new antibiotics.

From the chemistry side, the compounds are certainly a valuable resource as leading structures for medicinal chemistry programs. Natural products tend to occupy unfilled chemical spaces that prompt new synthetic strategies and diversity-oriented chemical libraries for screening (Cordier et al. 2008). Applications of fragment-based design and creation of new natural product-derived scaffolds for elaboration by analog synthesis and SAR studies are other encouraging developments. Thus, multiple opportunities are available to discover new agents that target bacterial protein synthesis (as well as other MOAs). All of these potential developments indicate that inhibition of protein synthesis should continue to flourish as an important MOA for new antimicrobial agents.

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