1

ANTIBIOTICS: GROUPS AND PROPERTIES

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1.1 INTRODUCTION

The introduction of the sulfonamides in the 1930s and benzylpenicillin in the 1940s completely revolutionized medicine by reducing the morbidity and mortality of many infectious diseases. Today, antimicrobial drugs are used in food-producing animals to treat and prevent diseases and to enhance growth rate and feed efficiency. Such use is fundamental to animal health and well-being and to the economics of the livestock industry, and has seen the development of antimicrobials such as ceftiofur, florfenicol, tiamulin, tilmicosin, tulathromycin, and tylosin specifically for use in food-producing animals.[1,2] However, these uses may result in residues in foods and have been linked to the emergence of antibiotic-resistant strains of disease-causing bacteria with potential human health ramifications.[3] Antimicrobial drug resistance is not addressed in detail in this text, and the interested reader is referred to an excellent overview by Martinez and Silley.[4]

Many factors influence the residue profiles of antibiotics in animal-derived edible tissues (meat and offal) and products (milk and eggs), and in fish and honey. Among these factors are the approved uses, which vary markedly between antibiotic classes and to a lesser degree within classes. For instance, in some countries, residues of quinolones in animal tissues, milk, honey, shrimp, and fish are legally permitted (maximum residue limits [MRLs] have been established). By comparison, the approved uses of the macrolides are confined to the treatment of respiratory disease and for growth promotion (in some countries) in meat-producing animals (excluding fish), and to the treatment of American foulbrood disease in honeybees. As a consequence, residues of macrolides are legally permitted only in edible tissues derived from these food-producing species, and in honey in some countries. Although a MRL for tylosin in honey has not been established, some countries apply a safe working residue level, thereby permitting the presence of trace concentrations of tylosin to allow for its use. Substantial differences in the approved uses of antimicrobial agents also occur between countries. A second factor that influences residue profiles of antimicrobial drugs is their chemical nature and physicochemical properties, which impact pharmacokinetic behavior. Pharmacokinetics (PK), which describes the timecourse of drug concentration in the body, is introduced in this chapter and discussed further in Chapter 2.

Analytical chemists take numerous parameters into account when determining antibiotic residues in food of animal origin, some of which are discussed here.

1.1.1 Identification

A substance needs to be identified by a combination of the appropriate identification parameters including the name or other identifier of the substance, information related to molecular and structural formula, and composition of the substance.

International nonproprietary names (INNs) are used to identify pharmaceutical substances or active pharmaceutical ingredients. Each INN is a unique name that is internationally consistent and is recognized globally. As of October 2009, approximately 8100 INNs had been designated, and this number is growing every year by some 120–150 new INNs.[5] An example of an INN is tylosin, a macrolide antibiotic.

Chemical Analysis of Antibiotic Residues in Food, First Edition. Edited by Jian Wang, James D. MacNeil, and Jack F. Kay.

International Union of Pure and Applied Chemistry (IUPAC) names are based on a method that involves selecting the longest continuous chain of carbon atoms, and then identifying the groups attached to that chain and systematically indicating where they are attached. Continuing with tylosin as an example, the IUPAC name is [(2*R*,3*R*,4*E*,6*E*,9*R*,11*R*,12*S*,13*S*,14*R*)-12-{[3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-ribohexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy}-2-ethyl-14-hydroxy-5,9,13-trimethyl-8,16-dioxo-11-(2-oxoethyl)oxacyclohexadeca-4,6-dien-3-yl]methyl 6-deoxy-2,3-di-*O*-methyl-β-D-allopyranoside.

The Chemical Abstract Service (CAS) Registry Number is the universally recognized unique identifier of chemical substances. The CAS Registry Number for tylosin is 1401-69-0.

Synonyms are used for establishing a molecule's unique identity. For the tylosin example, there are numerous synonyms, one of which is Tylan.

1.1.2 Chemical Structure

For the great majority of drugs, action on the body is dependent on chemical structure, so that a very small change can markedly alter the potency of the drug, even to the point of loss of activity.[6] In the case of antimicrobial drugs, it was the work of Ehrlich in the early 1900s that led to the introduction of molecules selectively toxic for microbes and relatively safe for the animal host. In addition, the presence of different sidechains confers different pharmacokinetic behavior on a molecule. Chemical structures also provide the context to some of the extraction, separation, and detection strategies used in the development of analytical methods. Certain antibiotics consist of several components with distinct chemical structures. Tylosin, for example, is a mixture of four derivatives produced by a strain of *Streptomyces fradiae*. The chemical structures of the antimicrobial agents described in this chapter are presented in Tables 1.2–1.15.

1.1.3 Molecular Formula

By identifying the functional groups present in a molecule, a molecular formula provides insight into numerous properties. These include the molecule's water and lipid solubility, the presence of fracture points for gas chromatography (GC) determinations, sources of potential markers such as chromophores, an indication as to the molecule's UV absorbance, whether derivatization is likely to be required when quantifying residues of the compound, and the form of ionization such as protonated ions or adduct ions when using electrospray ionization. The molecular formulas of the antimicrobial agents described in this chapter are shown in Tables 1.2–1.15.

1.1.4 Composition of the Substance

Regulatory authorities conduct risk assessments on the chemistry and manufacture of new and generic antimicrobial medicines (formulated products) prior to granting marketing approvals. Typically, a compositional standard is developed for a new chemical entity or will already exist for a generic drug. A compositional standard specifies the minimum purity of the active ingredient, the ratio of isomers to diastereoisomers (if relevant), and the maximum permitted concentration of impurities, including those of toxicological concern. The risk assessment considers the manufacturing process (the toxicological profiles of impurities resulting from the synthesis are of particular interest), purity, and composition to ensure compliance with the relevant standard. The relevant test procedures described in pharmacopoeia and similar texts apply to the active ingredient and excipients present in the formulation. The overall risk assessment conducted by regulatory authorities ensures that antimicrobial drugs originating from different manufacturing sources, and for different batches from the same manufacturing source, have profiles that are consistently acceptable in terms of efficacy and safety to target animals, public health, and environmental health.

1.1.5 pK_a

The symbol pK_a is used to represent the negative logarithm of the acid dissociation constant K_a, which is defined as $[H^+][B]/[HB]$, where B is the conjugate base of the acid HB. By convention, the acid dissociation constant (pK_a) is used for weak bases (rather than the pK_b) as well as weak organic acids. Therefore, a weak acid with a high pK_a will be poorly ionized, and a weak base with a high pK_a will be highly ionized at blood pH. The pK_a value is the principal property of an electrolyte that defines its biological and chemical behavior. Because the majority of drugs are weak acids or bases, they exist in both ionized and un-ionized forms, depending on pH. The proportion of ionized and un-ionized species at a particular pH is calculated using the Henderson–Hasselbalch equation. In biological terms, pK_a is important in determining whether a molecule will be taken up by aqueous tissue components or lipid membranes and is related to the partition coefficient log *P*. The pK_a of an antimicrobial drug has implications for both the fate of the drug in the body and the action of the drug on microorganisms. From a chemical perspective, ionization will increase the likelihood of a species being taken up into aqueous solution (because water is a very polar solvent). By contrast, an organic molecule that does not readily ionize will often tend to stay in a non-polar solvent. This partitioning behavior affects the efficiency of extraction and clean-up of analytes and is an important consideration when developing enrichment methods. The pK_a values for many

of the antimicrobial agents described in this chapter are presented in Tables 1.2–1.15. The consequences of pK_a for the biological and chemical properties of antimicrobial agents are discussed later in this text.

1.1.6 UV Absorbance

The electrons of unsaturated bonds in many organic drug molecules undergo energy transitions when UV light is absorbed. The intensity of absorption may be quantitatively expressed as an extinction coefficient ε, which has significance in analytical application of spectrophotometric methods.

1.1.7 Solubility

From an *in vitro* perspective, solubility in water and in organic solvents determines the choice of solvent, which, in turn, influences the choice of extraction procedure and analytical method. Solubility can also indirectly impact the timeframe of an assay for compounds that are unstable in solution. From an *in vivo* perspective, the solubility of a compound influences its absorption, distribution, metabolism, and excretion. Both water solubility and lipid solubility are necessary for the absorption of orally administered antimicrobial drugs from the gastrointestinal tract. This is an important consideration when selecting a pharmaceutical salt during formulation development. Lipid solubility is necessary for passive diffusion of drugs in the distributive phase, whereas water solubility is critical for the excretion of antimicrobial drugs and/or their metabolites by the kidneys.

1.1.8 Stability

In terms of residues in food, stability is an important parameter as it relates to (1) residues in biological matrices during storage, (2) analytical reference standards, (3) analytes in specified solvents, (4) samples prepared for residue analysis in an interrupted assay run such as might occur with the breakdown of an analytical instrument, and (5) residues being degraded during chromatography as a result of an incompatible stationary phase.

Stability is also an important property of formulated drug products since all formulations decompose with time.[7] Because instabilities are often detectable only after considerable storage periods under normal conditions, stability testing utilizes high-stress conditions (conditions of temperature, humidity, and light intensity, which are known to be likely causes of breakdown). Adoption of this approach reduces the amount of time required when determining shelf life. Accelerated stability studies involving the storage of products at elevated temperatures are commonly conducted to allow unsatisfactory formulations to be eliminated early in development and for a successful product to reach market sooner. The concept of accelerated stability is based on the Arrhenius equation:

$$k = Ae^{(-E_a/RT)}$$

where k is the rate constant of the chemical reaction; A, a pre-exponential factor; E_a, activation energy; R, gas constant; and T, absolute temperature.

In practical terms, the Arrhenius equation supports the generalization that, for many common chemical reactions at room temperature, the reaction rate doubles for every 10°C increase in temperature. Regulatory authorities generally accept accelerated stability data as an interim measure while real-time stability data are being generated.

1.2 ANTIBIOTIC GROUPS AND PROPERTIES

1.2.1 Terminology

Traditionally, the term *antibiotic* refers to substances produced by microorganisms that at low concentration kill or inhibit the growth of other microorganisms but cause little or no host damage. The term *antimicrobial agent* refers to any substance of natural, synthetic, or semisynthetic origin that at low concentration kills or inhibits the growth of microorganisms but causes little or no host damage. Neither antibiotics nor antimicrobial agents have activity against viruses. Today, the terms *antibiotic* and *antimicrobial agent* are often used interchangeably.

The term *microorganism* or *microbe* refers to (for the purpose of this chapter) prokaryotes, which, by definition, are single-cell organisms that do not possess a true nucleus. Both typical bacteria and atypical bacteria (rickettsiae, chlamydiae, mycoplasmas, and actinomycetes) are included. Bacteria range in size from 0.75 to 5 μm and most commonly are found in the shape of a sphere (coccus) or a rod (bacillus). Bacteria are unique in that they possess peptidoglycan in their cell walls, which is the site of action of antibiotics such as penicillin, bacitracin, and vancomycin. Differences in the composition of bacterial cell walls allow bacteria to be broadly classified using differential staining procedures. In this respect, the Gram stain developed by Christian Gram in 1884 (and later modified) is by far the most important differential stain used in microbiology.[8] Bacteria can be divided into two broad groups—Gram-positive and Gram-negative—using the Gram staining procedure. This classification is based on the ability of cells to retain the dye methyl violet after washing with a decolorizing agent such as absolute alcohol or acetone. Gram-positive cells retain the stain, whereas Gram-negative cells do not. Examples of Gram-positive bacteria are *Bacillus, Clostridium, Corynebacterium, Enterococcus,*

Erysipelothrix, Pneumococcus, Staphylococcus, and *Streptococcus*. Examples of Gram-negative bacteria are *Bordetella, Brucella, Escherichia coli, Haemophilus, Leptospira, Neisseria, Pasteurella, Proteus, Pseudomonas, Salmonella, Serpulina hyodysenteriae, Shigella*, and *Vibrio*. Differential sensitivity of Gram-positive and Gram-negative bacteria to antimicrobial drugs is discussed later in this chapter.

1.2.2 Fundamental Concepts

From the definitions above, it is apparent that a critically important element of antimicrobial therapy is the selective toxicity of a drug for invading organisms rather than mammalian cells. The effectiveness of antimicrobial therapy depends on a triad of bacterial susceptibility, the drug's disposition in the body, and the dosage regimen. An additional factor that influences therapeutic outcomes is the competence of host defence mechanisms. This property is most relevant when clinical improvement relies on the inhibition of bacterial cell growth rather than bacterial cell death. Irrespective of the mechanism of action, the use of antimicrobial drugs in food-producing species may result in residues.

The importance of antibacterial drug pharmacokinetics (PK) and pharmacodynamics (PD) in determining clinical efficacy and safety was appreciated many years ago when the relationship between the magnitude of drug response and drug concentration in the fluids bathing the infection site(s) was recognized. PK describes the timecourse of drug absorption, distribution, metabolism, and excretion (what the *body does to the drug*) and therefore the relationship between the dose of drug administered and the concentration of non-protein-bound drug at the site of action. PD describes the relationship between the concentration of non-protein-bound drug at the site of action and the drug response (ultimately the therapeutic effect) (what the *drug does to the body*).[9]

In conceptualizing the relationships between the host animal, drug, and target pathogens, the chemotherapeutic triangle (Fig. 1.1) alludes to antimicrobial drug PK and PD. The relationship between the host animal and the drug reflects the PK properties of the drug, whereas drug action against the target pathogens reflects the PD properties of the drug. The clinical efficacy of antimicrobial therapy is depicted by the relationship between the host animal and target pathogens.

1.2.3 Pharmacokinetics of Antimicrobial Drugs

The pharmacokinetics of antimicrobial drugs is discussed in Chapter 2. The purpose of the following discussion, then, is to introduce the concept of pharmacokinetics and, in particular, to address the consequences of an antimicrobial drug's pK_a value for both action on the target pathogen and fate in the body.

The absorption, distribution, metabolism, and excretion of an antimicrobial drug are governed largely by the drug's chemical nature and physicochemical properties. Molecular size and shape, lipid solubility, and the degree of ionization are of particular importance, although the degree of ionization is not an important consideration for amphoteric compounds such as fluoroquinolones, tetracyclines, and rifampin.[10] The majority of antimicrobial agents are weak acids and bases for which the degree of ionization depends

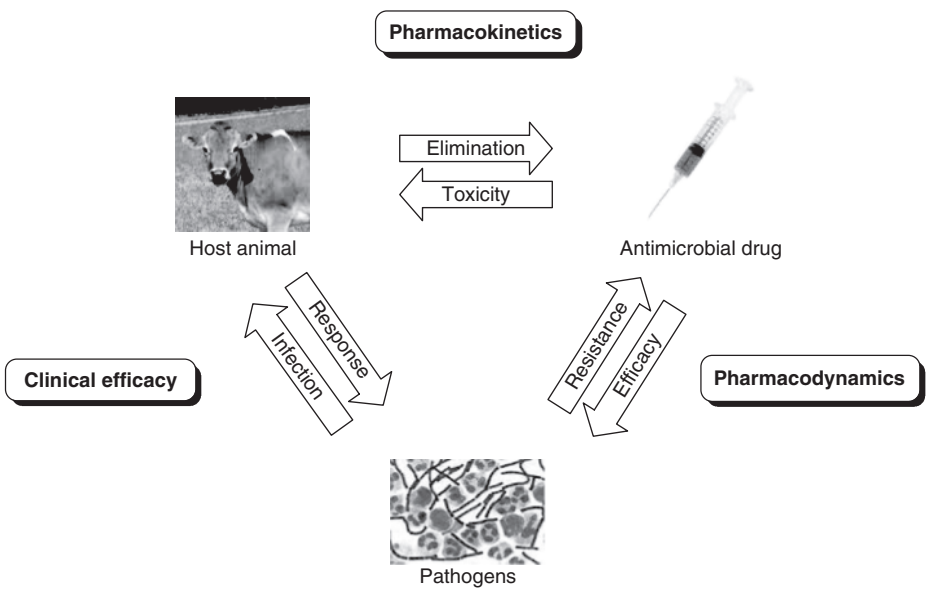

Figure 1.1 Schematic of the chemotherapeutic triangle depicting the relationships between the host animal, antimicrobial drug, and target pathogens.

on the pK_a of the drug and the pH of the biological environment. Only the un-ionized form of these drugs is lipid-soluble and able to cross cell membranes by passive diffusion. Two examples from Baggot and Brown[11] are presented here to demonstrate the implications of pK_a for the distributive phase of drug disposition. However, the same principles of passive diffusion apply to the absorption, metabolism, and excretion of drugs in the body and to the partitioning of drugs into microorganisms.

The first example relates to the sodium salt of a weak acid (with pK_a 4.4) that is infused into the mammary glands of dairy animals to treat mastitis. The pH of the normal mammary gland can be as low as 6.4, and at this pH, the Henderson–Hasselbalch equation predicts that the ratio of un-ionized to ionized drug is 1 : 100. Mastitic milk is more alkaline (with pH $\sim$ 7.4) and the ratio of un-ionized to ionized drug, as calculated by the Henderson–Hasselbalch equation, is 1 : 1000. This is identical to the ratio for plasma, which also has a pH of 7.4. This example demonstrates that, when compared to the normal mammary gland, the mastitic gland will have more drug "trapped" in the ionized form. The second example involves the injection of a lipid-soluble, organic base that diffuses from the systemic circulation (with pH 7.4) into ruminal fluid (pH 5.5–6.5) during the distributive phase of a drug. Again, the ionized form becomes trapped in the acidic fluid of the rumen; the extent of trapping will be determined by the pK_a of the organic base. In summary, weakly acidic drugs are trapped in alkaline environments and, vice versa, weakly basic drugs are trapped in acidic fluids.

A second PK issue is the concentration of antimicrobial drug at the site of infection. This value reflects the drug's distributive behavior and is critically important in terms of efficacy. Furthermore, the optimization of dosage regimens is dependent on the availability of quality information relating to drug concentration at the infection site. It raises questions regarding the choice of sampling site for measuring the concentration of antimicrobial drugs in the body and the effect, if any, that the extent of plasma protein binding has on the choice of sampling site. These matters are addressed below.

More often than not, the infection site (the biophase) is remote from the circulating blood that is commonly sampled to measure drug concentration. Several authors[12–14] have reported that plasma concentrations of free (non-protein-bound) drug are generally the best predictors of the clinical success of antimicrobial therapy. The biophase in most infections comprises extracellular fluid (plasma + interstitial fluids). Most pathogens of clinical interest are located extracellularly and as a result, plasma concentrations of free drug are generally representative of tissue concentrations; however, there are some notable exceptions:

1. Intracellular microbes such as *Lawsonia intracellularis*, the causative agent of proliferative enteropathy in pigs, are not exposed to plasma concentrations of antimicrobial drugs.
2. Anatomic barriers to the passive diffusion of antimicrobial drugs are encountered in certain tissues, including the central nervous system, the eye, and the prostate gland.
3. Pathological barriers such as abscesses impede the passive diffusion of drugs.
4. Certain antimicrobial drugs are preferentially accumulated inside cells. Macrolides, for instance, are known to accumulate within phagocytes.[15]
5. Certain antimicrobial drugs are actively transported into infection sites. The active transport of fluoroquinolones and tetracyclines by gingival fibroblasts into gingival fluid is an example.[16]

With regard to the effect of plasma protein binding on the choice of sampling site, Toutain and coworkers[14] reported that plasma drug concentrations of antimicrobial drugs that are >80% bound to plasma protein are unlikely to be representative of tissue concentrations. Those antimicrobial drugs that are highly bound to plasma protein include clindamycin, cloxacillin, doxycycline, and some sulfonamides.[17,18]

The most useful PK parameters for studying antimicrobial drugs are discussed in Chapter 2.

1.2.4 Pharmacodynamics of Antimicrobial Drugs

The PD of antimicrobial drugs against microorganisms comprises three main aspects: spectrum of activity, bactericidal and bacteriostatic activity, and the type of killing action (i.e., concentration-dependent, time-dependent, or co-dependent). Each of these is discussed below. Also described are the PD indices—minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)—and the mechanisms of action of antimicrobial drugs.

1.2.4.1 Spectrum of Activity

Antibacterial agents may be classified according to the class of target microorganism. Accordingly, antibacterial agents that inhibit only bacteria are described as narrow- or medium-spectrum, whereas those that also inhibit mycoplasma, rickettsia, and chlamydia (so-called atypical bacteria) are described as broad-spectrum. The spectrum of activity of common antibacterial drugs is shown in Table 1.1.

A different classification describes those antimicrobial agents that inhibit only Gram-positive or Gram-negative bacteria as narrow-spectrum, and those that are active against a range of both Gram-positive and Gram-negative bacteria as broad-spectrum. However, this distinction is not always absolute.

TABLE 1.1 Spectrum of Activity of Common Antibacterial Drugs

	Class of Microorganism				
Antibacterial Drug	Bacteria	Mycoplasma	Rickettsia	Chlamydia	Protozoa
Aminoglycosides	+	+	−	−	−
β-Lactams	+	−	−	−	−
Chloramphenicol	+	+	+	+	−
Fluoroquinolones	+	+	+	+	−
Lincosamides	+	+	−	−	+/−
Macrolides	+	+	−	+	+/−
Oxazolidinones	+	+	−	−	−
Pleuromutilins	+	+	−	+	−
Tetracyclines	+	+	+	+	−
Streptogramins	+	+	−	+	+/−
Sulfonamides	+	+	−	+	+
Trimethoprim	+	−	−	−	+

Notation: Presence or absence of activity against certain protozoa is indicated by plus or minus sign (+/−).

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The differential sensitivity of Gram-positive and Gram-negative bacteria to many antimicrobials is due to differences in cell wall composition. Gram-positive bacteria have a thicker outer wall composed of a number of layers of peptidoglycan, while Gram-negative bacteria have a lipophilic outer membrane that protects a thin peptidoglycan layer. Antibiotics that interfere with peptidoglycan syntheses more easily reach their site of action in Gram-positive bacteria. Gram-negative bacteria have protein channels (porins) in their outer membranes that allow the passage of small hydrophilic molecules. The outer membrane contains a lipopolysaccharide component that can be shed from the wall on cell death. It contains a highly heat-resistant molecule known as *endotoxin*, which has a number of toxic effects on the host animal, including fever and shock.

Antibiotic sensitivity also differs between aerobic and anaerobic organisms. Anaerobic organisms are further classified as facultative and obligate. Facultative anaerobic bacteria derive energy by aerobic respiration if oxygen is present but are also capable of switching to fermentation. Examples of facultative anaerobic bacteria are *Staphylococcus* (Gram-positive), *Escherichia coli* (Gram-negative), and *Listeria* (Gram-positive). In contrast, obligate anaerobes die in the presence of oxygen. Anaerobic organisms are resistant to antimicrobials that require oxygen-dependent mechanisms to enter bacterial cells. Anaerobic organisms may elaborate a variety of toxins and enzymes that can cause extensive tissue necrosis, limiting the penetration of antimicrobials into the site of infection, or inactivating them once they are present.

1.2.4.2 Bactericidal and Bacteriostatic Activity

The activity of antimicrobial drugs has also been described as being bacteriostatic or bactericidal, although this distinction depends on both the drug concentration at the site of infection and the microorganism involved. Bacteriostatic drugs (tetracyclines, phenicols, sulfonamides, lincosamides, macrolides) inhibit the growth of organisms at the MIC but require a significantly higher concentration, the MBC, to kill the organisms (MIC and MBC are discussed further below). By comparison, bactericidal drugs (penicillins, cephalosporins, aminoglycosides, fluoroquinolones) cause death of the organism at a concentration near the same drug concentration that inhibits its growth. Bactericidal drugs are required for effectively treating infections in immunocompromised patients and in immunoincompetent environments in the body.

1.2.4.3 Type of Killing Action

A further classification of antimicrobial drugs is based on their killing action, which may be time-dependent, concentration-dependent, or co-dependent. For time-dependent drugs, it is the duration of exposure (as reflected in time exceeding MIC for plasma concentration) that best correlates with bacteriological cure. For drugs characterized by concentration-dependent killing, it is the maximum plasma concentration and/or area under the plasma concentration–time curve that correlates with outcome. For drugs with a co-dependent killing effect, both the concentration achieved and the duration of exposure determine outcome (see Chapter 2 for further discussion).

Growth inhibition–time curves are used to define the type of killing action and steepness of the concentration–effect curve. Typically, reduction of the initial bacterial count (response) is plotted against antimicrobial drug concentration. The killing action (time-, concentration-, or co-dependent) of an antibacterial drug is determined largely by the slope of the curve. Antibacterial drugs that demonstrate time-dependent killing activity include the β-lactams, macrolides, tetracyclines, trimethoprim–sulfonamide

combinations, chloramphenicol, and glycopeptides. A concentration-dependent killing action is demonstrated by the aminoglycosides, fluoroquinolones, and metronidazole. The antibacterial response is less sensitive to increasing drug concentration when the slope is steep and vice versa.

1.2.4.4 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The most important indices for describing the PD of antimicrobial drugs are MIC and MBC. The MIC is the lowest concentration of antimicrobial agent that prevents visible growth after an 18- or 24-h incubation. It is a measure of the intrinsic antimicrobial activity (potency) of an antimicrobial drug. Because an MIC is an absolute value that is not based on comparison with a reference standard, it is critically important to standardize experimental factors that may influence the result, including the strain of bacteria, the size of the inocula, and the culture media used, according to internationally accepted methods (e.g., CLSI[19] or EUCAST[20]). The MIC is determined from culture broth containing antibiotics in serial two-fold dilutions that encompass the concentrations normally achieved *in vivo*. Positive and negative controls are included to demonstrate viability of the inocula and suitability of the medium for their growth, and that contamination with other organisms has not occurred during preparation, respectively.

After the MIC has been determined, it is necessary to decide whether the results suggest whether the organisms are susceptible to the tested antimicrobial *in vivo*. This decision requires an understanding of the PK of the drug (see Chapter 2 for discussion) and other factors. For example, *in vitro* assessments of activity may underestimate the *in vivo* activity because of a post-antibiotic effect and post-antibiotic leukocyte enhancement. The *post-antibiotic effect* (PAE) refers to a persistent antibacterial effect at subinhibitory concentrations, whereas the term *post-antibiotic leukocyte enhancement term* (PALE) refers to the increased susceptibility to phagocytosis and intracellular killing demonstrated by bacteria following exposure to an antimicrobial agent.[21]

The MIC test procedure described above can be extended to determine the MBC. The MBC is the minimal concentration that kills 99.9% of the microbial cells. Samples from the antibiotic-containing tubes used in the MIC determination in which microbial growth was not visible are plated on agar with no added antibiotic. The lowest concentration of antibiotic from which bacteria do not grow when plated on agar is the MBC.

1.2.4.5 Mechanisms of Action

Antimicrobial agents demonstrate five major mechanisms of action.[22] These mechanisms, with examples of each type, are as follows:

1. Inhibition of cell wall synthesis (β-lactam antibiotics, bacitracin, vancomycin)
2. Damage to cell membrane function (polymyxins)
3. Inhibition of nucleic acid synthesis or function (nitroimidazoles, nitrofurans, quinolones, fluoroquinolones)
4. Inhibition of protein synthesis (aminoglycosides, phenicols, lincosamides, macrolides, streptogramins, pleuromutilins, tetracyclines)
5. Inhibition of folic and folinic acid synthesis (sulfonamides, trimethoprim)

1.2.5 Antimicrobial Drug Combinations

The use of antimicrobial combinations is indicated in some situations. For instance, mixed infections may respond better to the use of two or more antimicrobial agents. A separate example is fixed combinations such as the potentiated sulfonamides (comprising a sulfonamide and a diaminopyrimidine such as trimethoprim) that display synergism of antimicrobial activity. Other examples include the sequential inhibition of cell wall synthesis; facilitation of one antibiotic's entry to a microbe by another; inhibition of inactivating enzymes; and the prevention of emergence of resistant populations.[2] Another potential advantage of using antimicrobial drugs in combination is that the dose, and therefore the toxicity, of drugs may be reduced when a particular drug is used in combination with another drug(s).

Disadvantages from combining antimicrobial drugs in therapy also arise, and to address this possibility, combinations should be justified from both pharmacokinetic and pharmacodynamic perspectives.[23] For example, with a fixed combination of an aminoglycoside and a β-lactam, the former displays a concentration-dependent killing action and should be administered once daily, while the latter displays time-dependent killing and should be administered more frequently in order to ensure that the plasma concentration is maintained above the MIC of the organism for the majority of the dosing interval. One way to achieve this is to combine an aminoglycoside and the procaine salt of benzylpenicillin. The former requires a high C_{max} : MIC ratio, while the procaine salt of benzylpenicillin gives prolonged absorption to maintain plasma concentrations above MIC for most of the interdose interval. Similarly, a bacteriostatic drug may prevent some classes of bactericidal drugs from being efficacious.[23]

1.2.6 Clinical Toxicities

Animals may experience adverse effects when treated with veterinary antimicrobial drugs. These effects may reflect the pharmacological or toxicological properties of the substances or may involve hypersensitivity reactions or anaphylaxis. The major adverse effects to the various classes of antibiotics used in animals are described later in this chapter.

1.2.7 Dosage Forms

Antimicrobials are available as a range of pharmaceutical formulation types for food-producing animals, and of these, oral and parenteral dosage forms are the most common. Pharmaceutical formulations are designed to ensure the stability of the active ingredient up to the expiry date (when the product is stored in accordance with label recommendations), to control the rate of release of the active ingredient, and to achieve a desirable PK profile for the active ingredient. When mixed with feed or drinking water, veterinary antimicrobials must be stable, and those incorporated in feed should (ideally) be evenly dispersed in the feed. Antimicrobial products, including generic products, should be manufactured in accordance with current good manufacturing practices (GMP) and following the specifications described in the licensing application approved by the relevant authority. Generic products should normally have been shown to be bioequivalent to the reference (usually the pioneer) product.

1.2.8 Occupational Health and Safety Issues

Occupational health and safety considerations are paramount for manufacturing staff and for veterinarians and farmers administering antimicrobials to food-producing animals. In the period 1985–2001, antimicrobial drugs accounted for 2% of all suspected adverse reactions to have occurred in humans that were reported to the UK Veterinary Medicines Directorate.[24] The major problem following human exposure to antimicrobial drugs is sensitization and subsequent hypersensitivity reactions, and these are well recognized with β-lactam antibiotics.[25] Dust inhalation and sensitization to active ingredients are major concerns in manufacturing sites and are addressed by containment and the use of protective personal equipment. Other conditions that occur in those occupationally exposed to antimicrobials include dermatitis, bronchial asthma, accidental needlesticks, and accidental self-administration of injectable formulations. The occupational health and safety issues associated with specific classes of antimicrobial drugs are discussed later in this chapter.

1.2.9 Environmental Issues

Subject to the type of animal production system being considered, antimicrobial agents used in the livestock industries may enter the environment (for a review, see Boxall[26]). In the case of manure or slurry, which is typically stored before being applied to land, anaerobic degradation of antimicrobials occurs to differing degrees during storage. For example, β-lactam antibiotics rapidly dissipate in a range of manure types whereas tetracyclines are likely to persist for months. Compared to the situation in manure or slurry, the degradation of antimicrobials in soil is more likely to involve aerobic organisms. In fish production systems, medicated food pellets are added directly to pens or cages to treat bacterial infections in fish.[27–29] This practice results in the sediment under cages becoming contaminated with antimicrobials.[30–32] More recently, the literature has described tetracycline[33] and chloramphenicol[34] produced by soil organisms being taken up by plants. This raises the possibility that food-producing species may consume naturally derived antimicrobials when grazing herbs and grasses. The effects of the various classes of antibiotics on the environment are introduced later in this chapter to provide a foundation for the discussion that follows in Chapter 3.

1.3 MAJOR GROUPS OF ANTIBIOTICS

There are hundreds of antimicrobial agents in human and veterinary use, most of which belong to a few major classes; however, only some of these drugs are approved for use in food-producing species. Many factors contribute to this situation, one of which is concern over the transfer of antimicrobial resistance from animals to humans. In 1969, the Swann report in the United Kingdom recommended against the use of antimicrobial drugs already approved as therapeutic agents in humans or animals for growth promotion in animals.[35] This recommendation was only partially implemented in Britain at the time. Since then, the use of additional drugs for growth promotion has been prohibited in several countries. In addition, the World Health Organization (WHO), Codex Alimentarius Commission (CAC), the World Organization for Animal Health [Office International des Epizooties (OIE)], and national authorities are now developing strategies for reducing losses resulting from antimicrobial resistance, of those antimicrobial agents considered to be of critical importance to human medicine. When implemented, the recommendations from these important initiatives are certain to further restrict the availability of antimicrobial drugs for prophylactic and therapeutic uses in food-producing species.

An antimicrobial class comprises compounds with a related molecular structure and generally with similar modes of action. Variations in the properties of antimicrobials within a class often arise as a result of the presence of different sidechains of the molecule, which confer different patterns of PK and PD behavior on the molecule.[36] The major classes of antimicrobial drugs are discussed below.

1.3.1 Aminoglycosides

Streptomycin, the first aminogylcoside, was isolated from a strain of *Streptomyces griseus* and became available

in 1944. Over the next 20 years, other aminoglycosides were isolated from streptomycetes (neomycin and kanamycin) and *Micromonospora purpurea* (gentamicin). Semi-synthetic derivatives have subsequently been produced, including amikacin from kanamycin.

Aminoglycosides are bactericidal antibiotics with a concentration-dependent killing action, active against aerobic Gram-negative bacteria and some Gram-positive bacteria, but have little or no activity against anaerobic bacteria. Aminoglycosides are actively pumped into Gram-negative cells through an oxygen-dependent interaction between the negatively charged surface of the outer cell membrane and the aminoglycoside cations. This results in altered bacterial cell membrane permeability. The aminoglycosides then bind to the 30*S* ribosomal subunit and cause misreading of the messenger RNA, resulting in disruption of bacterial protein synthesis. This further affects cell membrane permeability, allowing more aminoglycoside uptake leading to more cell disruption and finally cell death.[37] Different aminoglycosides have slightly different effects. Streptomycin and its dihydro derivatives act at a single site on the ribosome, but other aminoglycosides act at several sites. The action of aminoglycosides is bactericidal and dose-dependent, and there is a significant post-antibiotic effect. While theoretically one would expect interaction with β-lactam antibiotics to enhance penetration of aminoglycosides into bacterial cells as a result of the interference with cell wall synthesis, human efficacy and toxicity studies now dispute that there is any therapeutic justification for this type of combination.[38] However, it would appear that some of the formulation types used in animals, such as a combination of an aminoglycoside and the procaine salt of benzylpenicillin (see discussion above), do provide enhanced antibacterial activity.

Bacterial resistance to aminoglycosides is mediated through bacterial enzymes (phosphotransferases, acetyltransferases, adenyltransferases), which inactivate aminoglycosides and prevent their binding to the ribosome. Genes encoding these enzymes are frequently located on plasmids, facilitating rapid transfer of resistance to other bacteria.

Aminoglycosides are not well absorbed from the gastrointestinal tract but are well absorbed after intramuscular or subcutaneous injection. Effective concentrations are achieved in synovial, pleural, peritoneal, and pericardial fluids. Intrauterine and intramammary administration is also effective, but significant tissue residues result. Aminoglycosides do not bind significantly to plasma proteins, and as they are large polar molecules, they are poorly lipid-soluble and do not readily enter cells or penetrate cellular barriers. This means that therapeutic concentrations are not easily achieved in cerebrospinal or ocular fluids. Their volumes of distribution are small, and the half-lives in plasma are relatively short (1–2 h).[39] Elimination is entirely via the kidney.

Aminoglycosides tend to be reserved for more serious infections because of their toxicity. The more toxic members such as neomycin are restricted to topical or oral use; the less toxic aminoglycosides such as gentamicin are used parenterally for treatment of Gram-negative sepsis. Oral preparations of neomycin and streptomycin preparations are available for treatment of bacterial enteritis in calves, ophthalmic preparations of framycetin are used in sheep and cattle, and neomycin preparations (some in combination with β-lactams) are used in the treatment of bovine mastitis. Systemic use of streptomycin, neomycin, and spectinomycin is often restricted in food-producing animals because of widespread resistance and because of extended persistence of residues in kidney tissues. Aminoglycosides are used to treat individual animals for therapeutic purposes rather than metaphylaxis or prophylaxis. An exception is the use of neomycin as a dry-cow treatment at the end of lactation in dairy cows. No aminoglycosides are used as antimicrobial growth promotants.

All aminoglycosides display ototoxicity and nephrotoxicity. Streptomycin is the most ototoxic but the least nephrotoxic; neomycin is the most nephrotoxic. Nephrotoxicity is associated with accumulation of aminoglycosides in the renal proximal tubule cells, where the drugs accumulate within the lysosomes and are released into the cytoplasm, causing damage to cellular organelles and cell death. Risk factors for aminoglycoside toxicity include prolonged therapy ($>7-10$ days), more than once daily treatment, acidosis and electrolyte disturbances, age (neonates, geriatrics) and pre-existing renal disease. As toxicity to aminoglycosides is related to the trough concentration of drug, once-daily high-dose treatment is used to allow drug concentration during the trough period to fall below the threshold that causes toxicity.[40] Once-daily dosing is effective because aminoglycosides display concentration-dependent killing activity and a long post-antibiotic effect. In the case of animals with impaired renal function, this may not apply as aminoglycosides are generally contraindicated or administered with extended dosing intervals.[41]

The limited information available suggests that aminoglycoside residues persist at trace levels in the environment (see also discussion in Chapter 3).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated toxicological and residue depletion data for dihydrostreptomycin and streptomycin, gentamicin, kanamycin, neomycin, and spectinomycin (see list in Table 1.2). On the basis of the risk assessments carried out by the JECFA, ADIs were allocated for all of these substances except kanamycin.[42] In addition, on the basis of JECFA recommendations, CAC MRLs were established for dihydrostreptomycin and streptomycin in muscle, liver, kidney, and fat of cattle, sheep, pigs, and chickens, and in cow's milk and sheep's milk; for

gentamicin in muscle, liver, kidney, and fat of cattle and pigs, and in cow's milk; for neomycin in muscle, liver, kidney, and fat of cattle, sheep, pigs, chickens, goats, ducks, and turkeys, and in cow's milk and chicken eggs; and for spectinomycin in muscle, liver, kidney, and fat of cattle, sheep, pigs, and chickens, and in cow's milk and chicken eggs.[43] Details of residue studies considered by JECFA in recommending MRLs for adoption by the CAC, after review by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF), are contained in monographs dealing with dihydrostreptomycin and streptomycin,[44–47] gentamicin,[48,49] neomycin,[50–53] and spectinomycin.[54,55]

1.3.2 β-Lactams

The discovery by Fleming in 1929 that cultures of *Penicillium notatum* produced an antibacterial substance and the subsequent purification of penicillin and its use by Florey, Chain, and others a decade later to successfully treat infections in human patients launched the chemotherapeutic revolution. In 1945, Fleming, Florey, and Chain were jointly awarded the Nobel Prize in Physiology or Medicine for this work.

There are a number of classes of β-lactam antibiotics, on the basis of their chemical structure. All are bactericidal and act by disrupting peptidoglycan synthesis in

TABLE 1.2 Aminoglycosides and Aminocyclitols

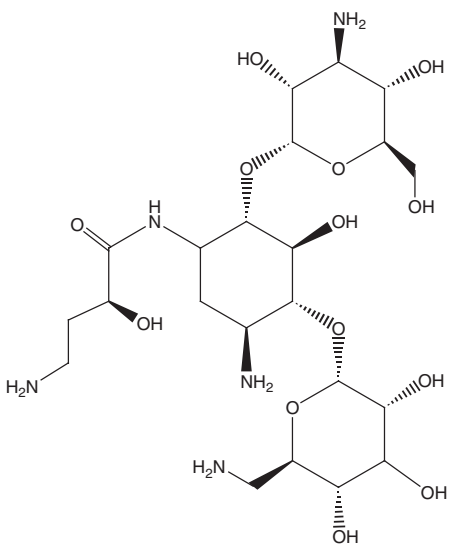
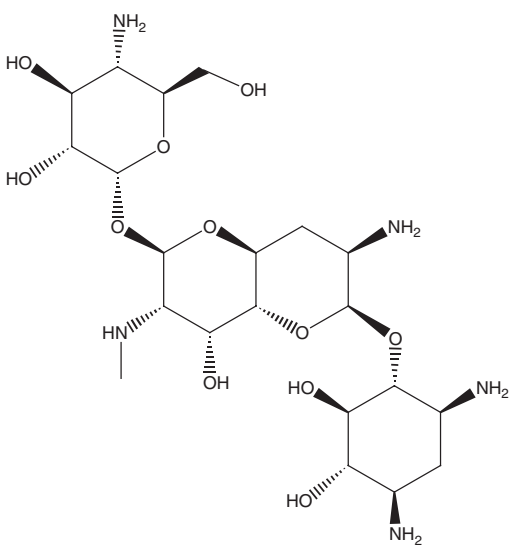
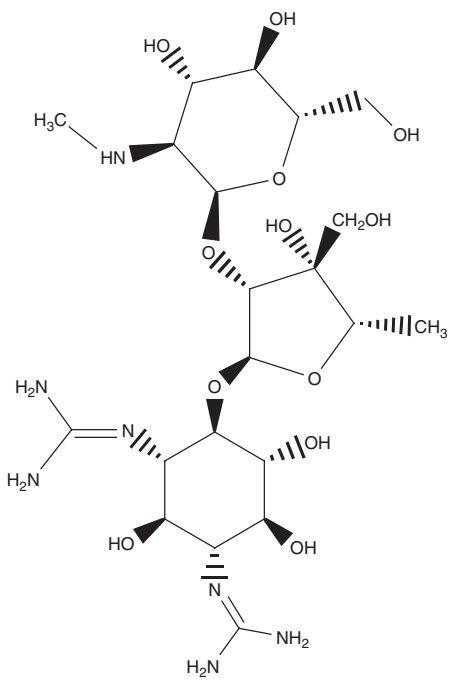
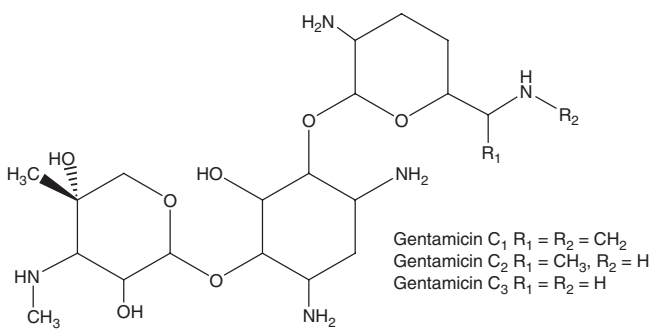
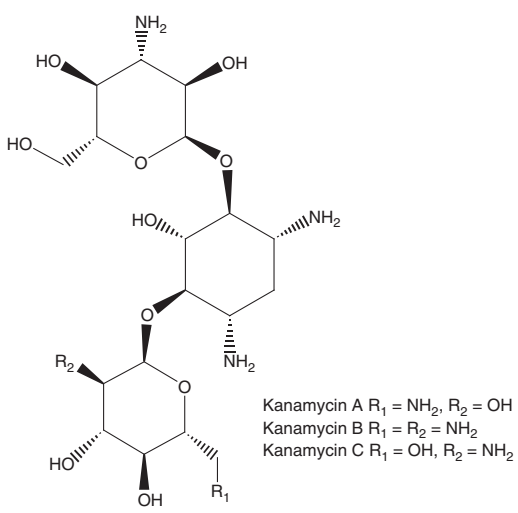
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
	Aminoglycosides		
Amikacin	(2*S*)-4-Amino-*N*-[(1*R*,2*S*,3S,4*R*,5*S*)-5-amino-2-[(2*S*,3*R*,4*S*,5*S*,6*R*)-4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4-[(2*R*,3*R*,4*S*,5*S*,6*R*)-6-(aminomethyl)-3,4,5-trihydroxyoxan-2-yl]oxy-3-hydroxycyclohexyl]-2-hydroxybutanamide $C_{22}H_{43}N_5O_{13}$ 37517-28-5		HB^+ 8.1[56]
Apramycin	(2*R*,3*R*,4*S*,5*S*,6*S*)-2-[[(2*S*,3*R*,4a*S*,6*R*,7*S*,8*R*,8a*R*)-3-Amino-2-[(1*R*,2*R*,3*S*,4*R*,6*S*)-4,6-diamino-2,3-dihydroxycyclohexyl]oxy-8-hydroxy-7-methylamino-2,3,4,4a,6,7,8,8a-octahydropyrano[2,3-*e*]pyran-6-yl]oxy]-5-amino-6-(hydroxymethyl)oxane-3,4-diol $C_{21}H_{41}N_5O_{11}$ 37321-09-08		HB^+ 8.5[57]

TABLE 1.2 (***Continued***)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Dihydrostreptomycin	2-[(1*S*,2*R*,3*R*,4*S*,5*R*,6*R*)-5-(Diaminomethylideneamino)-2-[(2*R*,3*R*,4*R*,5*S*)-3-[(2*S*,3*S*,4*S*,5*R*,6*S*)-4,5-dihydroxy-6-(hydroxymethyl)-3-methylaminooxan-2-yl]oxy-4-hydroxy-4-(hydroxymethyl)-5-methyloxolan-2-yl]oxy-3,4,6-trihydroxycyclohexyl]guanidine $C_{21}H_{41}N_7O_{12}$ 128-46-1		HB^+ 7.8[56]
Gentamicin	2-[4,6-Diamino-3-[3-amino-6-(1-methylaminoethyl)oxan-2-yl]oxy-2-hydroxycyclohexyl]-oxy-5-methyl-4-methylamino-oxane-3,5-diol $C_{21}H_{43}N_5O_7$ (gentamicin C_1) 1403-66-3	 Gentamicin C_1 $R_1 = R_2 = CH_2$ Gentamicin C_2 $R_1 = CH_3$, $R_2 = H$ Gentamicin C_3 $R_1 = R_2 = H$	HB^+ 8.2[56]
Kanamycin	(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(Aminomethyl)-6-[(1*R*,2*R*,3*S*,4*R*,6*S*)-4,6-diamino-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-4-amino-3,5-dihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2-hydroxycyclohexyl]-oxyoxane-3,4,5-triol $C_{18}H_{36}N_4O_{11}$ (kanamycin A) 59-01-8	 Kanamycin A $R_1 = NH_2$, $R_2 = OH$ Kanamycin B $R_1 = R_2 = NH_2$ Kanamycin C $R_1 = OH$, $R_2 = NH_2$	HB^+ 6.4[56] HB^+ 7.6[56] HB^+ 8.4[56] HB^+ 9.4[56]

(*continued*)

TABLE 1.2 (*Continued*)

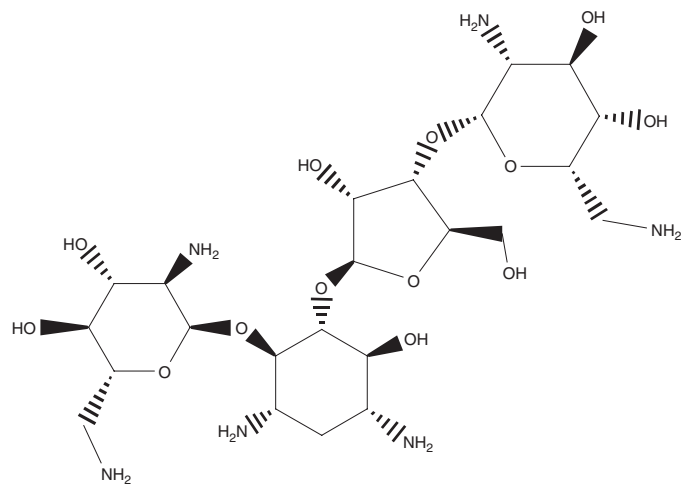
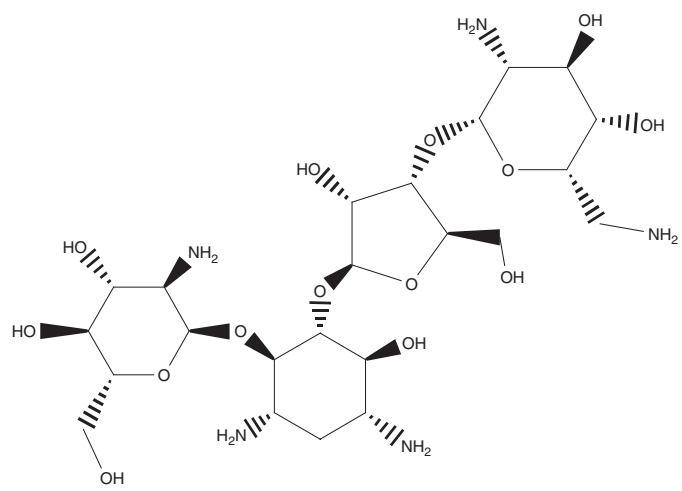
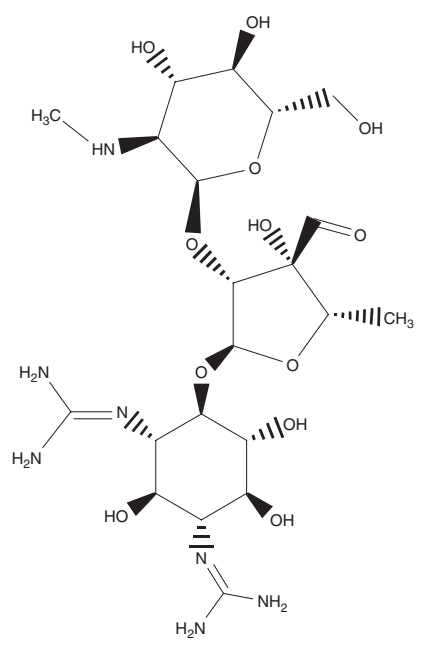
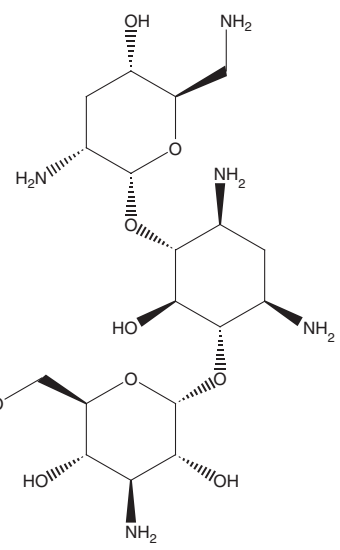
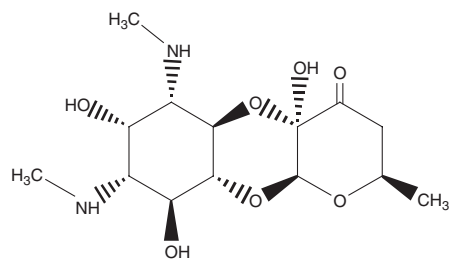
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Neomycin B	(2*R*,3*S*,4*R*,5*R*,6*R*)-5-Amino-2-(aminomethyl)-6-[(1*R*,2*R*,3*S*,4*R*,6*S*)-4,6-diamino-2-[(2*S*,3*R*,4*S*,5*R*)-4-[(2*R*,3*R*,4*R*,5*S*,6*S*)-3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5-(hydroxymethyl)-oxolan-2-yl]oxy-3-hydroxy-cyclohexyl]oxyoxane-3,4-diol $C_{23}H_{46}N_6O_{13}$ 1404-04-2		HB^+ 8.3[58]
Paromomycin	(2*R*,3*S*,4*R*,5*R*,6*S*)-5-Amino-6-[(1*R*,2*S*,3*S*,4*R*,6*S*)-4,6-diamino-2-[(2*S*,3*R*,4*R*,5*R*)-4-[(2*R*,3*R*,4*R*,5*R*,6*S*)-3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5-(hydroxymethyl)oxolan-2-yl]oxy-3-hydroxy-cyclohexyl]-oxy-2-(hydroxymethyl)oxane-3,4-diol $C_{23}H_{45}N_5O_{14}$ 1263-89-4		HB^+ 6.0[56] HB^+ 7.1[56] HB^+ 7.6[56] HB^+ 8.2[56] HB^+ 8.9[56]
Streptomycin A	2-[(1*S*,2*R*,3*R*,4*S*,5*R*,6*R*)-5-(Diaminomethylideneamino)-2-[(2*R*,3*R*,4*R*,5*S*)-3-[(2*S*,3*S*,4*S*,5*R*,6*S*)-4,5-dihydroxy-6-(hydroxymethyl)-3-methylaminooxan-2-yl]oxy-4-formyl-4-hydroxy-5-methyloxolan-2-yl]oxy-3,4,6-trihydroxycyclohexyl] guanidine $C_{21}H_{39}N_7O_{12}$ 57-92-1		HB^+ 7.8[56] HB^+ 11.5[56] $HB^+ > 12$[56]

TABLE 1.2 (***Continued***)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Tobramycin	4-Amino-2-[4,6-diamino-3-[3-amino-6-(aminomethyl)-5-hydroxyoxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-6-(hydroxymethyl)oxane-3,5-diol $C_{18}H_{37}N_5O_9$ 32986-56-4		HB^+ 6.7[56] HB^+ 8.3[56] HB^+ 9.9[56]
	Aminocyclitols		
Spectinomycin	Decahydro-4α,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one $C_{14}H_{24}N_2O_7$ 1695-77-8		HB^+ 7.0[56] HB^+ 8.7[56]

actively multiplying bacteria.[59] β-Lactams bind to proteins in the cell membrane [penicillin-binding proteins (PBPs)], which are enzymes that catalyze cross-linkages between the peptide chains on the *N*-acetylmuramic acid-*N*-acetylglucosamine backbone of the peptidoglycan molecule. Lack of cross-linkages results in the formation of a weak cell wall and can lead to lysis of growing cells. The differences in susceptibility of Gram-positive and Gram-negative bacteria to β-lactams are due to the larger amount of peptidoglycan in the cell wall, differences in PBPs between organisms, and the fact that it is difficult for some β-lactams to penetrate the outer lipopolysaccharide layer of the Gram-negative cell wall. Antimicrobial resistance to β-lactams is due to the action of β-lactamase enzymes that break the β-lactam ring and modification of PBPs, resulting in reduced binding affinity of the β-lactam for the peptide chain. Many Gram-negative bacteria are naturally resistant to some of the β-lactams because the β-lactam cannot penetrate the outer lipopolysaccharide membrane of the cell wall.

β-Lactams have a slower kill rate than do fluoroquinolones and aminoglycosides, and killing activity starts after a lag phase. Antimicrobial activity is usually time-dependent, not concentration-dependent. The β-lactams generally are wholly ionized in plasma and have relatively small volumes of distribution and short half-lives. They do not cross biological membranes well but are widely distributed in extracellular fluids. Elimination is generally through the kidneys.

The penicillins are characterized by their 6-aminopenicillanic acid (6-APA) core. This is a thiazolidone ring linked to a β-lactam ring and a sidechain at position C6, which allows them to be distinguished from one another. Penicillins can be separated into six groups on the basis of their activity. Benzylpenicillin (penicillin G) was the first β-lactam purified for clinical use from *Penicillium* cultures. Clinical limitations were soon recognized, with instability in the presence of gastric acids, susceptibility to β-lactamase enzymes, and ineffectiveness against many Gram-negative organisms. It also has a short terminal

half-life of around 30–60 min. However, benzylpenicillin is still the best antibiotic to use against most Gram-positive organisms (except resistant staphylococci and enterococci) and some Gram-negative bacteria. Most commonly now it is administered by deep intramuscular injection as procaine penicillin, where procaine provides a depot effect as a result of slow absorption. The first modification to the 6-APA core was acylation to produce phenoxymethylpenicillin (penicillin V),[60] which is more acid-stable and active orally. This development led to the ability to produce a wide range of semi-synthetic penicillins by adding sidechains to the 6-APA core. The first group were the anti-staphylococcal penicillins such as methicillin,[61] which are resistant to staphylococcal β-lactamases. Of these, cloxacillin is commonly used to treat mastitis in dairy cows. The extended or broad-spectrum penicillins, such as ampicillin, which is active against Gram-negative bacteria, including *Escherichia coli*, was the next class of penicillins. These antibiotics are susceptible to the action of β-lactamases. However, amoxicillin and amoxicillin plus clavulanate (a β-lactamase inhibitor) are widely used in livestock and companion animals to treat Gram-negative infections, particularly those caused by enteric Enterobacteriaceae. The next development was the anti-pseudomonal penicillins such as carbenicillin. These antibiotics are not commonly used in animals. The final class is the (Gram-negative) β-lactamase resistant penicillins such as temocillin. At this time, these are not registered for use in animals.

Shortly after the development of benzypenicillin, cephalosporin C was isolated from the fungus *Cephalosporium acremonium*. Cephalosporins have a 7-aminocephalosporanic acid core that includes the β-lactam ring and were of early interest because of activity against Gram-negative bacteria. In addition, these antibiotics are less susceptible to the action of β-lactamases. Over the years the cephalosporin core molecule was also modified to provide a series of classes (generations) of semi-synthetic cephalosporins with differing activities. The first-generation cephalosporins (e.g., cephalothin) were introduced to treat β-lactamase-resistant staphylococcal infections but also demonstrated activity against Gram-negative bacteria. They are no longer used commonly in companion animals but are still used in dry-cow therapies in dairy cows. Second-generation cephalosporins (e.g., cephalexin) are active against both Gram-positive and Gram-negative organisms. Oral preparations are widely used to treat companion animals. Products are registered for use in mastitis control in dairy cows. Third-generation cephalosporins (e.g., ceftiofur) demonstrate reduced activity against Gram-positive bacteria but increased activity against Gram-negative organisms. Because of their importance in human medicine, these products should be reserved for serious infections where other therapy has failed. They are used to treat both livestock and companion animals. Fourth-generation cephalosporins (e.g., cefquinome) have increased activity against both Gram-positive and Gram-negative bacteria.[62] These are reserve drugs in human medicine but in some countries are registered for use in cattle and horses.

Other β-lactams with natural origins include carbapenems (from *Streptomyces* spp.) and monobactams. These classes of β-lactams are not registered for use in food-producing animals but are used off-label in companion animals. Carbapenems have a wide range of activity against Gram-positive and Gram-negative bacteria and are resistant to most β-lactamases. Monobactams such as aztreonam are resistant to most β-lactamases and have a narrow spectrum of activity with good activity against many Gram-negative bacteria.

β-Lactam antibiotics are largely free of toxic effects, and the margin of safety is substantial. The major adverse effect is acute anaphylaxis, which is uncommon and associated mostly with penicillins; urticaria, angioneurotic edema, and fever occur more commonly. Penicillin-induced immunity-mediated hemolytic anemia in horses has also been reported.[63] The administration of procaine penicillin has led to pyrexia, lethargy, vomiting, inappetance, and cyanosis in pigs[64] and to signs of procaine toxicity, including death in horses.[65,66]

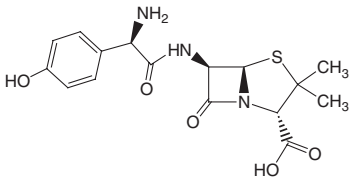
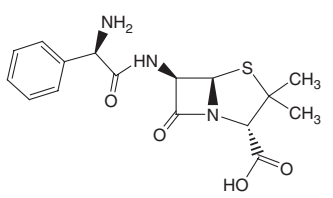
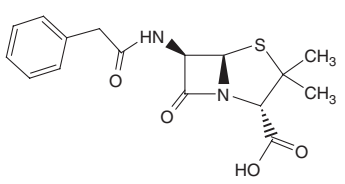
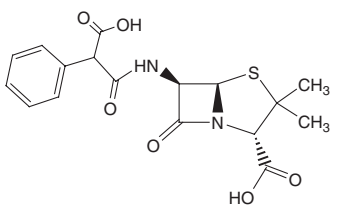
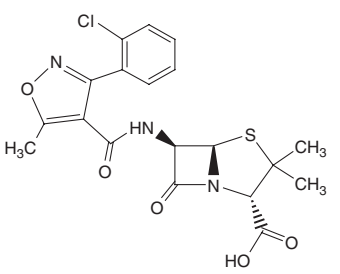
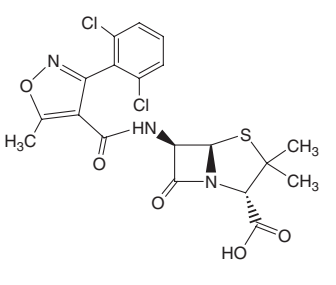
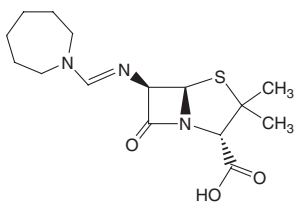
In humans, sensitization and subsequent hypersensitivity reactions to penicillin are relatively common during treatment. By comparison, adverse reactions attributed to occupational exposure to penicillin or the ingestion of food containing residues of penicillin are now seldom reported.

The concentrations of β-lactams reportedly present in the environment are negligible. This is consistent with β-lactam antibiotics being hydrolyzed shortly after they are excreted[67] and rapidly dissipating in a range of manure types.[26]

The CAC MRLs have been established on the basis of risk assessments carried out by the JECFA for benzylpenicillin,[42,68] procaine pencillin,[69] and ceftiofur.[70] The CAC MRLs established are for benzylpenicillin in muscle, liver, kidney, and milk of all food-producing species; for procaine penicillin in muscle, liver, and kidney of pigs and chickens; and for ceftiofur (expressed as desfuroyl-ceftiofur) in muscle, liver, kidney, and fat of cattle and pigs.[43] Details of residue studies considered by JECFA in recommending MRLs for CAC adoption are contained in monographs prepared for benzylpenicillin,[71] procaine penicillin,[72] and ceftiofur.[73,74]

From an analytical perspective, β-lactam antibiotics (Table 1.3) are stable under neutral or slightly basic conditions. These drugs degrade significantly as a result of the composition of some buffers (see Chapter 6 for further discussion).

TABLE 1.3 β-Lactams

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
	Penicillins		
Amoxicillin	(2*S*,5*R*,6*R*)-6-{[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{16}H_{19}N_3O_5S$ 26787-78-0		HA 2.6;[56] HB^+, HA 7.3;[56] HA, HB^+ 9.5[56]
Ampicillin	(2*S*,5*R*,6*R*)-6-{[(2*R*)-Aminophenylacetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{16}H_{19}N_3O_4S$ 69-53-4		HA 2.5,[56] HB^+ 7.3[56]
Benzylpenicillin (penicillin G)	(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(2-phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{16}H_{18}N_2O_4S$ 61-33-6		HA 2.7[56]
Carbenicillin	(2*S*,5*R*,6*R*)-6-[(3-Hydroxy-3-oxo-2-phenylpropanoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{17}H_{18}N_2O_6S$ 4697-36-3		HA 2.2,[56] HA 3.3[56]
Cloxacillin	(2*S*,5*R*,6*R*)-6-[[[3-(2-Chlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{19}H_{18}ClN_3O_5S$ 61-72-3		HA 2.7[56]
Dicloxacillin	(2*S*,5*R*,6*R*)-6-[[3-(2,6-Dichlorophenyl)-5-methyl-1,2-oxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{19}H_{17}Cl_2N_3O_5S$ 3116-76-5		HA 2.7[56]
Mecillinam	(2*S*,5*R*,6*R*)-6-(Azepan-1-ylmethylideneamino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{15}H_{23}N_3O_3S$ 32887-01-7		HA 2.7[56] HB^+ 8.8[56]

(*continued*)

TABLE 1.3 (*Continued*)

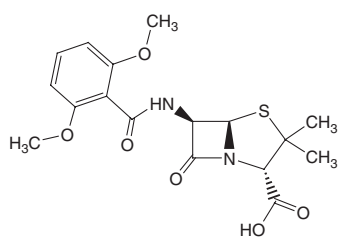
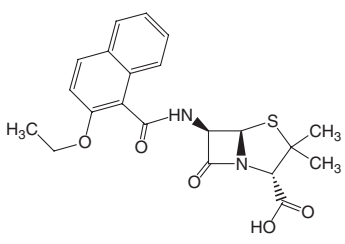
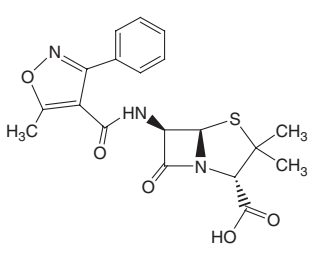
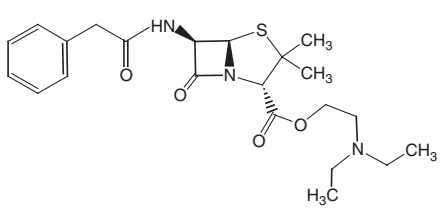
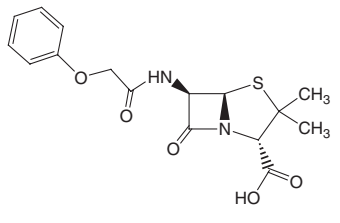
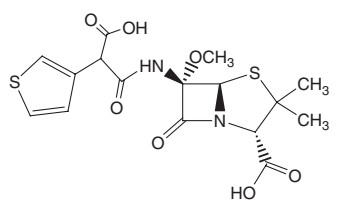
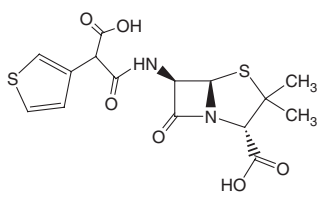
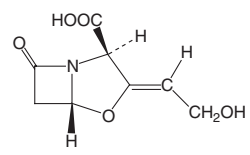
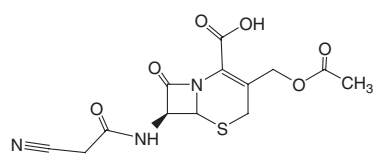
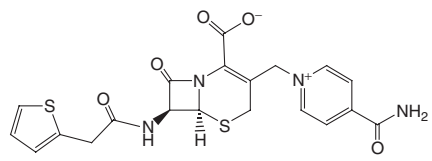
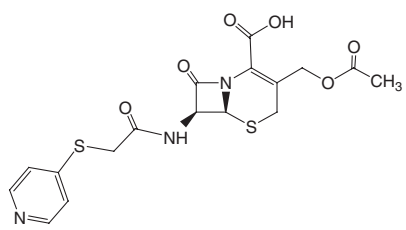
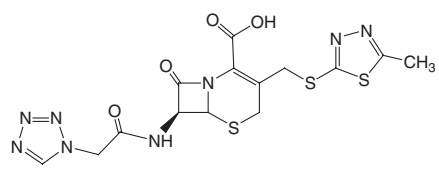
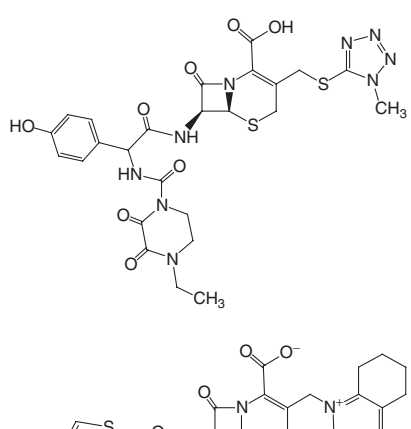
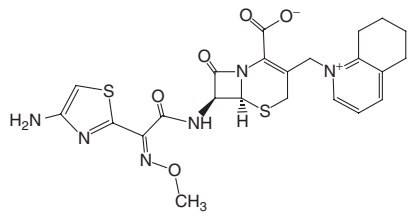
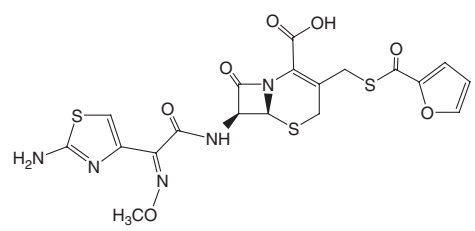
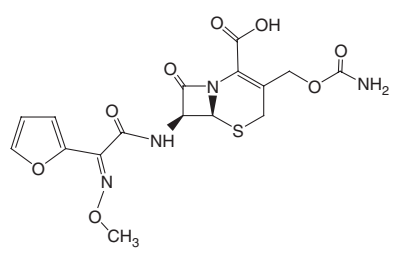
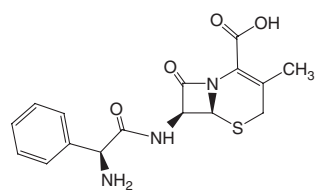
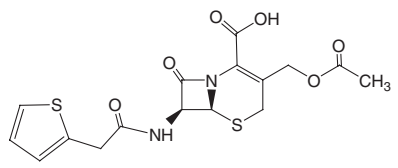
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Methicillin	(2*S*,5*R*,6*R*)-6-[(2,6-Dimethoxybenzoyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{17}H_{20}N_2O_6S$ 61-32-5		HA 2.8[56]
Nafcillin	2*S*,5*R*,6*R*)-6-[(2-Ethoxynaphthalene-1-carbonyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{21}H_{22}N_2O_5S$ 985-16-0		HA 2.7[56]
Oxacillin	(2*S*,5*R*,6*R*)-3,3-Dimethyl-6-[(5-methyl-3-phenyl,1,2-oxazole-4-carbonyl)amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{19}H_{19}N_3O_5S$ 66-79-5		HA 2.7[56]
Penethamate	(2*S*,5*R*)-3,3-Dimethyl-7-oxo-6α-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2β-carboxylic acid 2-(diethylamino)ethyl ester; (6α-[(phenylacetyl)amino]penicillanic acid 2-(diethylamino)ethyl)ester $C_{22}H_{31}N_3O_4S$ 3689-73-4		N/A[a]
Phenoxymethyl penicillin (penicillin V)	(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[[2-(phenoxy)acetyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{16}H_{18}N_2O_5S$ 87-08-1		HA 2.7[56]
Temocillin	(2*S*,5*R*,6*S*)-6-[(Carboxy-3-thienylacetyl)amino]-6-methoxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{16}H_{18}N_2O_7S_2$ 66148-78-5		N/A[a]
Ticarcillin	(2*S*,5*R*,6*R*)-6-[[(2*R*)-3-Hydroxy-3-oxo-2-thiophen-3-ylpropanoyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_{15}H_{16}N_2O_6S_2$ 34787-01-4		HA 2.9,[56] HB^+ 3.3[56]

TABLE 1.3 (*Continued*)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
	β-Lactamase Inhibitors		
Clavulanic acid	[2*R*-(2α,3*Z*,5α)]-3-(2-Hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid $C_8H_9NO_5$ 58001-44-8		2.7[74]
	Cephalosporins		
Cefacetrile	(6*R*,7*R*)-3-(Acetyloxymethyl)-7-[(2-cyanoacetyl)amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid $C_{13}H_{13}N_3O_6S$ 10206-21-0		HA 2.0[56]
Cefalonium	(6*R*,7*R*)-3-[(4-Carbamoylpyridin-1-ium-1-yl)methyl]-8-oxo-7-[(2-thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate $C_{20}H_{18}N_4O_5S_2$ 5575-21-3		N/A[a]
Cefaprin (cephapirin)	(6*R*,7*R*)-3-(Acetyloxymethyl)-8-oxo-7-[(2-pyridin-4-ylsulfanylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid $C_{17}H_{17}N_3O_6S_2$ 21593-23-7		HA 1.8,[56] HB$^+$ 5.6[56]
Cefazolin	(7*R*)-3-[(5-Methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid $C_{14}H_{14}N_8O_4S_3$ 25953-19-9		HA 2.8[56]
Cefoperazone	(6*R*,7*R*)-7-[[2-[(4-Ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-(4-hydroxyphenyl)acetyl]amino]-3-[(1-methyltetrazol-5-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid $C_{25}H_{27}N_9O_8S_2$ 62893-19-0		HA 2.6[56]
Cefquinome	1-[[(6*R*,7*R*)-7-[[(2*Z*)-(2-Amino-4-thiazolyl)-(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-en-3-yl]methyl]-5,6,7,8-tetrahydroquinolinium inner salt $C_{23}H_{24}N_6O_5S_2$ 84957-30-2		N/A[a]

(*continued*)

TABLE 1.3 (*Continued*)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Ceftiofur	(6*R*,7*R*)-7-[[(2*Z*)-(2-Amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[(2-furanylcarbonyl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid $C_{19}H_{17}N_5O_7S_3$ 80370-57-6		N/A[a]
Cefuroxime	(6*R*,7*R*)-3-(Carbamoyloxymethyl)-7-[[(2*E*)-2-furan-2-yl-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid $C_{16}H_{16}N_4O_8S$ 55268-75-2		HA 2.5[56]
Cephalexin	(6*R*,7*R*)-7-[[(2*R*)-2-Amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid $C_{16}H_{17}N_3O_4S$ 15686-71-2		HA 2.5,[56] HB^+ 7.1[56]
Cephalothin	(6*R*,7*R*)-3-(Acetyloxymethyl)-8-oxo-7-[(2-thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid $C_{16}H_{16}N_2O_6S_2$ 153-61-7		HA 2.4[56]

[a]The author was not able to find a pK_a value for the substance in the public literature (N/A = data not available).

1.3.3 Quinoxalines

The quinoxaline-1,4-di-*N*-oxides were originally investigated for potential antagonism to vitamin K activity. Quindoxin (quinoxaline-1,4-dioxide) was later used as a growth promoter in animal husbandry before being withdrawn because of its photoallergic properties. In the 1970s, three synthetic derivatives of quindoxin—carbadox, cyadox, and olaquindox—became available as antimicrobial growth promoters. These substances are active against Gram-positive and some Gram-negative bacteria as well as some chlamydiae and protozoa. Their antimicrobial activity is attributed to the inhibition of DNA synthesis by a mechanism that is not completely understood. On the basis of studies conducted in *E. coli*, Suter et al.[75] postulated that free radicals produced by the intracellular reduction of quinoxalines damage existing DNA and inhibit the synthesis of new DNA. Resistance to olaquindox has been reported in *E. coli* to be *R*-plasmid-mediated.

Carbadox is well absorbed when administered as a feed additive to pigs. Nonetheless, concentrations of carbadox in the stomach and duodenum of pigs following in-feed administration of 50 mg/kg are adequate to provide effective prophylaxis against *Brachyspira hyodysenteriae*, the causative agent in swine dysentery.[76] The major metabolites of carbadox are its aldehyde, desoxycarbadox, and quinoxaline-2-carboxylic acid. Urinary excretion accounts for two-thirds of a carbadox dose within 24 h of administration. Olaquindox is rapidly and extensively absorbed following oral administration to pigs and undergoes oxidative and/or reductive metabolism. Urinary excretion of unchanged olaquindox and a mono-*N*-oxide of olaquindox accounts for approximately 70% and 16%, respectively, of a dose within 24 h of administration.

Van der Molen et al.[77] and Nabuurs et al.[78] investigated the toxicity of quinoxalines in pigs. A dose of 50 mg/kg carbadox was demonstrated to cause increased fecal dryness, reduced appetite, dehydration, and disturbances in electrolyte homeostasis. These signs are attributable principally

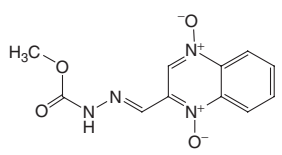
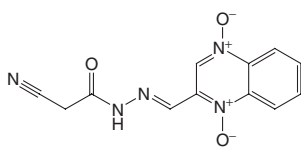
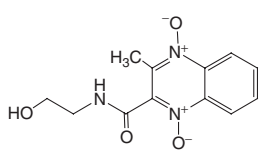
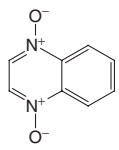
to hypoaldosteronism, a manifestation of carbadox-induced damage of the adrenal glands. The accidental feeding of high doses (331–363 mg/kg) of carbadox to weaner pigs resulted in inappetance, ill thrift, posterior paresis, and deaths.[79] The toxic effect of olaquindox is comparable with that of carbadox, whereas cyadox is less toxic.

Carbadox is used in feed at a dose of 10–25 mg/kg as an antimicrobial growth-promoting agent for improving weight gain and feed efficiency in pigs. The commercial product is used in starter and/or grower rations but not in finisher rations. A dose of 50–55 mg/kg carbadox is administered as a feed additive for the prevention and control of (1) swine dysentery caused by the anaerobic intestinal spirochaetal bacterium, *Brachyspira hyodysenteriae* and (2) bacterial enteritis caused by susceptible organisms. Carbadox is also used in pigs to treat nasal infections caused by *Bordetella bronchiseptica*. Olaquindox is administered as medicated feed to pigs for improving feed conversion efficiency and for the prevention of porcine proliferative enteritis caused by *Campylobacter* species. Cyadox has been used as a feed additive for pigs, calves, and poultry to promote growth.

Occupational exposure of farmworkers to the quinoxaline class of antimicrobials may result in dermal photosensitivity reactions. In general terms, photosensitivity may take the form of phototoxic reactions, whereby a drug absorbs energy from ultraviolet A light and releases it into the skin, causing cellular damage; or photoallergic reactions, whereby light causes a structural change in a drug so that it acts as a hapten, possibly binding to proteins in the skin. Olaquindox causes photoallergic reactions in humans and animals. On exposure to light, olaquindox forms a reactive oxaziridine derivative, and this imino-*N*-oxide reacts with protein to form a photoallergen. In 1999, the use of carbadox and olaquindox was banned in the European Union in response to concerns of toxicity to humans from occupational exposure.[80] More recently, the health concerns with carbadox and olaquindox identified by the JECFA were noted at the 18th Session of the CCRVDF, as was the ongoing use of these substances in some countries.[81]

In addition to the concerns relating to occupational exposure described above, the use of quinoxalines (see list in Table 1.4) in food-producing species is associated with food safety concerns. The genotoxic and carcinogenic nature of carbadox and its metabolites and the presence of relatively persistent residues in edible tissues of pigs treated with carbadox resulted in the JECFA not allocating an acceptable daily intake (ADI).[82,83] In the case of olaquindox, the JECFA[84] concluded that the substance is potentially genotoxic and that the toxicity of its metabolites is inadequately understood. For these reasons, the JECFA was unable to determine the amount of residues in food that did not cause an appreciable risk to human health, and thus MRLs were not established for these compounds by the CAC (see Chapter 3 for further discussion). Details

TABLE 1.4 Quinoxalines

INN	IUPAC Name, Molecular Formula, CAS Registry No.	Chemical Structure	pK_a
Carbadox	Methyl (2*E*)-2-[(1,4-dioxidoquinoxalin-2-yl)-methylene]hydrazine carboxylate $C_{11}H_{10}N_4O_4$ 6804-07-5		N/A[a]
Cyadox	2-Cyano-*N*-[(*E*)-(1-hydroxy-4-oxido-quinoxalin-2-ylidene)methyl]iminoacetamide $C_{12}H_9N_5O_3$ 65884-46-0		N/A[a]
Olaquindox	*N*-(2-Hydroxyethyl)-3-methyl-4-oxido-1-oxoquinoxalin-1-ium-2-carboxamide $C_{12}H_{13}N_3O_4$ 23696-28-8		N/A[a]
Quindoxin	Quinoxaline-1,4-dioxide $C_8H_6N_2O_2$ 2423-66-7		N/A[a]

[a]The author was not able to find a pK_a value for the substance in the public literature.

of residue studies on olaquindox reviewed by JECFA are available in monographs prepared for the 36th[85] and 42nd[86] meetings of the committee.

1.3.4 Lincosamides

The lincosamide class of antimicrobial drugs includes lincomycin, clindamycin, and pirlimycin; two of these drugs—lincomycin and pirlimycin—are approved for use in food-producing species. Lincosamides are derivatives of an amino acid and a sulfur-containing galactoside. Lincomycin was isolated in 1962 from the fermentation product of *Streptomyces lincolnensis* subsp. *lincolnensis*. Clindamycin is a semi-synthetic derivative of lincomycin, and pirlimycin is an analog of clindamycin.

The lincosamides inhibit protein synthesis in susceptible bacteria by binding to the 50*S* subunits of bacterial ribosomes and inhibiting peptidyltransferases; interference with the incorporation of amino acids into peptides occurs thereby. Lincosamides may be bacteriostatic or bactericidal depending on the concentration of drug at the infection site, bacterial species and bacterial strain. These drugs have activity against many Gram-positive bacteria and most obligate anaerobes but are not effective against most Gram-negative organisms. Clindamycin, which is not approved for use in food-producing animals, has a wider spectrum of activity than does lincomycin.

Resistance specific to lincosamides results from the enzymatic inactivation of these drugs. More common, however, is cross-resistance among macrolides, lincosamides, and streptogramin group B antibiotics (MLSB resistance). With this form of resistance, binding of the drug to the target is prevented on account of methylation of the adenine residues in the 23*S* ribosomal RNA of the 50*S* ribosomal subunit (the target).[87] Complete cross-resistance between lincomycin and clindamycin occurs with both forms of resistance.

Lincomycin is effective against *Staphylococcus* species, *Streptococcus* species (except *Streptococcus faecalis*), *Erysipelothrix insidiosa, Leptospira pomona*, and *Mycoplasma* species. Lincomycin hydrochloride is added to feed or drinking water to treat and control swine dysentery in pigs and to control necrotic enteritis in chickens. It is used also in medicated feed for growth promotion and to increase feed efficiency in chickens and pigs, the control of porcine proliferative enteropathies caused by *Lawsonia intracellularis* in pigs, and the treatment of pneumonia caused by *Mycoplasma* species in pigs. An injectable formulation of lincomycin is used in pigs to treat joint infections and pneumonia.

Several combination products containing lincomycin are approved for use in food-producing species. A lincomycin–spectinomycin product administered in drinking water is used for the treatment and control of respiratory disease and for improving weight gains in poultry. A product containing the same active ingredients is available for in-feed or drinking water administration to pigs for the treatment and control of enteric and respiratory disease, treatment of infectious arthritis, and increasing weight gain. Injectable combination products containing lincomycin and spectinomycin are used for the treatment of bacterial enteric and respiratory disease in pigs and calves, treatment of arthritis in pigs, and treatment of contagious foot-rot in sheep. A lincomycin–sulfadiazine combination product administered in-feed is used for the treatment of atrophic rhinitis and enzootic pneumonia in pigs. Lincomycin–neomycin combination products are used for treating acute mastitis in lactating dairy cattle.

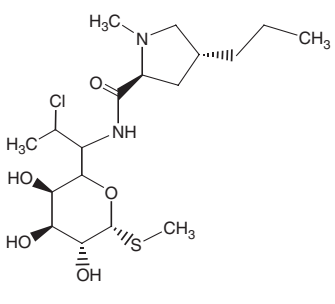
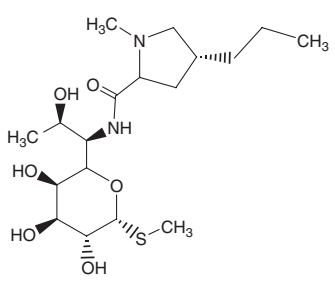
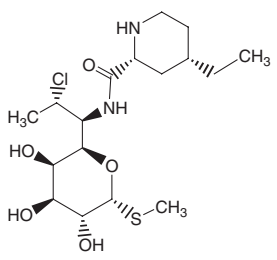
Pirlimycin is approved as an intramammary infusion for the treatment of mastitis in lactating dairy cattle. It is active against sensitive organisms such as *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae*, and some enterococci. Pirlimycin exhibits a post-antibiotic effect *in vitro* against *Staphylococcus aureus* isolated from bovine mastitis, and exposure of pathogens to subinhibitory concentrations increases their susceptibility to phagocytosis by polymorphonuclear leukocytes. Many species of anaerobic bacteria are extremely sensitive to pirlimycin.

The use of lincosamides (see list in Table 1.5) is contraindicated in horses because of the potential risk of serious or fatal enterocolitis and diarrhea. This commonly involves overgrowth of the normal microflora by nonsusceptible bacteria such as *Clostridium* species. Oral administration of lincomycin to ruminants has also been associated with adverse side effects such as anorexia, ketosis, and diarrhea. Such use is therefore contraindicated in ruminants.

The limited information available suggests that lincomycin does not pose a risk to organisms in those environments where the drug is known to be used. A 2006 UK study that used targeted monitoring detected a maximum concentration of 21.1 μg lincomycin per liter of streamwater, which compares with the predicted no-effect concentration for lincomycin of 379.4 μg per liter.[88]

From a food safety perspective, the JECFA has allocated ADI values for lincomycin[89] and pirlimycin.[89] On the basis of JECFA recommendations, CAC MRLs for lincomycin in muscle, liver, kidney, and fat of pigs and chickens, and in cow's milk and for pirlimycin in muscle, liver, kidney, and fat of cattle and in cow's milk have also been established.[43] Details of residue studies reviewed by JECFA to develop MRL recommendations for CCRVDF may be found in monographs published for lincomycin[91–93] and pirlimycin.[94]

TABLE 1.5 Lincosamides

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Clindamycin	(2*S*,4*R*)-*N*-[2-chloro-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-methyl-sulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide $C_{18}H_{33}ClN_2O_5S$ 18323-44-9		HB^+ 7.7[56]
Lincomycin	(4*R*)-*N*-[(1*R*,2*R*)-2-hydroxy-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-methylsulfanyloxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide $C_{18}H_{34}N_2O_6S$ 154-21-2		HB^+ 7.5[56]
Pirlimycin	Methyl(2*S*-*cis*)-7-chloro-6,7,8-trideoxy-6[[(4-ethyl-2-piperidinyl)-carbonyl]amino]-1-thio-L-*threo*-α-D-galactooctopyranoside $C_{17}H_{31}ClN_2O_5S$ 79548-73-5		8.5[74]

1.3.5 Macrolides and Pleuromutilins

The macrolide class of antibiotics consists of natural products isolated from fungi and their semi-synthetic derivatives. The macrolide structure is characterized by a 12–16-atom lactone ring; however, none of the 12-member ring macrolides are used clinically. Erythromycin and oleandomycin are 14-member ring macrolides derived from strains of *Saccharopolyspora erythreus* (formerly *Streptomyces erythreus*) and *Streptomyces antibioticus*, respectively. Clarithromycin and azithromycin are semi-synthetic derivatives of erythromycin. Spiramycin and tylosin are 16-member ring macrolides derived from strains of *Ambofaciens streptomyces* and the actinomycete *Streptomyces fradiae*, respectively. Tilmicosin is a 16-member ring macrolide produced semi-synthetically by chemical modification of desmycosin. Tulathromycin, a semi-synthetic macrolide, is a mixture of a 13-member ring macrolide (10%) and a 15-member ring macrolide (90%) (shown in Table 1.6). Macrolide drugs are complex mixtures of closely related antibiotics that differ from one another with respect to the chemical substitutions on the various carbon atoms in the structure, and in the aminosugars and neutral sugars. Erythromycin, for example, consists primarily of erythromycin A (shown in Table 1.6), but the B, C, D, and E forms may also be present. It was not until 1981 that erythromycin A was chemically synthesized. Two pleuromutilins, tiamulin and valnemulin, are used in animals, and these compounds are semi-synthetic derivatives of the naturally occurring diterpene antibiotic, pleuromutilin.

The antimicrobial activity of the macrolides is attributed to the inhibition of protein synthesis. Macrolides bind to the 50*S* subunit of the ribosome, resulting in blockage of the transpeptidation or translocation reactions, inhibition of protein synthesis, and thus the inhibition of cell growth. These drugs are active against most aerobic and anaerobic Gram-positive bacteria, Gram-negative cocci, and also *Haemophilus, Actinobacillus, Bordetella, Pasteurella, Campylobacter*, and *Helicobacter*. However, they are not active against most Gram-negative bacilli. The macrolides display activity against atypical mycobacteria, mycobacteria, mycoplasma, chlamydia, and rickettsia species. They are predominantly bacteriostatic, however, high concentrations are slowly bactericidal against more sensitive organisms. In human medicine, erythromycin,

TABLE 1.6 Macrolides and Pleuromutilins

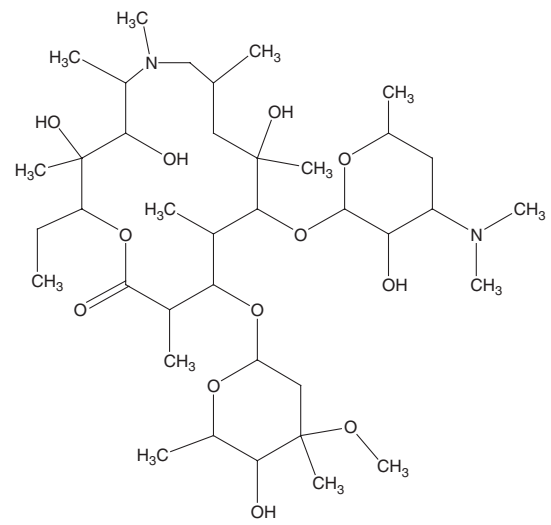
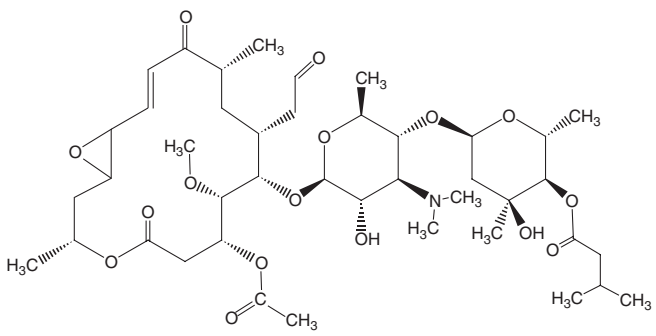
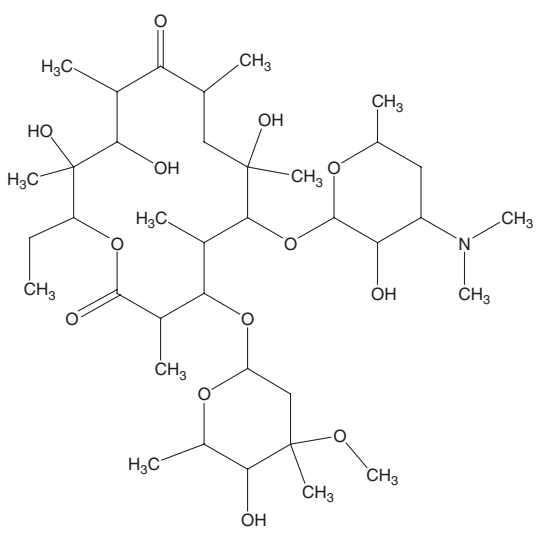
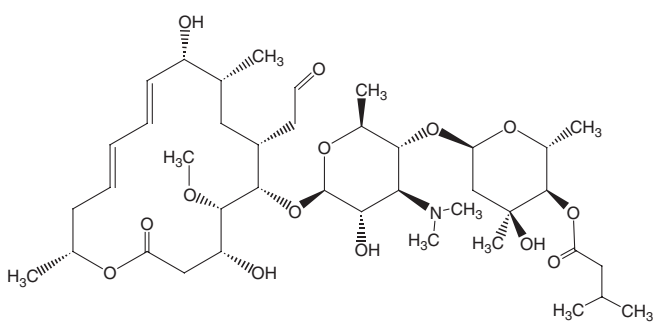
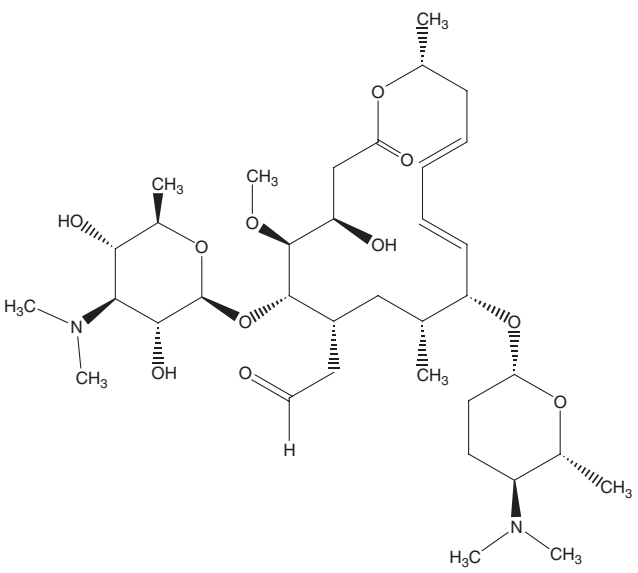
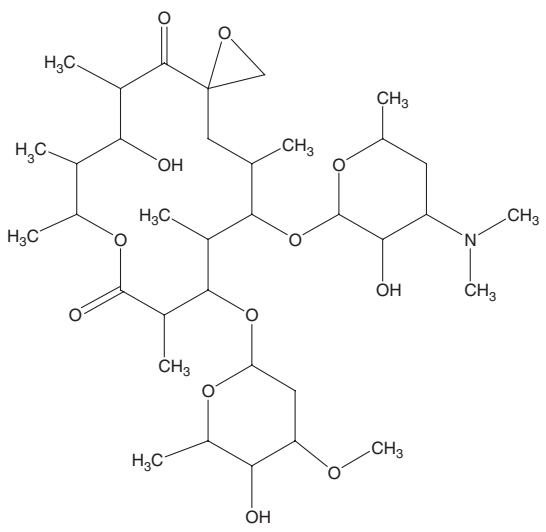
INN	IUPAC Name Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
		Macrolides	
Azithromycin	[2*R*-(2*R**,3*S**,4*R**,5*R**,8*R**,10*R**,11*R**,12*S**,13*S**,14*R**)]-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribohexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylohexopyranosyl]oxy]1-oxa-6-azacyclopentadecan-15-one $C_{38}H_{72}N_2O_{12}$ 83905-01-5		HB^+ 8.7,[56] HB^+ 9.5[56]
Carbomycin	[(2*S*,3*S*,4*R*,6*S*)-6-[(2*R*,3*S*,4*R*,5*R*,6*S*)-6-[[(3*R*,7*R*,8*S*,9*S*,10*R*,12*R*,14*E*)-7-acetyloxy-8-methoxy-3,12-dimethyl-5,13-dioxo-10-(2-oxoethyl)-4,17-dioxabicyclo[14.1.0]heptadec-14-en-9-yl]oxy]-4-(dimethylamino)-5-hydroxy-2-methyloxan-3-yl]oxy-4-hydroxy-2,4-dimethyloxan-3-yl]3-methylbutanoate $C_{42}H_{67}NO_{16}$ 4564-87-8		HB^+ 7.6[56]
Erythromycin A	(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-Dimethylamino-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione $C_{37}H_{67}NO_{13}$ 114-07-8		HB^+ 8.6[56]

TABLE 1.6 (***Continued***)

INN	IUPAC Name Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Kitasamycin (Leucomycin A_1)	[(2*S*,3*S*,4*R*,6*S*)-6-[(2*R*,3*S*,4*R*,5*R*,6*S*)-6-[[(4*R*,5*S*,6*S*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-4-acetyloxy-10-Hydroxy-5-methoxy-9,16-dimethyl-2-oxo-7-(2-oxoethyl)-1-oxacyclohexadeca-11,13-dien-6-yl]oxy]-4-dimethylamino-5-hydroxy-2-methyloxan-3-yl]oxy-4-hydroxy-2,4-dimethyloxan-3-yl]-3-methylbutanoate $C_{40}H_{67}NO_{14}$ 1392-21-8		N/A[a]
Neospiramycin	2-[(1*R*,3*R*,4*R*,5*E*,7*E*,10*R*,14*R*,15*S*,16*S*)-16-[(2*S*,3*R*,4*S*,5*S*,6*R*)-4-(Dimethylamino)-3,5-dihydroxy-6-methyloxan-2-yl]oxy-4-[(2*r*,5*s*,6*r*)-5-(dimethylamino)-6-methyloxan-2-yl]oxy-14-hydroxy-15-methoxy-3,10-dimethyl-12-oxo-11-oxacyclohexadeca-5,7-dien-1-yl]acetaldehyde $C_{36}H_{62}N_2O_{11}$ 102418-06-4		N/A[a]
Oleandomycin	(3*R*,5*R*,6*S*,7*R*,8*R*,11*R*,12*S*,13*R*,14*S*,15*S*)-14-((2*S*,3*R*,4*S*,6*R*)-4-(Dimethylamino)-3-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yloxy)-6-hydroxy-12-((2*R*,4*S*,5*S*,6*S*)-5-hydroxy-4-methoxy-6-methyltetrahydro-2*H*-pyran-2-yloxy)-5,7,8,11,13,15-hexamethyl-1,9-dioxaspiro[2.13]hexadecane-4,10-dione $C_{35}H_{61}NO_{12}$ 3922-90-5		HB$^+$ 8.5[56]

(*continued*)

TABLE 1.6 (*Continued*)

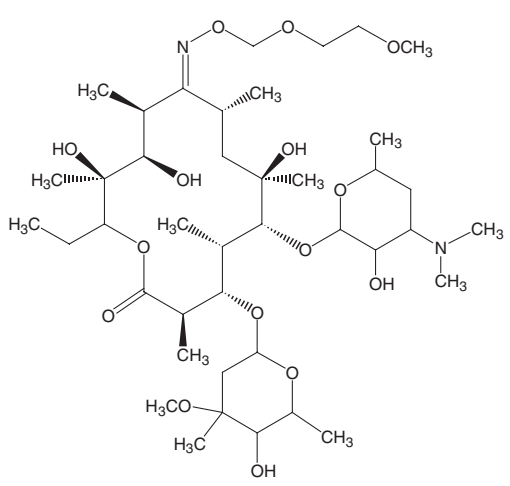
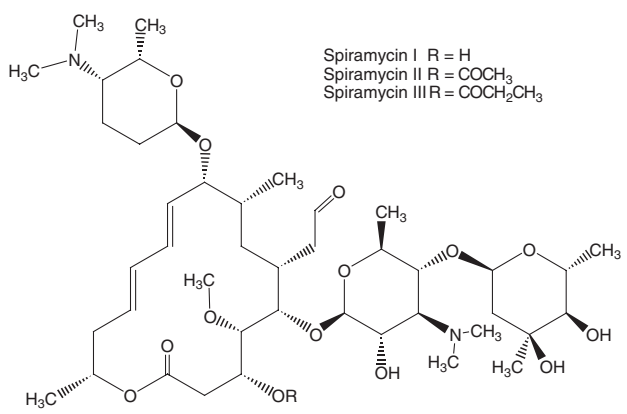
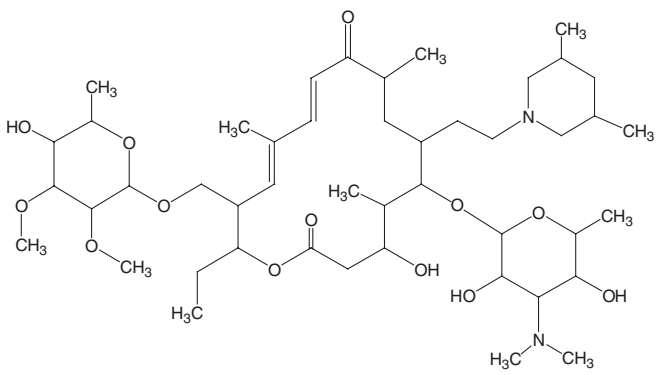
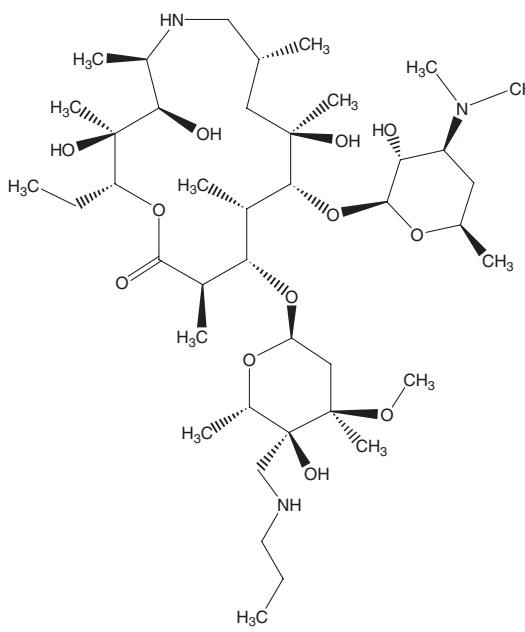
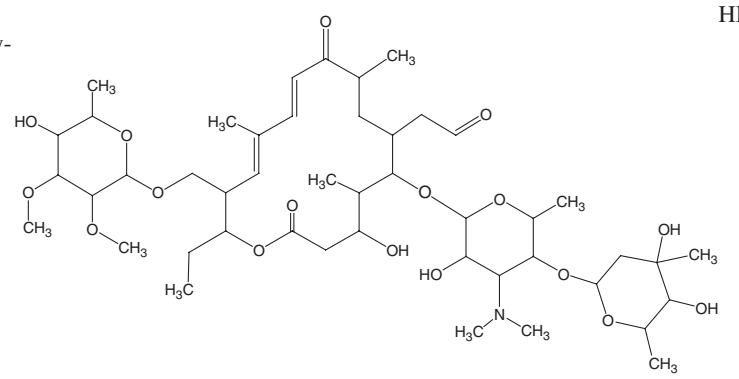
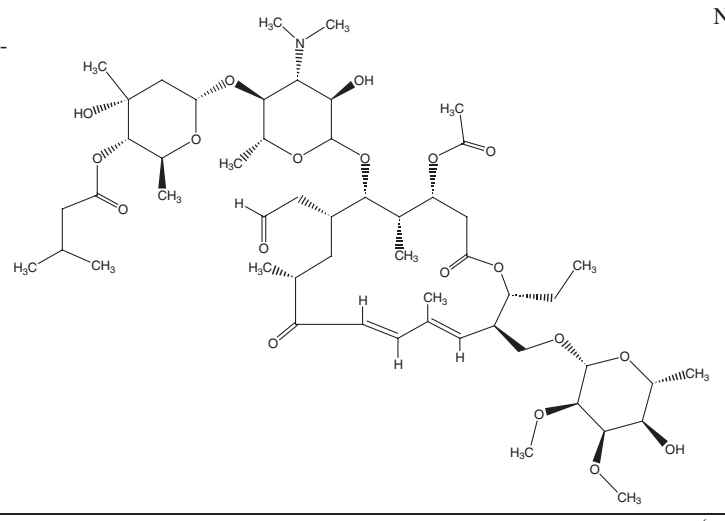
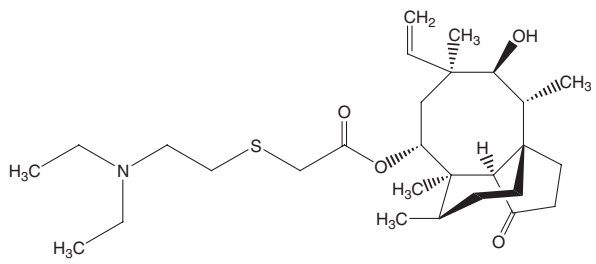
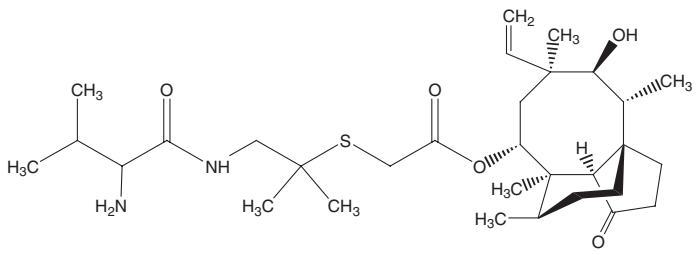
INN	IUPAC Name Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Roxithromycin	(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*)-6-[(2*S*,3*R*,4*S*,6*R*)-4-Dimethylamino-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-10-(2-methoxyethoxy-methoxyimino)-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecan-2-one $C_{41}H_{76}N_2O_{15}$ 80214-83-1		N/A[a]
Spiramycin	(4*R*,5*S*,6*R*,7*R*,9*R*,10*R*,11*E*,13*E*,16*R*)-10-{[(2*R*,5*S*,6*R*)-5-(Dimethylamino)-6-methyltetrahydro-2*H*-pyran-2-yl]oxy}-9,16-dimethyl-5-methoxy-2-oxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-6-yl 3,6-dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-(dimethylamino)-α-D-glucopyranoside $C_{43}H_{74}N_2O_{14}$ (spiramycin I) 8025-81-8		8.2[15]
Tilmicosin	(10*E*,12*E*)-(3*R*,4*S*,5*S*,6*R*,8*R*,14*R*,15*R*)-14-(6-Deoxy-2,3-di-*O*-methyl-*b*-*d*-*allo*-hexopyranosyoxymethyl)-5-(3,6-dideoxy-3-dimethylamino-*b*-*d*-glucohexapyranosyloxy)-6-[2-(*cis*-3,5-dimethylpiperidino)ethyl]-3-hydroxy-4,8,12-trimethyl-9-oxoheptadeca-10,12-dien-15-olide $C_{46}H_{80}N_2O_{13}$ 108050-54-0		HB^+ 8.2,[56] HB^+ 9.6[56]

TABLE 1.6 (***Continued***)

INN	IUPAC Name Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Tulathromycin	(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[[2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-4-*C*-[(propylamino)methyl]-α-L-ribo-hexopyrano-syl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylohexopyranosyl]-oxy]-1-oxa-6-azacyclopentadecan-15-one $C_{41}H_{79}N_3O_{12}$ 217500-96-4		8.5[120] 9.3[120] 9.8[120] (90% isomer A)
Tylosin	[(2*R*,3*R*,4*E*,6*E*,9*R*,11*R*,12*S*,13*S*,14*R*)-12-{[3,6-Dideoxy-4-*O*-(2,6-dideoxy-3-*C*-methyl-α-L-ribo-hexopyranosyl)-3-(dimethylamino)-β-D-glucopyranosyl]oxy}-2-ethyl-14-hydroxy-5, 9,13-trimethyl-8, 16-dioxo-11-(2-oxoethyl)oxacyclohexadeca-4,6-dien-3-yl]methyl 6-deoxy-2,3-di-*O*-methyl-β-D-allopyranoside $C_{46}H_{77}NO_{17}$ 1401-69-0		HB^+ 7.7[56]
Tylvalosin (acetyliso-valeryltylosin)	(4*R*,5*S*,6*S*,7*R*,9*R*,11*E*,13*E*,15*R*,16*R*)-15-{[(6-Deoxy-2,3-di-*O*-methyl-β-D-allopyranosyl)oxy]methyl}-6-({3,6-dideoxy-4-*O*-[2,6-dideoxy-3-*C*-methyl-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl]-3-(dimethylamino)-β-D-glucopyranosyl}oxy)-16-ethyl-5,9,13-trimethyl-2,10-dioxo-7-(2-oxoethyl)oxacyclohexadeca-11,13-dien-4-yl acetate (2*R*,3*R*)-2,3-dihydroxybutanedioate $C_{53}H_{87}NO_{19}$ 63409-12-1		N/A[a]

(*continued*)

TABLE 1.6 (*Continued*)

INN	IUPAC Name Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
		Pleuromutilins	
Tiamulin	(4*R*,5*S*,6*S*,8*R*,9a*R*,10*R*)-5-Hydroxy-4,6,9,10-tetramethyl-1-oxo-6-vinyldecahydro-3a,9-propano-cyclopenta[8]annulen-8-yl {[2-(diethylamino)ethyl] sulfanyl}acetate $C_{28}H_{47}NO_4S$ 55297-95-5		7.6[87]
Valnemulin	(3a*S*,4*R*,5*S*,6*S*,8*R*,9*R*,9a*R*,10*R*)-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro-3a,9-propano-3a*H*-cyclopenta[8]annulen-8-yl-[(*R*)-2-(2-amino-3-methylbutanoylamino)-1,1-dimethtylethylsulfanyl]acetate $C_{31}H_{52}N_2O_5S$ 101312-92-9		N/A[a]

[a]The author was not able to find a pK_a value for the substance in the public literature.

which is the most widely used of the macrolide class of antimicrobials, is used as an alternative to penicillin in many infections, especially in patients who are allergic to penicillin. Macrolides are significantly more active at higher pH ranges (pH 7.8–8.0).

Bacterial resistance to macrolides results from alterations in ribosomal structure with loss of macrolide binding affinity. The structural alteration very often involves methylation of ribosomal RNA and is attributed to enzymatic activity expressed by plasmids. Cross-resistance between macrolides, lincosamides, and streptogramins occurs as a result of these drugs sharing a common binding site on the ribosome.

Macrolides are used in a variety of dosage forms, including medicated feed, a water-soluble powder for the addition to drinking water, tablets, and injections for the treatment of systemic and local infections in animals. Erythromycin and/or tylosin are indicated for the prophylaxis of hepatic abscesses and the treatment of diphtheria, metritis, bacterial pneumonia, pododermatitis, and bovine respiratory disease in cattle. These drugs are also used in pigs for the prophylaxis and treatment of atrophic rhinitis, infectious arthritis, enteritis, erysipelas, respiratory syndrome, and bacterial respiratory infections, and in farrowing sows for leptospirosis. Erythromycin is indicated for the prophylaxis of enterotoxemia in lambs, while erythromycin and tylosin are used in the treatment of pneumonia and upper respiratory disease in sheep. Erythromycin is administered to chickens and turkeys for the prophylaxis of infectious coryza, chronic respiratory disease, and infectious synovitis, and to turkeys for the treatment of enteritis. Tylosin is approved in the United States for the control of American foulbrood disease in honeybees. This drug is also used in some countries to improve feed efficiency in pigs and chickens. Erythromycin is used for the treatment of *Campylobacter* enteritis and pyoderma in dogs. Although erythromycin is used in the treatment of pneumonia caused by *Rhodococcus equi* in foals, azithromycin combined with rifampicin is now more commonly used.

As mentioned above, two pleuromutilins are used in veterinary medicine. Tiamulin is available as a pre-mix and a water-soluble powder for addition to drinking water for pigs and poultry, and as an injection for pigs. It is indicated for the prophylaxis and treatment of dysentery, pneumonia, and mycoplasmal infections in pigs and poultry. In the European Union (EU), valnemulin is approved for oral administration in the treatment and prevention of swine enzootic pneumonia, swine dysentery, and proliferative ileitis in pigs.

Although the incidence of serious adverse effects to the macrolides is relatively low in animals, notable reactions do occur with some formulations and in certain animal species. For example, the irritancy of some parenteral

formulations causes severe pain on intramuscular injection, thrombophlebitis at the injection site after intravenous injection, and inflammatory reactions following intramammary infusion. Macrolide-induced gastrointestinal disturbances have occurred in most species but are more serious in horses. Dosing horses with erythromycin, for instance, has resulted in fatalities from enterocolitis caused by *Clostridium difficile*.

Reports of macrolides causing adverse reactions in humans relate primarily to medicated stockfeed and parenteral formulations for injection. Farmworkers exposed to stockfeed medicated with spiramycin and tylosin have developed dermatitis and bronchial asthma.[95] In addition, accidental needlesticks with needles contaminated with tilmicosin have caused minor local reactions,[96] whereas accidental self-administration of injectable formulations of tilmicosin has resulted in serious cardiac effects and death.[97–99]

Some of the macrolides used in veterinary medicine have been detected at trace levels in the environment.[100] An investigation into the sorption behavior of a range of veterinary drugs found tylosin to be slightly mobile and slightly persistent in soil, whereas erythromycin was nonmobile and persistent.[101] Macrolides have also been shown to rapidly dissipate in a range of manure types.[102–104]

The JECFA has allocated ADIs for erythromycin,[105] spiramycin,[106] tilmicosin,[107] and tylosin,[108] with those values for erythromycin, spiramycin, and tylosin based on microbiological endpoints. The CAC also established MRLs for erythromycin in muscle, liver, kidney, and fat of chickens and turkeys, and in chicken eggs; for spiramycin in muscle, liver, kidney, and fat of cattle, pigs, and chickens; for tilmicosin in muscle, liver, kidney, fat (or fat/skin) of cattle, sheep, pigs, chickens, and turkeys; and MRLs for tylosin in muscle, liver, kidney, fat of cattle, pigs and chickens, and in chicken eggs.[43] Details of residue studies considered by JECFA are contained in monographs prepared for erythromycin,[109] spiramycin,[110–113] tilmicosin,[114,115] and tylosin.[116,117]

The properties of the macrolides from an analytical perspective were discussed in a recent review[118] and are addressed in Chapters 4–6. Some of the macrolides are pH-sensitive and degrade under acidic conditions.[119] For example, erythromycin is completely transformed to erythromycin-H_2O with the loss of one molecule of water at pH 4.[67] Erythromycin exists principally in the degraded form in aquatic environments and is measured as erythromycin-H_2O in environmental samples following pH adjustment to achieve total conversion of erythromycin to erythromycin-H_2O. Tylosin A is also unstable under acidic conditions, which accounts for its slow degradation to tylosin B in honey.[117]

1.3.6 Nitrofurans

Furans are five-membered ring heterocycles, and it is the presence of a nitro group in the 5 position of the furan ring that confers antibacterial activity on many 2-substituted furans. Although the use of nitrofurans in food-producing species is prohibited because of their carcinogenicity, nitrofurantoin, nitrofurazone, furazolidone, and nifuroxazide are used in small animals and horses.

The mechanism of antibacterial action of the furan derivatives is unknown. However, the reduced forms of nitrofurans are highly reactive and are thought to inhibit many bacterial enzyme systems, including the oxidative decarboxylation of pyruvate to acetylcoenzyme A. Nitrofurans (see list in Table 1.7) are bacteriostatic but, at high concentrations, can be bactericidal to sensitive organisms. Both chromosomal and plasmid-mediated mechanisms of resistance to nitrofurantoin occur, and these most commonly involve the inhibition of nitrofuran reductase.

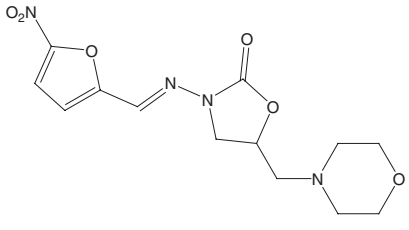
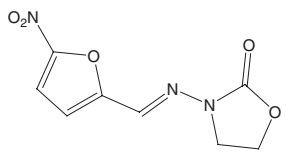
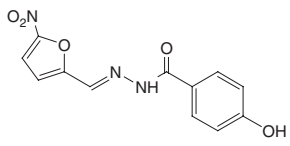
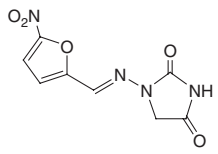
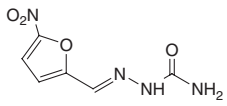
Following the administration of safe doses of nitrofurantoin, effective plasma concentrations are not achieved because of its rapid elimination, and for this reason, the drug cannot be used to treat systemic infections. However, nitrofurantoin is a useful lower-urinary-tract disinfectant in small animals and occasionally in horses. The antibacterial activity observed is attributed to approximately 40% of a dose being excreted unchanged in urine, and antibacterial activity is greater in acidic urine. Nitrofurantoin has activity against several Gram-negative and some Gram-positive organisms, including many strains of *E. coli*, *Klebsiella*, *Enterobacter*, *Enterococci*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Citrobacter*, *Salmonella*, *Shigella*, and *Corynebacterium*. It has little or no activity against most strains of *Proteus, Serratia*, or *Acinetobacter* and no activity against *Pseudomonas* species.

Nitrofurazone is used in small animals and horses as a broad-spectrum topical antibacterial agent in the prevention and treatment of bacterial skin infections and in the treatment of mixed infections in superficial wounds. It exhibits bacteriostatic activity against a variety of Gram-positive and Gram-negative microorganisms and, at high concentrations, bactericidal activity to sensitive organisms. Nitrofurazone is available as a cream, ointment, powder, soluble dressing, and topical solution. The systemic toxicity of nitrofurazone is relatively low when applied topically because absorption is not significant.

Furazolidone is occasionally used in small animals to treat enteric infections. It has activity against *Giardia, Vibrio cholera, Trichomonas*, coccidia, and many strains of *Escherichi coli, Enterobacter, Campylobacter, Salmonella*, and *Shigella*. Another nitrofuran, nifuroxazide, is used for treating acute bacterial enteritis.

There is a paucity of information describing nitrofurans in the environment. This may reflect the fact that the

TABLE 1.7 Nitrofurans

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Furaltadone	5-(4-Morpholinomethyl)-3-(5-nitro-2-furfurylideneamino)-2-oxazolidinone $C_{13}H_{16}N_4O_6$ 139-91-3		HB^+ 5.0[56]
Furazolidone	3-{[(5-Nitro-2-furyl)methylene]amino}-1,3-oxazolidin-2-one $C_8H_7N_3O_5$ 67-45-8		N/A[a]
Nifuroxazide	4-Hydroxybenzoic acid [(5-nitro-2-furanyl)methylene]hydrazide $C_{12}H_9N_3O_5$ 965-52-6		N/A[a]
Nitrofurantoin	1-[(5-Nitrofuran-2-yl)methylideneamino]imidazolidine-2,4-dione $C_8H_6N_4O_5$ 67-20-9		HA 7.0[56]
Nitrofurazone	[(5-Nitrofuran-2-yl)methylideneamino]urea $C_6H_6N_4O_4$ 59-87-0		HA 9.3[56]

[a]The author was not able to find a pK_a value for the substance in the public literature.

use of these drugs in food-producing species is prohibited and is minor in small animals and horses. Consequently, the quantities of nitrofurans released into the environment will be small or negligible. Furthermore, furazolidone is unstable on exposure to light[121] and degrades very quickly in marine aquaculture sediment.[122]

Following its evaluation, the JECFA concluded that nitrofurazone was carcinogenic but not genotoxic whereas furazolidone was a genotoxic carcinogen.[121] Consequently, JECFA did not establish ADIs, and CAC MRLs have not been established for any of the nitrofurans. The carcinogenicity of the nitrofurans has led to the prohibition of their use in food-producing species in many regions, including Australia, Canada, EU, and the United States.

1.3.7 Nitroimidazoles

The chemical synthesis and biological testing of numerous nitroimidazoles occurred following the discovery in 1955 of azomycin, a 2-nitroimidazole compound, and the demonstration of its trichomonacidal properties a year later. The trichomonacidal activity of metronidazole, a 5-nitroimidazole, was reported in 1960. The chemical synthesis of other 5-nitroimidazole compounds, including dimetridazole, ipronidazole, ronidazole, and tinidazole, followed. In addition to antiprotozoal activity, these compounds display concentration-dependent activity against anaerobic bacteria. Both activities are utilized in human and veterinary medicine, although the use of nitroimidazoles in food-producing species is prohibited in Australia, Canada, the EU, and the United States.

The antimicrobial activity of the 5-nitroimidazoles involves the reduction *in vivo* of the 5-nitro group with the formation of an unstable hydroxylamine derivative that covalently binds to various cellular macromolecules. The interaction of this unstable intermediate with DNA results in a loss of helical structure and strand breakage and, in turn, the inhibition of DNA synthesis and cell death. It is via this mechanism that nitroimidazoles display antiprotozoal activity and antibacterial activity against obligate anaerobes, including penicillinase-producing strains of *Bacteroides*. They are not effective against facultative anaerobes or obligate aerobes.

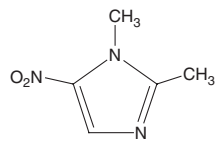
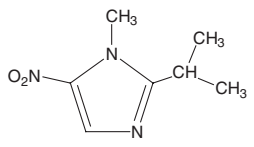
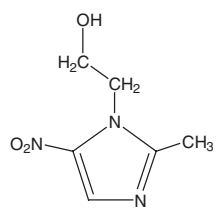
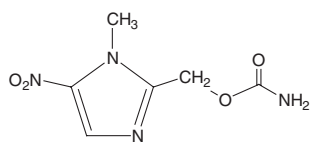
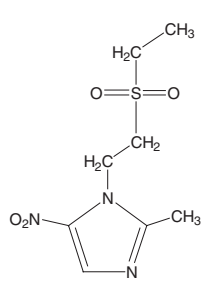
The emergence of resistance to 5-nitroimidazoles is rare. When it does emerge, resistance is generally attributed to a decrease in the reduction of the 5-nitro group to form an unstable intermediate.

Metronidazole is used in dogs, cats, horses, and birds for the treatment of protozoal infections and anaerobic bacterial infections caused by susceptible organisms. The drug is effective against *Trichomonas, Entamoeba, Giardia*, and *Balantidium* species. It is used, for example, in dogs and cats with giardiasis to eliminate the shedding of giardial cysts and treat the associated diarrhea. Metronidazole is also used for the treatment of conditions such as peritonitis, empyema, and periodontal disease caused by susceptible anaerobic bacteria, and for the prevention of infection following colonic surgery. Formulations that combine metronidazole and an antimicrobial agent active against aerobic bacteria are also available. One example is a tablet for dogs and cats that combines metronidazole and erythromycin. Oral and parenteral dosage forms of metronidazole (as the sole active ingredient) are commercially available in some countries. Dimetridazole is available as a soluble powder for administration in drinking water to birds not producing meat or eggs for human consumption, and for the control of blackhead caused by *Histomonas melagridis*.

Clinical toxicity in animals treated with metronidazole at the recommended dose rate is uncommon. However, high doses lead to neurological signs including seizures, head tilt, paresis, ataxia, vertical nystagmus, tremors, and rigidity in cats, dogs, and horses. A common occurrence in animals treated with metronidazole is the voiding of reddish brown urine. This does not require medical intervention.

Residues of nitroimidazoles in the environment have not been reported. (See list of nitroimidazoles in Table 1.8).

TABLE 1.8 Nitroimidazoles

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Dimetridazole	1,2-Dimethyl-5-nitro-1*H*-imidazole $C_5H_7N_3O_2$ 551-92-8		N/A[a]
Ipronidazole	1-Methyl-2-(1-methylethyl)-5-nitro-1*H*-imidazole $C_7H_{11}N_3O_2$ 14885-29-1		HB^+ 2.7[56]
Metronidazole	2-(2-Methyl-5-nitroimidazol-1-yl)ethanol $C_6H_9N_3O_3$ 443-48-1		HB^+ 2.6[56]
Ronidazole	1-Methyl-5-nitroimidazole-2-methanol carbamate (ester) $C_6H_8N_4O_4$ 7681-76-7		N/A[a]
Tinidazole	1-(2-Ethylsulfonylethyl)-2-methyl-5-nitroimidazole $C_8H_{13}N_3O_4S$ 19387-91-8		N/A[a]

[a]The author was not able to find a pK_a value for the substance in the public literature.

Although JECFA has not established ADI values for metronidazole, dimetridazole, or ipronidazole, they did allocate a temporary ADI for ronidazole in 1989[123] but it was withdrawn in 1995.[124]

1.3.8 Phenicols

In 1947, Ehrlich and coworkers reported the isolation of chloramphenicol (known at that time as *chloromycetin*) from *Streptomyces venezuelae*, a Gram-positive soil-dwelling actinomycete.[125] Today, the drug is produced for commercial use by chemical synthesis. Chloramphenicol was the first broad-spectrum antibiotic developed. It demonstrates a time-dependent bacterial effect and is bacteriostatic for most Gram-positive and many Gram-negative aerobic bacteria, although at higher concentrations, it can be bactericidal against some very sensitive organisms. Many strains of *Salmonella* species are susceptible to chloramphenicol, while most strains of *Pseudomonas aeruginosa* are resistant. The drug is also very effective against all obligate anaerobes and suppresses the growth of rickettsia and chlamydia species. Other members of the phenicol class are thiamphenicol and florfenicol. The antibacterial activity of thiamphenicol is less than that of chloramphenicol. The activity spectrum of florfenicol, which is not approved for use in humans, is similar to that of chloramphenicol but is more active.

The phenicols are transported into bacterial cells by passive or facilitated diffusion. They bind to the 50*S* subunit of the 70*S* bacterial ribosome and impair peptidyltransferase activity, thereby interfering with the incorporation of amino acids into newly formed peptides. Chloramphenicol also inhibits mitochondrial protein synthesis in mammalian bone marrow cells but does not significantly affect other intact cells.

Chloramphenicol is available as a bitter-tasting free base and as two esters—a neutral-tasting palmitate for oral administration and a water-soluble sodium succinate for injection. Other forms are available for topical and ophthalmic use. Chloramphenicol base is rapidly absorbed following oral administration to non-ruminant animals. In ruminants, however, reduction of the nitro moiety of chloramphenicol by ruminal microflora results in inactivation and very low bioavailability. Chloramphenicol sodium succinate may be injected intravenously or intramuscularly and is activated on hydrolysis to the free base. Chloramphenicol is un-ionized at physiological pH and is lipophilic; it readily crosses membranes. The drug is widely distributed to virtually all tissues and body fluids, including the central nervous system, cerebrospinal fluid, and the eye. The principal metabolic pathway for chloramphenicol is hepatic metabolism to the inactive metabolite, chloramphenicol glucuronide. Urinary excretion of unchanged chloramphenicol accounts for approximately 5–15% of a dose. Florfenicol also penetrates most body tissues but to a lesser extent than does chloramphenicol in the case of cerebrospinal fluid and the eye. In cattle, urinary excretion of unchanged florfenicol accounts for approximately 64% of a dose. Thiamphenicol does not undergo significant metabolism and is excreted unchanged in urine.

Chloramphenicol causes two distinct forms of toxicity in humans. The most serious form is an irreversible aplastic anaemia. This rare idiosyncratic response (the incidence is $\approx$1 : 25,000–60,000) may have an immunological component; however, the mechanism of chloramphenicol-induced aplastic anemia remains unknown. Neither a dose–response relationship nor a threshold dose for the induction of aplastic anaemia has been established. Aplastic anemia is associated with reduced numbers of erythrocytes, leukocytes, and platelets (pancytopaenia), with resultant bleeding disorders and secondary infections. The condition tends to be irreversible and fatal. By comparison, leukemia may be a sequel of hypoplastic anemia. Because thiamphenicol and florfenicol lack the *p*-nitro moiety, they do not induce irreversible aplastic anemia in humans.

The second form of chloramphenicol toxicity in humans involves dose-dependent and reversible bone marrow suppression. With this toxicity, erythroid and myeloid precursors do not mature normally, serum iron concentration is increased, and phenylalanine concentrations are decreased. These signs of toxicity usually disappear when chloramphenicol is discontinued. Chronic dosing with thiamphenicol or florfenicol may also cause dose-dependent bone marrow suppression.

Bacteria develop resistance to chloramphenicol by four main mechanisms: (1) mutation of the 50*S* ribosomal subunit; (2) decreased membrane permeability to chloramphenicol; (3) elaboration of the inactivating enzyme, chloramphenicolacetyltransferase (CAT); and (4) increased expression of efflux pumps. Mechanism 3 is the most frequent cause of resistance to chloramphenicol. It involves CAT catalyzing the covalent binding of one or two acetyl groups derived from acetyl CoA to the hydroxyl moieties on the chloramphenicol molecule. The (di)acetylated product is unable to bind to the 50*S* subunit of the 70*S* bacterial ribosome and lacks antibacterial activity. This form of resistance may involve endogenous CAT or alternatively, CAT expressed by plasmids that are transferred during bacterial conjugation. Florfenicol is less susceptible to resistance from CAT inactivation because the hydroxyl moiety is replaced with a fluorine moiety that is less susceptible to CAT inactivation. Resistance to florfenicol in Gram-negative bacteria is attributed to increased expression of efflux pumps.[126] The findings of an Australian study indicate that cross-resistance with chloramphenicol is very important. The study found that 60% of *E. coli* isolates from pigs were resistant to florfenicol when the antimicrobial was introduced onto the Australian market in 2003.[1]

It was proposed that the past use of chloramphenicol may have selected for strains carrying *cml* A gene that had persisted for more than 20 years in the absence of selection pressure (chloramphenicol was last used in food-producing species in Australia in 1982).

Chloramphenicol is used to treat a variety of local and systemic infections in small animals and horses. Its use in food-producing species is banned in most countries because of human health implications (discussed in Chapter 3). Therapeutic uses include chronic respiratory infections, bacterial meningoencephalitis, brain abscesses, ophthalmitis and intraocular infections pododermatitis, dermal infections, and otitis externa. The drug is effective against *Salmonellosis* and *Bacteroides* sepsis. Its poor efficacy against lower-urinary-tract infections reflects the small amount of unchanged drug excreted in urine. Florfenicol is an effective therapy for bovine respiratory disease in cattle caused by *Mannheimia, Pasteurella*, and *Histophilus*. The drug is also approved in some countries for use in pigs and fish. Thiamphenicol is approved for use in Europe and Japan.

The possibility of chloramphenicol detected in food samples collected in national monitoring programs in the early 2000s being attributed to environmental exposure was the subject of a 2004 review.[127] Two aspects—natural synthesis of chloramphenicol in soil and the persistence of chloramphenicol in the environment after historical veterinary use—were considered. The review found that although the possibility of food being occasionally contaminated from environmental sources could not be completely ruled out, it was highly unlikely. More recently, Berendsen and coworkers[34] reported that non-compliant residues of chloramphenicol in animal-derived food products may, in part, be due to the natural occurrence of chloramphenicol in herbs and grasses grazed by food-producing species.

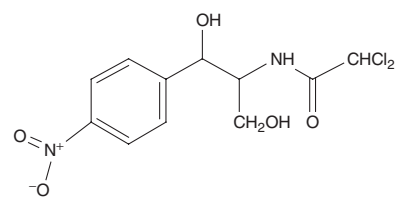
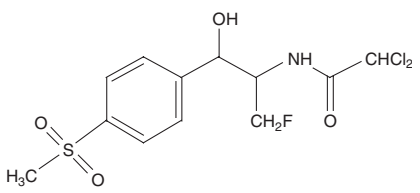
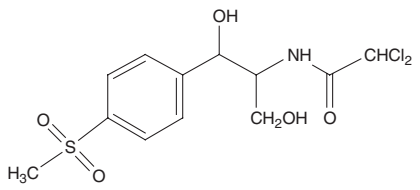
As mentioned above, in order to protect the health of consumers, few countries permit the use of chloramphenicol in food-producing animals. In addition to epidemiological studies in humans showing that treatment with chloramphenicol is associated with the induction of aplastic anemia, chloramphenicol is a genotoxin *in vivo* and may cause adverse effects in humans[127] (discussed further in Chapter 3). The use of thiamphenicol and florfenicol is permitted in food-producing species in some countries. JECFA has established an ADI for thiamphenicol[128] and recommended temporary MRLs for thiamphenicol residues that were withdrawn when additional residue data requested for evaluation were not provided.[129] Two reviews of residue studies on thiamphenicol provided for evaluation by JECFA have been published.[130,131] The CAC does not currently list MRLs for florfenicol or thiamphenicol.[43]

Properties of three phenicols are listed in Table 1.9.

1.3.9 Polyether Antibiotics (Ionophores)

The polyether ionophore class of antibiotics includes lasalocid, maduramicin, monensin, narasin, salinomycin, and semduramicin. These drugs are used exclusively in

TABLE 1.9 Phenicols

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Chloramphenicol	2,2-Dichloro-*N*-[1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide $C_{11}H_{12}Cl_2N_2O_5$ 56-75-7		N/A[a]
Florfenicol	2,2-Dichloro-*N*-[(1*S*,2*R*)-1-(fluoromethyl)-2-hydroxy-2-[4-(methylsulfonyl)phenyl]ethyl]acetamide $C_{12}H_{14}Cl_2FNO_4S$ 73231-34-2		N/A[a]
Thiamphenicol	2,2-Dichloro-*N*-{(1*R*,2*R*)-2-hydroxy-1-(hydroxymethyl)-2-[4-(methylsulfonyl)phenyl]ethyl}acetamide $C_{12}H_{15}Cl_2NO_5S$ 15318-45-3		N/A[a]

[a]The author was not able to find a pK_a value for the substance in the public literature.

veterinary medicine for their antibacterial and anticoccidial activities. The first ionophore to be discovered was lasalocid in 1951. This drug, which is a fermentation product of *Streptomyces lasaliensis*, is a divalent polyether ionophore. The discovery of monensin, a fermentation product of *Streptomyces cinnamonensis* and a monovalent polyether ionophore, followed in 1967. The discoveries of salinomycin, a fermentation product of *Streptomyces albus* and its methyl analoge, narasin, a fermentation product of *Streptomyces aureofaciens*, were reported in 1972 and 1975, respectively. Both salinomycin and narasin are monovalent polyether ionophores. Maduramicin, a fermentation product of *Actinomadura yumaense*, and semduramicin, a fermentation product of *Actinomadura roseorufa*, discovered in 1983 and 1988, respectively, are monovalent monoglycoside polyether ionophores.

Polyether ionophores (Table 1.10) have a distinctly different mode of action from therapeutic antibiotics.

TABLE 1.10 Polyether Antibiotics (Ionophores)

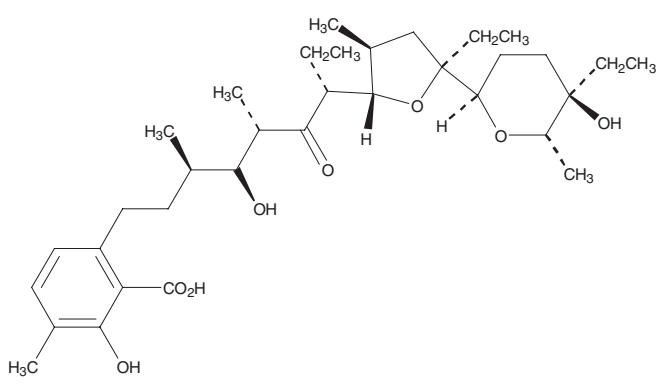
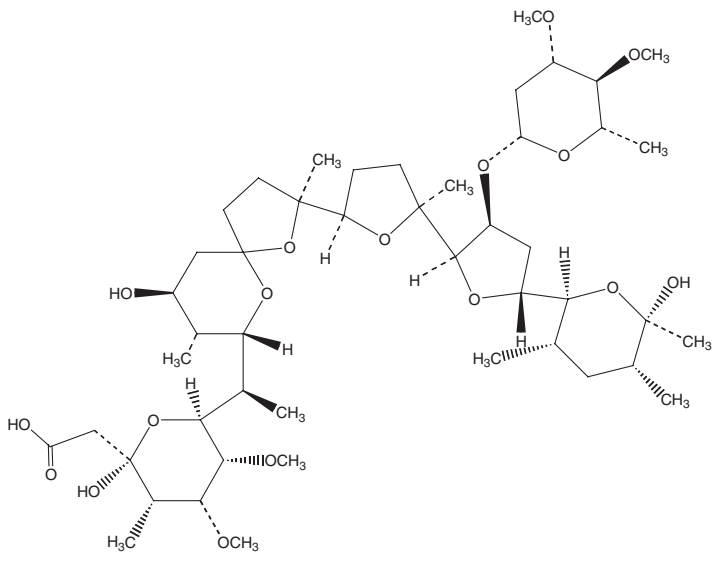
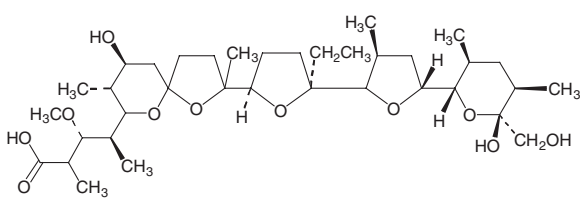
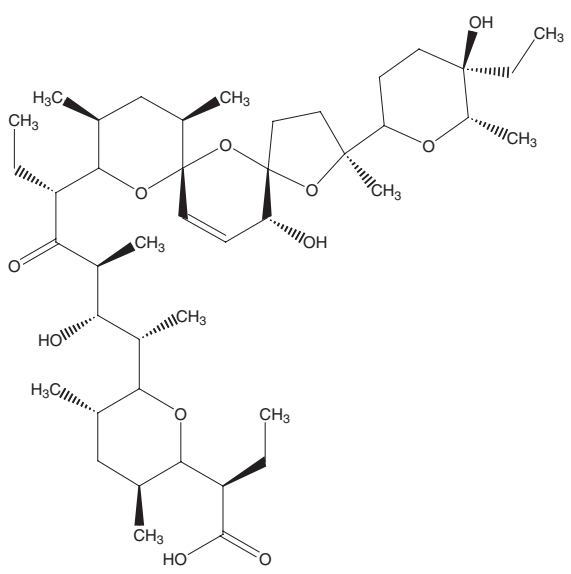
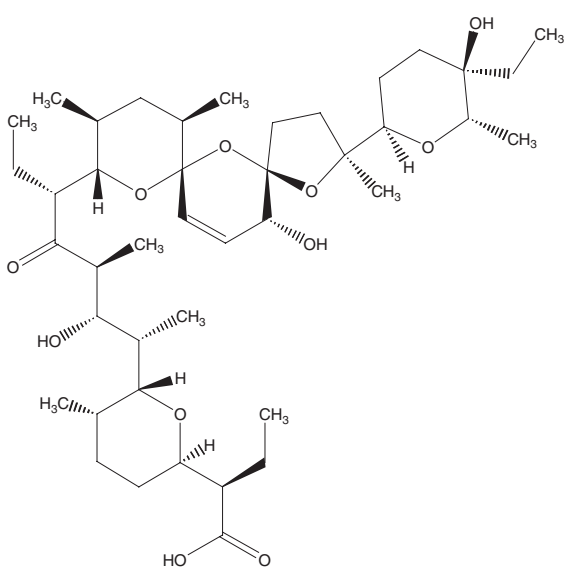
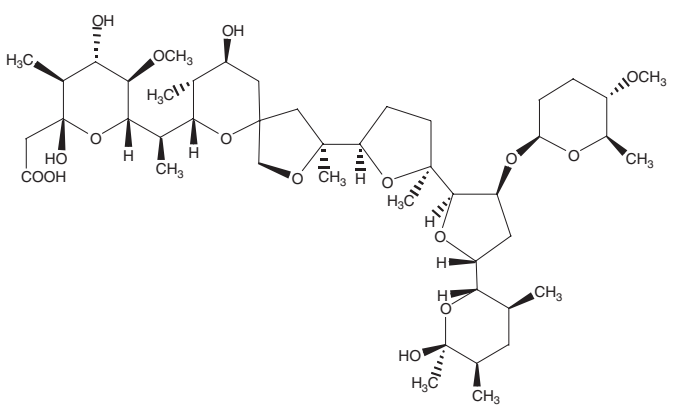
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Lasalocid A	6-[7*R*-[5*S*-ethyl-5-(5*R*-Ethyltetrahydro-5-hydroxy-6*S*-methyl-2*H*-pyran-2*R*-yl)tetrahydro-3*S*-methyl-2*S*-furanyl]-4*S*-hydroxy-3*R*,5*S*-dimethyl-6-oxononyl]-2-hydroxy-3-methylbenzoic acid $C_{34}H_{54}O_8$ 25999-31-9		4.4[153]
Maduramicin	(2*R*,3*S*,4*S*,5*R*,6*S*)-6-[(1*R*)-1-[(2*S*,5*R*,7*S*,8*R*,9*S*)-2-[(2*S*,2′*R*,3′S,5*R*,5′*R*)-3′-[(2,6-Dideoxy-3,4-di-*O*-methyl-*b*-L-*arabino*-hexopyranosyl)oxy]-octahydro-2-methyl-5′-[(2*S*,3*S*,5*R*,6*S*)-tetrahydro-6-hydroxy-3,5,6-trimethyl-2*H*-pyran-2-yl][2,2′-bifuran]-5-yl]-9-hydroxy-2,8-dimethyl-1,6-dioxaspiro[4.5]dec-7-yl]ethyl]tetrahydro-2-hydroxy-4,5-dimethoxy-3-methyl-2*H*-pyran-2-acetic acid $C_{47}H_{80}O_{17}$ 61991-54-6		4.2[154]
Monensin	2-[5-Ethyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2*H*-pyran-2-yl]-2-furyl]-2-furyl]-9-hydroxy-β-methoxy-α,γ,2,8-tetramethyl-1,6-dioxaspiro[4.5]decane-7-butyric acid $C_{36}H_{62}O_{11}$ 17090-79-8		6.7[153]

TABLE 1.10 (*Continued*)

INN	IUPAC Name Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Narasin	(αβ,2β,3α,5α,6α)-α-Ethyl-6-[5-[5-(5α-ethyltetrahydro-5β-hydroxy-6α-methyl-2*H*-pyran-2β-yl)-3″α,4,4″,5,5″α,6″-hexahydro-3′β-hydroxy-3″β,5α,5″β-trimethylspiro]furan-2(3H),2′-[2*H*]pyan-6′(3′H),2″-[2*H*]pyran]6″α-yl]2α-hydroxy-1α,3β-dimethyl-4-oxoheptyl]-tetrahydro-3,5-dimethyl-2*H*-pyran-2-acetic acid $C_{43}H_{72}O_{11}$ 55134-13-9		7.9[153]
Salinomycin	(2*R*)-2-((5S)-6-{5-[(10*S*,12*R*)-2-((6*S*,5*R*)-5-Ethyl-5-hydroxy-6-methylperhydro-2*H*-pyran-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro[4.1.5.3]pentadec-13-en-9-yl](1*S*,2*S*,3*S*,5*R*)-2-hydroxy-1,3-dimethyl-4-oxoheptyl}-5-methylperhydro-2*H*-pyran-2-yl)butanoic acid $C_{42}H_{70}O_{11}$ 53003-10-4		4.5[153] 6.4[153]
Semduramicin	(2*R*,3*S*,4*S*,5*R*,6*S*)-Tetrahydro-2,4-dihydroxy-6-[(1*R*)-1-[(2*S*,5*R*,7*S*,8*R*,9*S*)-9-hydroxy-2,8-dimethyl-2-[(2*R*,6*S*)-tetrahydro-5-methyl-5-[(2*R*,3*S*,5*R*)-tetrahydro-5[(2*S*,3*S*,5*R*,6*S*)-tetrahydro-6-hydroxy-3,5,6-trimethyl-2*H*-pyran-2-yl]-3-[[(2*S*,5*S*,6*R*)-tetrahydro-5-methoxy-6-methyl-2*H*-pyran-2-yl]oxy]-2-furyl{}-2-furyl]-1,6-dixoaspirol[4.5]dec-7-yl]ethyl]-5-methoxy-3-methyl-2*H*-pyran-2-acetic acid $C_{45}H_{76}O_{16}$ 113378-31-7		4.2[154]

Their structures involve an alkyl-rich, lipid-soluble exterior and a cagelike interior that is capable of binding and shielding monovalent metal ions (e.g., sodium, potassium) and divalent metal ions (e.g., magnesium, calcium). The ionophores are highly lipophilic and able to transport cations across cell membranes of susceptible bacteria.[132] They are most effective against Gram-positive bacteria because the peptidoglycan layer is porous, allowing them to pass through to reach the cytoplasmic membrane, where they rapidly dissolve into the membrane. The exchange of intracellular potassium for extracellular protons, and extracellular sodium for intracellular protons, disrupts ion gradients.[133] Because the potassium gradient is greater than the sodium gradient, the net effect of these exchanges is the accumulation of protons inside the bacterium.[134] The cellular response to this homeostatic disturbance is the activation of ATP-dependent processes, which in turn, exhausts cellular energy sources and leads to cell death.[133,135] Because ionophores selectively affect Gram-positive organisms, the rumen microflora shifts toward a more Gram-negative population and results in changes in the patterns of diet fermentation. The proportions of acetic acid and butyric acid in the volatile fatty acids are decreased, while the proportion of propionic acid is increased. The result is reduced energy losses per unit of feed consumed.[136] The anticoccidial activity of ionophores is thought to alter membrane integrity and internal osmolality of extracellular sporozoites and merozoites. Because coccidia have no osmoregulatory organelles, perturbances of internal osmotic conditions lead to cell death.[137]

Ionophore resistance appears to be mediated by extracellular polysaccharides (glycocalyx) that exclude ionophores from the cell membrane.[138] This is believed to involve physiological selection rather than a mutation *per se* because cattle that are not receiving ionophores can have large populations of resistant ruminal bacteria. To date, genes conferring ionophore resistance in ruminal bacteria have not been identified. Ionophore resistance is not restricted to bacteria for it is common with chicken *Eimeria* species in the United States.[137]

The use of lasalocid, monensin, and salinomycin as growth promoters was phased out in the European Union in 2006. In other regions, ionophores are used for improving production efficiency by altering the gastrointestinal microflora of animals. Ruminal fermentation is inherently inefficient, with the conversion of $\leq$12% of dietary carbon and energy to methane and heat that are unusable by the animal,[139] and $\leq$50% of dietary protein is degraded to ammonia and lost in the urine. Ionophore-induced improvements in productivity result from changes in the proportion of volatile fatty acids produced during ruminal digestion. The administration of monensin to cattle, for example, results in improvements in liveweight gain of $\leq$10%, increases in feed conversion efficiency of $\leq$7%, and decreases in food consumption of $\leq$6%. Ionophores also have a profound impact on ruminal nitrogen retention, a phenomenon referred to as a *protein-sparing effect*. Monensin is used in feedlot cattle to reduce the incidence of acute and subacute ruminal acidosis resulting from rapid fermentation of carbohydrates in the rumen and the accumulation of lactic acid. Monensin is administered by controlled-release capsules for its anti-bloat effects. The latter are mediated via a dual mechanism—the inhibition of *slime-producing bacteria* and a decrease in overall ruminal gas production.[140] Monensin is also used for decreasing the incidence of acute pneumonia caused by the eructation and inhalation of 3-methylindole, a by-product of L-tryptophan fermentation.[141] The efficacy of monensin in this condition is due to its direct inhibition of the lactobacilli producing 3-methylindole. The ionophores are also approved for use as coccidiostats in poultry, cattle, sheep, goats, and rabbits.

Ionophore toxicity has been widely reported in many species of animals, including rabbits, dogs, cats, pigeons, quail, chickens, turkeys, ostriches, goats, pigs, sheep, cattle, camels, and horses, sometimes with fatal consequences.[142] Toxicity is most often attributed to dosing errors, accidental ingestion including contaminated rations prepared by feedmills, the ingestion by ruminants of litter from ionophore-treated poultry flocks, and the concurrent administration of other agents and, in particular, tiamulin. The mechanism of ionophore toxicity generally involves cellular electrolyte imbalance, with skeletal and cardiac muscle affected most severely. Horses are particularly sensitive to ionophore toxicity; the LD_{50} for monensin in horses is 2–3 mg/kg, compared with LD_{50} values of 20 mg/kg for dogs and 200 mg/kg for chickens.[143] Food contaminated with salinomycin has resulted in polyneuropathy in cats.[144]

Kouyoumdjian and coworkers[145] reported the case of a 17-year-old male who developed myoglobinemia and renal failure and died 11 days after ingesting sodium monensin. The findings in this case were similar to those seen in animals following accidental intoxication.

Relatively few reports in the literature describe environmental concentrations, fate, and transport of monensin. Compared with tetracyclines and macrolides, monensin is not tightly adsorbed to soil and has been detected in river water and aquatic sediments in Colorado[146] and in streams in southern Ontario.[147]

The JECFA has allocated ADIs for monensin[148] and narasin.[149] The CAC has established MRLs for monensin in muscle, liver, kidney, and fat of cattle, sheep, chickens, goats, turkeys, and quails,[43] based on the residue evaluation conducted by JECFA.[150] The CAC MRLs for narasin in muscle, liver, kidney, and fat of pigs and chickens, and temporary MRLs for narasin in muscle, liver, kidney, and fat of cattle, have also been established,[43] on the basis of the JECFA evaluation.[151]

From an analytical perspective, ionophores are unstable in strongly acidic conditions. Moreover, weakly acidic extractants are not suitable for use with these substances.[152]

1.3.10 Polypeptides, Glycopeptides, and Streptogramins

The polypeptides include bacitracin A, colistin (polymyxin E), novobiocin, and polymyxin B. Bacitracin is a complex mixture of branched, cyclic decapeptides produced by *Bacillus subtilise*, which was first isolated in 1945. The polymyxins, discovered in 1947, are synthesized by various strains of *Bacillus polymyxa*. Colistin (polymyxin E) comprises a family of polymyxins and was known as colimycin when first isolated from a broth of *Bacillus polymyxa* var. *colistinus* in 1951. The polymyxins are cationic detergents. Novobiocin, first reported in 1955 as streptonivicin, is produced by the actinomycete *Streptomyces niveus*.

The glycopeptide antibiotics include avoparcin, teicoplanin, and vancomycin. Avoparcin is produced by *Amycolatopsis coloradensis*, while teicoplanin is a mixture of six closely related compounds produced by *Streptococcus teichomyetius*. Vancomycin is produced by *Streptococcus orientalis*.

The streptogramins include virginiamycin and pristinomycin. Virginiamycin is produced by a mutant strain of *Streptomyces virginiae*. It is a natural mixture of factor *M* and factor *S*, and its antibacterial activity is synergistically optimum when the $M:S$ ratio is approximately $4:1$.[155–157] Pristinamycin is a combination of quinupristin, a streptogramin B, and dalfopristin, a streptogramin A, in a $30:70$ ratio. Each of these compounds is a semi-synthetic derivative of naturally occurring pristinamycins produced by *Streptomyces pristinaespiralis*.

Bacitracin inhibits the synthesis of the bacterial cell wall by preventing the transport of peptidoglycan precursors through the cytoplasmic membrane. It is bactericidal to Gram-positive bacteria but exhibits little activity against Gram-negative organisms. The antibacterial activity of the polymyxins is attributed to their strong binding to phospholipids in cell membranes, which disrupts their structure and alters membrane permeability. These drugs are bactericidal and display activity against many species of Gram-negative bacteria, including *E. coli, Salmonella*, and *Pseudomonas aeruginosa* but not against *Proteus, Serratia*, or *Providencia*. The glycopeptide antibiotics inhibit cell wall synthesis by binding strongly with cell wall precursors. The antibacterial activity of the streptogramins is attributed to the inhibition of protein synthesis. This involves the *M* and *S* factors of virginiamycin binding to 50*S* ribosomal subunits and inhibiting the formation of peptide bonds during protein synthesis. Quinupristin and dalfopristin also inhibit protein synthesis. They bind to the 50*S* ribosomal subunit at different sites located in close proximity, thereby interfering with the formation of polypeptide chains.

Bacterial resistance to the polymyxins is rare; however, resistance is common in pig and chicken isolates of *Enterococcus* spp.[158] Interestingly, bacitracin administered to pigs and chickens has been shown to reduce the transfer of resistance plasmids among enteric *E. coli*. In the case of novobiocin, resistance has developed in many species of bacteria. Prior to the ban on its use in food-producing species, avoparcin was found to select for vancomycin-resistant enterococci (VRE). Bacterial resistance to vancomycin is generally uncommon, with the exception of *Enterococcus* species. Development of resistance to teicoplanin is also uncommon. In terms of pristinamycin, the mechanisms of resistance to class A streptogramins and class B streptogramins are different. With class A streptogramins, active efflux of drug from the bacterial cell as well as drug inactivation by acetyltransferases contribute to resistance. By comparison, resistance to class B streptogramins is most commonly due to methylation of the target 23*S* ribosomal RNA, while a less common mechanism involves enzymatic cleavage of a structural ring in the drug.

Bacitracin is used for the treatment of infections of the skin, eyes, and ears. Various topical dosage forms, including wound powders and ointments, and eye and ear ointments, are available. Bacitracin is used as a feed additive for pigs, poultry, and ruminants, except in the European Union, where use for growth promotion was banned in 1999. It improves growth rate and feed conversion efficiency of pigs, broilers, calves, sheep, and feedlot steers. Bacitracin is also used in the control of proliferative enteropathy in grower–finisher pigs, and to decrease the incidence and severity of clostridial enteritis in piglets born to sows treated during pregnancy. In the poultry industry, bacitracin is used for the prevention of necrotic enteritis in broilers and to improve the ability of broilers and layers to withstand heat stress. Novobiocin sodium is included with other agents in intramammary infusions for treating mastitis in dairy cattle.

The glycopeptides are not used in food-producing species. In humans, vancomycin is indicated for the treatment of life-threatening Gram-positive infections that are unresponsive to less toxic antibiotics. The worldwide emergence of vancomycin-resistant enterococci (VRE) is a major concern for public health and stimulated the debate concerning the use of avoparcin in agriculture and whether this contributed to VRE in humans. The agricultural use of avoparcin in many countries is now banned. A new glycopeptide antibiotic, teicoplanin, was developed against infections with resistant Gram-positive bacteria, especially bacteria resistant to vancomycin.

Virginiamycin is used to improve daily liveweight gain and feed conversion efficiency in feedlot and grazing cattle, broilers, turkeys, and pigs in several countries. However,

such use was discontinued in the European Union in 1999 and in Australia in 2005. In feedlot cattle, it also reduces the incidence and severity of liver abscessation. Virginiamycin reduces the risk of fermentative lactic acidosis in cattle and sheep fed high-concentrate diets. The drug is administered to horses on high grain diets to reduce the risk of laminitis.

Polymyxins are notably nephrotoxic and neurotoxic and cause intense pain if injected. Polymyxin B is a potent histamine releaser; however, hypersensitivity reactions to all polymyxins are seen occasionally. The incidence of adverse reactions to novobiocin sodium is also relatively frequent.

The limited literature available suggests that presently none of the polypeptide antibiotics, glycopeptide antibiotics, or streptogramins pose a risk to the environment.

On the basis of risk assessments carried out in 1968,[42] JECFA has allocated ADI values to bacitracin and novobiocin. MRLs were not established by the CAC because when administered to animals, these substances should not be allowed to give rise to detectable residues in food for human consumption. More recently, JECFA allocated an ADI for colistin based on a microbiological endpoint and recommended MRLs for colistin in muscle, liver, kidney, fat, and milk of cattle and sheep; and in muscle, liver, kidney and fat of pigs, rabbits, goats, turkeys, and chickens, and in chicken eggs.[159] These MRL recommendations were adopted by the CAC.[43] Details of the residue studies for colistin considered by JECFA were published in a monograph.[160] Properties of polypeptides, glycopeptides, and streptogramens are listed in Table 1.11.

1.3.11 Phosphoglycolipids

Flavophospholipol is the only phosphoglycolipid antibiotic that is approved for use in food-producing animals (see Table 1.12 for properties of this compound). It is produced by *Streptomyces* spp., including *S bambergiensis, S. ghanaensis, S. geyirensis*, and *S. ederensis* and was discovered in the mid-1950s. The product consists of a complex of similar components in which moenomycin A predominates. Flavophospholipol has a novel mode of action in that it inhibits peptidoglycan synthesis by interfering with transglycolase activity and prevents the formation of the murein backbone of the peptidoglycan molecule.[161] It is active mainly against Gram-positive organisms and has little activity against Gram-negative bacilli as it cannot penetrate the outer lipopolysaccharide cell membrane in these organisms. Flavophospholipol is absorbed poorly from the gastrointestinal tract and if administered parenterally, is strongly bound to plasma proteins and host cell membranes. It is slowly excreted unchanged in the urine.[162] Limited information is available on acquired resistance to flavophospholipol, but it seems that many *Enterococcus* species are intrinsically resistant.

For more than 30 years, flavophospholipol has been used in many countries, including Australia and European countries, solely as a growth-promoting antimicrobial in animal feeds. However, its use was banned in the EU in 2006. The most extensive use has been in pigs and poultry, although flavophopholipol also promotes growth in ruminants. The mechanism for growth promotion of flavophopholipol is unclear. Its mode of action on the rumen microbial population appears to differ from that of the ionophore class of antibiotics in that volatile fatty acid proportions are generally unchanged.[163] An interesting characteristic of flavophospholipol is its ability to inhibit transfer of plasmids carrying antibiotic resistance genes in *E. coli, Salmonella*, and *Enterococcus* spp.[162,164] Furthermore, it has been shown to reduce the shedding of salmonella in experimentally infected animals.[161] The PK and PD profiles of flavophospholipol make it unsuitable for use as a human antibiotic.

This author is not aware of any reports that describe the presence of flavophospholipol in the environment.

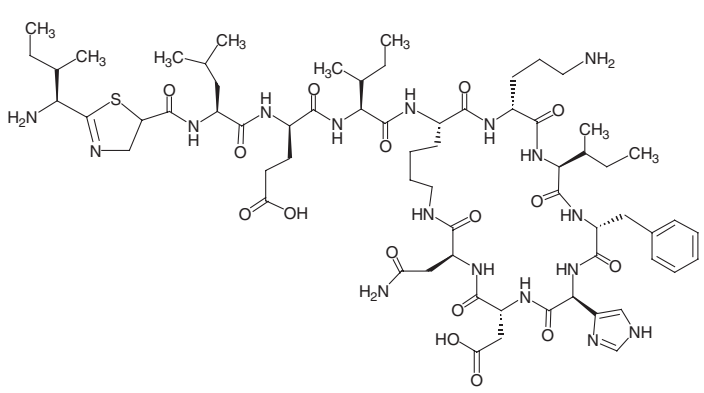
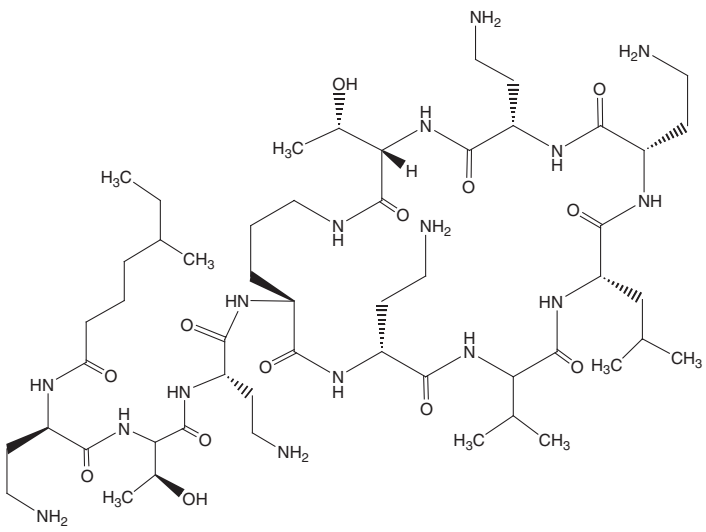
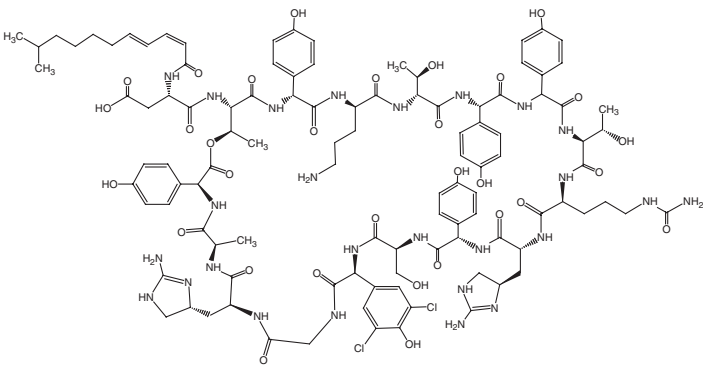
The JECFA has not evaluated toxicological or residue depletion data for flavophospholipol, and CAC MRLs for the substance have not been established.

1.3.12 Quinolones

The quinolones (Table 1.13) are a family of synthetic broad-spectrum antimicrobial drugs that comprise four generations; members of the first generation have a narrow spectrum of activity compared to those in later generations. The first quinolone to be used clinically for its antimicrobial activity was nalidixic acid in 1962; this drug is a derivative of chloroquine that was discovered by Lesher and coworkers. Today, naladixic acid and other first-generation quinolones such as flumequine and oxolinic acid are used primarily in aquaculture. Successive generations of quinolones have a fluorine atom in the quinolone ring structure, typically at the C6 position. Several fluoroquinolones, including danofloxacin, difloxacin, enrofloxacin (which is deethylated to form ciprofloxacin), marbofloxacin, orbifloxacin, and sarofloxacin, are used in veterinary but not human medicine. Conversely, some fluoroquinolones that are important in human medicine are not labeled for animal use.

The activity type of the fluoroquinolone antimicrobial drugs is concentration-dependent. Because quinolones accumulate in the cytosol of macrophages and neutrophils, they are often used to treat intracellular pathogens. The preponderance of macrophages and neutrophils in infected tissues compared to healthy tissues may explain the higher concentrations of fluoroquinolones attained in infected tissues.[62] Fluoroquinolones can produce a post-antibiotic effect, suppressing bacterial growth after local drug concentrations have fallen below the MIC of the target

TABLE 1.11 Polypeptides, Glycopeptides, and Streptogramins

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
		Polypeptides	
Bacitracin A	(4*R*)-4-[[(2*S*)-2-[[2-[(1*S*)-1-Amino-2-methylbutyl]4,5-dihydro-1,3-thiazole-5-carbonyl]amino]-4-methylpentanoyl]amino]-5-[[(2*S*)-1-[[(3*S*,6*R*,9*S*,12*R*,15*S*,18*R*,21*S*)-3-(2-amino-2-oxoethyl)-18-(3-aminopropyl)-15-butan-2-yl-6-(carboxymethyl)-9-(3*H*-imidazol-4-ylmethyl)-2,5,8,11,14,17,20-heptaoxo-12-(phenylmethyl)-1,4,7,10,13,16,19-heptazacyclopentacos-21-yl]amino]-3-methyl-1-oxopentan-2-yl]amino]-5-oxopentanoic acid $C_{66}H_{103}N_{17}O_{16}S$ 1405-87-4		N/A[a]
Colistin (polymyxin E)	*N*-[(2*S*)-4-Amino-1-[[(2*S*,3*R*)-1-[[(2*S*)-4-amino-1-oxo-1-[[(3*S*,6*S*,9*S*,12*S*,15*R*,18*S*,21*S*)-6,9,18-tris(2-aminoethyl)-3-(1-hydroxyethyl)-12,15-bis(2-methylpropyl)-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptazacyclotricos-21-yl]-amino]butan-2-yl]amino]-3-hydroxy-1-oxobutan-2-yl]amino]-1-oxobutan-2-yl]-5-methylheptanamide $C_{52}H_{98}N_{16}O_{13}$ 1066-17-7		N/A[a]
Enramycin (enduracidin)	IUPAC name not available $C_{107}H_{138}Cl_2N_{26}O_{31}$ 11115-82-5		N/A[a]

(*continued*)

TABLE 1.11 (*Continued*)

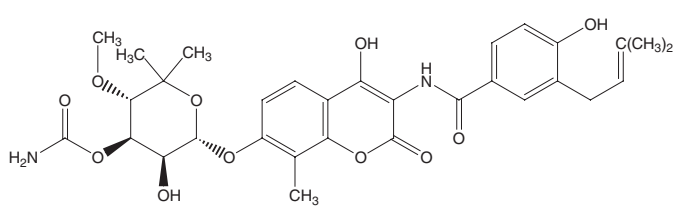
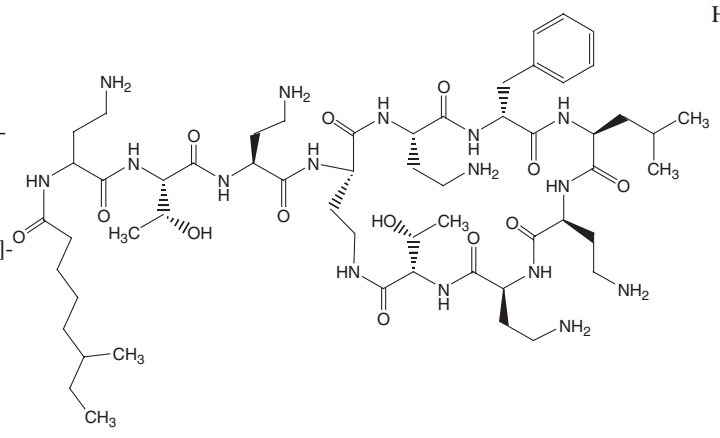
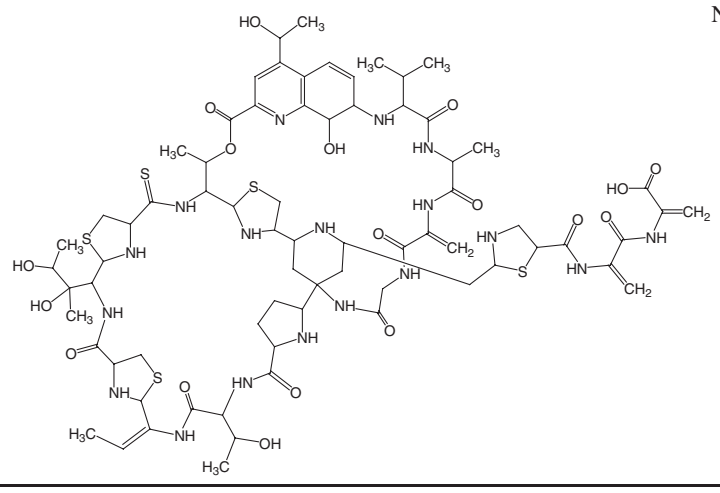
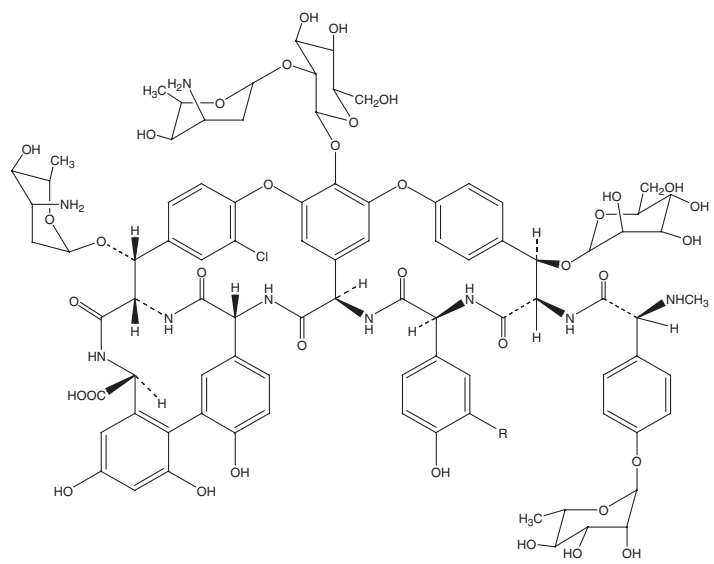
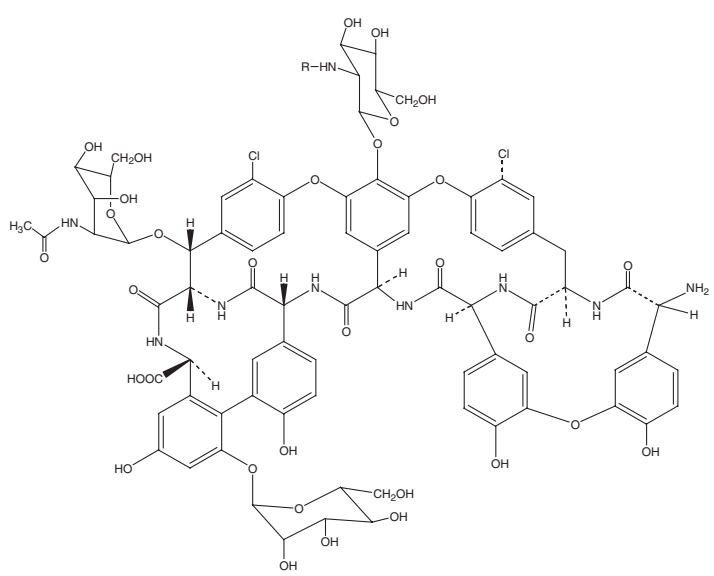
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Novobiocin	*N*-[7-[[3-*O*-(Aminocarbonyl)-6-deoxy-5-*C*-methyl-4-*O*-methyl-ß-L-lyxo-hexopyranosyl]oxy]-4-hydroxy-8-methyl-2-oxo-2*H*-1-benzopyran-3-yl]-4-hydroxy-3-(3-methyl-2-butenyl)benzamide $C_{31}H_{36}N_2O_{11}$ 303-81-1		HA 4.3,[56] HA 9.1[56]
Polymyxin B	*N*-[4-Amino-1-[[1-[[4-amino-1-oxo-1-[[6,9,18-tris(2-aminoethyl)-15-benzyl-3-(1-hydroxyethyl)-12-(2-methylpropyl)-2,5,8,11,14,17,20-heptaoxo-1,4,7,10,13,16,19-heptazacyclotricos-21-yl]amino]butan-2-yl]amino]-3-hydroxy-1-oxobutan-2-yl]amino]-1-oxobutan-2-yl]-6-methyloctanamide $C_{56}H_{98}N_{16}O_{13}$ 1405-20-5		HB^+ 8.9[56]
Thiopeptin B	IUPAC name not available $C_{72}H_{104}N_{18}O_{18}S_5$ 37339-66-5		N/A[a]

TABLE 1.11 (***Continued***)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
		Glycopeptides	
Avoparcin	IUPAC name not available $C_{89}H_{102}ClN_9O_{36}$ (α-avoparcin) $C_{89}H_{101}Cl_2N_9O_{36}$ (ß-avoparcin) 37332-99-3	 α-**avoparcin** R = H β-**avoparcin** R = Cl	N/A[a]
Teicoplanin	Ristomycin A: 34-*O*-[2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-22,31-dichloro-7-demethyl-64-*O*-demethyl-19-deoxy-56-*O*-[2-deoxy-2-[(8-methyl-1-oxononyl)amino]-β-D-glucopyranosyl]-42-O-α-D-mannopyranosyl $C_{88}H_{95}Cl_2N_9O_{33}$ (teicoplanin A_2—1) $C_{88}H_{97}Cl_2N_9O_{33}$ (teicoplanin A_2—2) $C_{88}H_{97}Cl_2N_9O_{33}$ (teicoplanin A_2—3) $C_{89}H_{99}Cl_2N_9O_{33}$ (teicoplanin A_2—4) $C_{89}H_{99}Cl_2N_9O_{33}$ (teicoplanin A_2—5) 61036-62-2	 A_2-1: R = (Z)-4-decanoic acid A_2-2: R = 8-methylnonanoic acid A_2-3: R = n-decanoic acid A_2-4: R = 8-methyldecanoic acid A_2-5: R = 9-methyldecanoic acid	N/A[a]

(*continued*)

TABLE 1.11 (*Continued*)

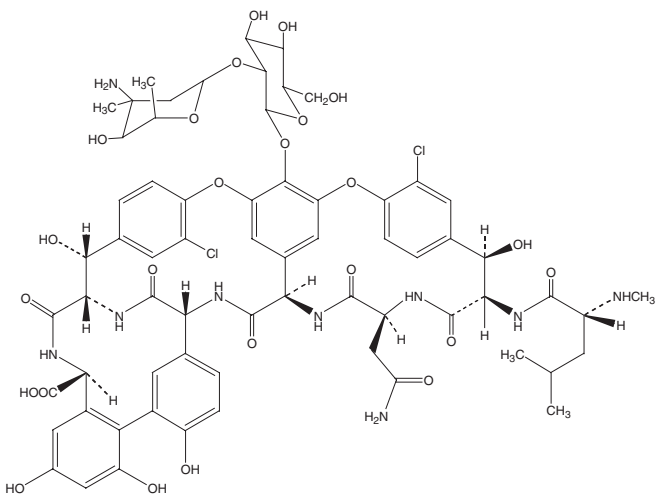
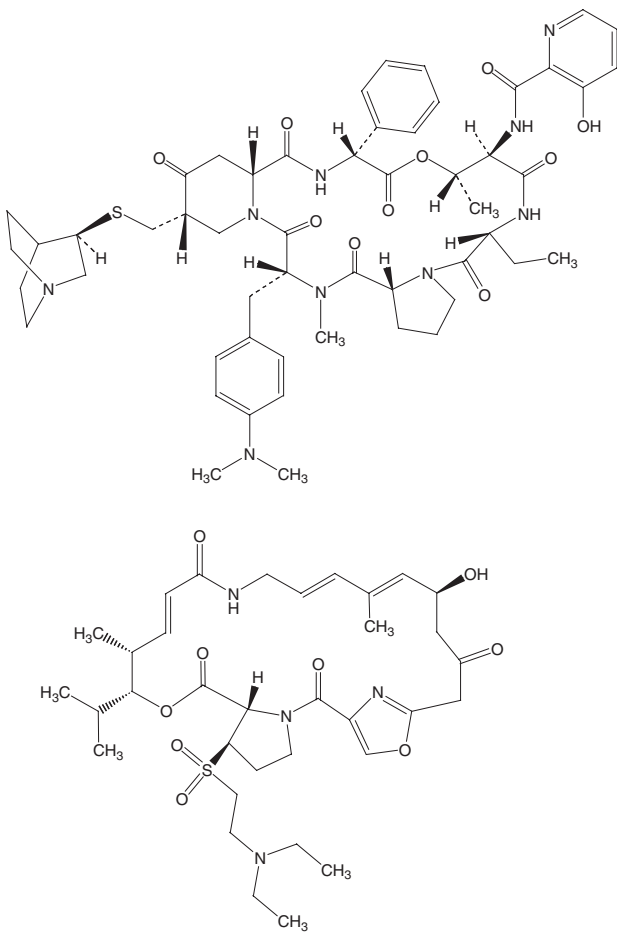
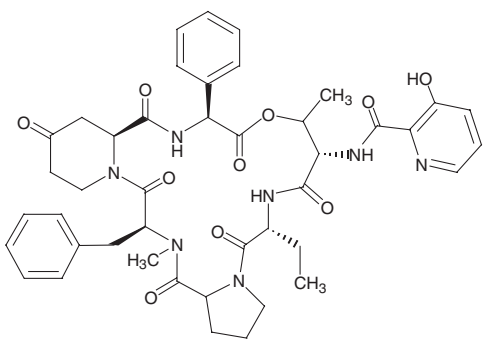
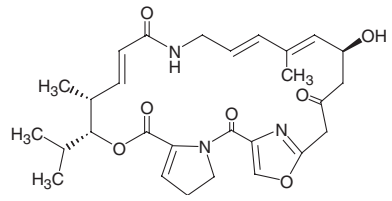
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Vancomycin	(3*S*,6*R*,7*R*,11*R*,23*S*,26*S*,30a*S*, 36*R*,38a*R*)-44-[2-*O*-(3-Amino-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyloxy]-3-(carbamoylmethyl)-10,19-dichloro-2,3,4,5,6,7,23,25,26,36,37,38,38a-tetradecahydro-7,22,28,30,32-pentahydroxy-6-(*N*-methyl-D-leucyl)-2,5,24,38,39-pentaoxo-1*H*,22*H*-23,36-(epiminomethano)-8,11:18,21-dietheno-13,16:31,35-di(metheno)^{1,6,9}oxadiazacyclohexadecino[4,5-*m*]^{10,2,16}benzoxadiazacyclotetracosine-26-carboxylic acid $C_{66}H_{75}Cl_2N_9O_{24}$ 1404-90-6		HA 2.2[56] (COOH), HB^+ 7.8[56] ($NHCH_3$) HB^+ 8.9[56] (NH_2), HA 9.6[56] (phenol), HA 10.4[56] (phenol), HA 12.0[56] (phenol)
	Streptogramins		
Quinupristin/ dalfopristin	Quinupristin: *N*-[(6*R*,9*S*,10*R*,13*S*,15a*S*,18*R*,22*S*,24a*S*)-22-[*p*-(Dimethylamino)benzyl]-6-ethyldocosahydro-10,23-dimethyl-5,8,12,15,17,21,24-heptaoxo-13-phenyl-18-[[(3*S*)-3-quinuclidinylthio] methyl]-12*H*-pyrido[2,1-*f*]pyrrolo-[2,1-*l*]^{1,4,7,10,13,16}oxapentaazacyclononadecin-9-yl]-3-hydroxypicolinamide $C_{53}H_{67}N_9O_{10}S$ 120138-50-3 Dalfopristin: (3*R*,4*R*,5*E*,10*E*,12*E*,14*S*,26*R*,26a*S*)-26-[[2-(Diethylamino)ethyl]sulfonyl]-8,9,14,15,24,25,26,26a-octahydro-14-hydroxy-3-isopropyl-4,12-dimethyl-3*H*-21,18-nitrilo-1*H*,22*H*-pyrrolo[2,1-*c*]^{1,8,4,19}-dioxadiazacyclotetracosine-1,7,16,22(4*H*,17*H*)-tetrone $C_{34}H_{50}N_4O_9S$ 112362-50-2 $C_{87}H_{117}N_{13}O_{19}S_2$ (combined) 126602-89-9 (combined)		N/A[a]

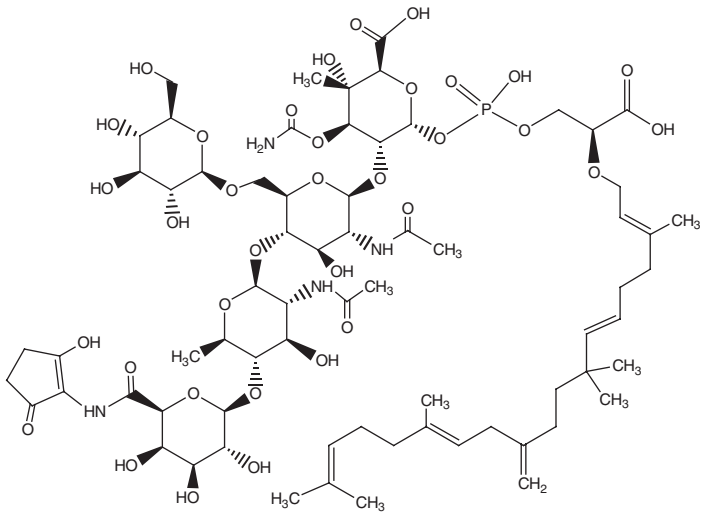
TABLE 1.11 (*Continued*)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Virginiamycin	Virginiamycin S_1: IUPAC name not available $C_{43}H_{49}N_7O_{10}$ 23152-29-6 Virginiamycin M_1: 8,9,14,15,24,25-Hexahydro-14-hydroxy-4,12-dimethyl-3-(1-methylethyl)(3*R*,4*R*,5*E*,10*E*,12*E*,14*S*)-3*H*-21,18-nitrolo-1*H*,22*H*-pyrrolo-[2,1-*c*][1,8,4,19] dioxadiazacyclotetracosine-1,7,16,22(4*H*,17*H*)-tetrone $C_{28}H_{35}N_3O_7$ 21411-53-0	 	N/A[a]

[a]The author was not able to find a pK_a value for the substance in the public literature.

pathogen. The fluoroquinolones enter bacterial cells via porins and inhibit bacterial DNA gyrase in many Gram-negative bacteria, or topoisomerase IV in many Gram-positive bacteria—thereby inhibiting DNA replication and transcription. Fluoroquinolones also cause the cessation of cellular respiration and disruption of membrane integrity. Although mammalian topoisomerase II is a target for a variety of quinolone-based drugs, concentrations approximately 100-fold higher than those recommended for bacterial activity are needed for the enzyme to be inhibited.

TABLE 1.12 Phosphoglycolipids

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Flavophospholipol (bambermycin, moenomycin A)	IUPAC name not available $C_{69}H_{107}N_4O_{35}P$ 11015-37-5		N/A[a]

[a]The author was not able to find a pK_a value for the substance in the public literature.

TABLE 1.13 Quinolones

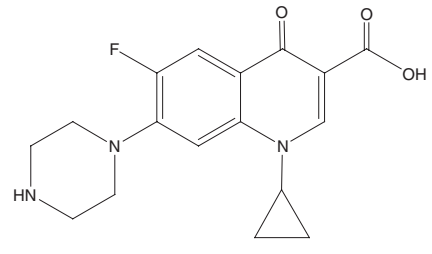
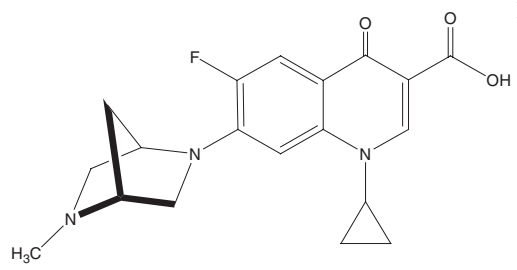
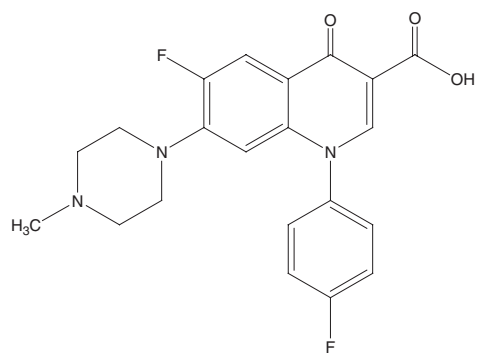
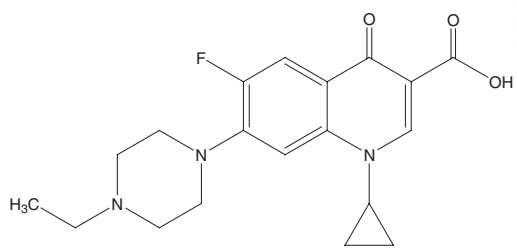
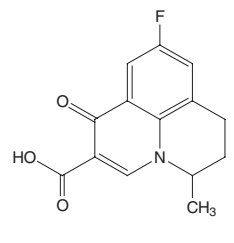
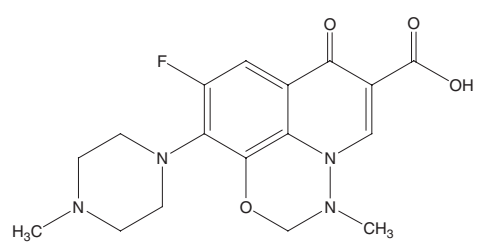
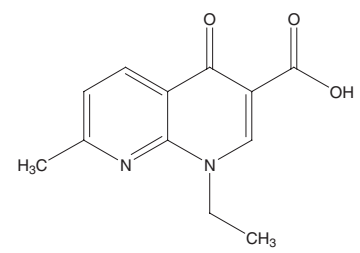
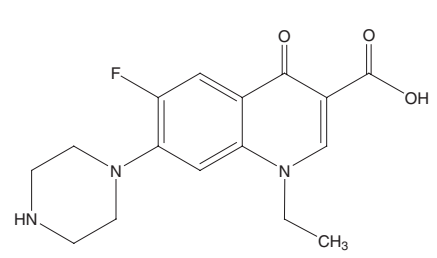
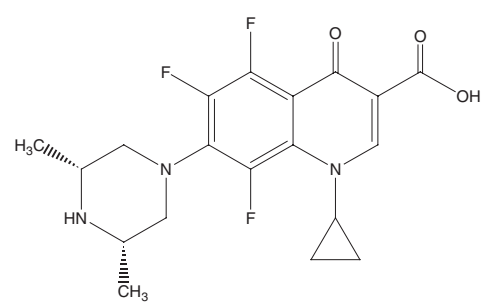
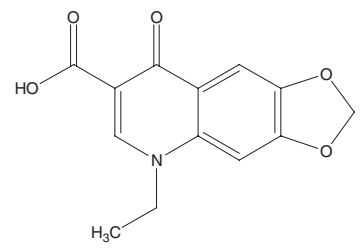
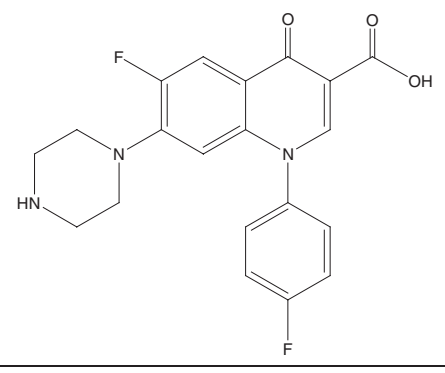
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Ciprofloxacin	1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid $C_{17}H_{18}FN_3O_3$ 85721-33-1		HA 6.2,[56] HB^+ 8.7[56]
Danofloxacin	(1*S*)-1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl)-4-oxo-3-quinoline carboxylic acid $C_{19}H_{20}FN_3O_3$ 112398-08-0		N/A[a]
Difloxacin	6-Fluoro-1-(4-fluorophenyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid $C_{21}H_{19}F_2N_3O_3$ 98106-17-3		HA 6.1,[56] HB^+ 7.6[56]
Enrofloxacin	1-Cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolonecarboxylic acid $C_{19}H_{22}FN_3O_3$ 93106-60-6		HA 6.0,[74] HB^+ 8.8[74]
Flumequine	9-Fluoro-6,7-dihydro-5-methyl-1-oxo-1*H*,5*H*-benzo[*ij*]-quinolizine-2-carboxylic acid. $C_{14}H_{12}FNO_3$ 42835-25-6		HA 6.4[56]
Marbofloxacin	9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyridol(3,2,1-ij)(4,2,1)benzoxadiazin-6-carboxylic acid $C_{17}H_{19}N_4O_4F$ 115550-35-1		N/A[a]

TABLE 1.13 (***Continued***)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Nalidixic acid	1-Ethyl-1,4-dihydro-7-methyl-4-oxo-^{1,8}naphthyridine-3-carboxylic acid $C_{12}H_{12}N_2O_3$ 389-08-2		HA 6.0[56]
Norfloxacin	1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid $C_{16}H_{18}FN_3O_3$ 70458-96-7		HA 6.3,[56] HB^+ 8.4
Orbifloxacin	1-Cyclopropyl-7-[(3*S*,5*R*)-3,5-dimethylpiperazin-1-yl]-5,6,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid $C_{19}H_{20}F_3N_3O_3$ 113617-63-3		HA ~6,[56] HB^+~9[56]
Oxolinic acid	5-Ethyl-5,8-dihydro-8-oxo^{1,3}dioxolo[4,5-*g*]quinoline-7-carboxylic acid $C_{13}H_{11}NO_5$ 14698-29-4		N/A[a]
Sarafloxacin	6-Fluoro-1-(4-fluorophenyl)-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid $C_{20}H_{17}F_2N_3O_3$ 98105-99-8		N/A[a]

[a]The author was not able to find a pK_a value for the substance in the public literature.

Resistance to quinolones can evolve rapidly; the most common mechanism involves mutation of DNA gyrase (topoisomerase II) in Gram-negative bacteria. A similar mechanism alters topoisomerase IV in Gram-positive bacteria. These mutations result in reduced binding affinity to quinolones, which decreases bactericidal activity. A second mechanism of resistance involves increased expression of efflux pumps that actively transport drug out of bacterial cells, resulting in decreased intracellular drug concentration. Plasmid-mediated resistance in Gram-negative bacteria results in the synthesis of proteins that bind to DNA gyrase, protecting it from the action of quinolones. At present, however, the clinical importance of this mechanism is unclear. The transfer of fluoroquinolone-resistant *Campylobacter* species and *Salmonella typhimurium*-type DT-104 from animals to humans is a major concern. As a consequence, some countries have established systems for monitoring and surveillance of antibiotic resistance in human and animal isolates. In many countries, approved and off-label uses of fluoroquinolones in food-producing species are either restricted or not permitted.

Fluoroquinolones are active against some Gram-negative bacteria, including *E. coli, Enterobacter* species, *Klebsiella* species, *Pasteurella* species, *Proteus* species, and *Salmonella* species. The susceptibility of *Pseudomonas aeruginosa* to fluoroquinolones is variable. These agents are also active against some Gram-positive bacteria and chlamydia, mycobacteria, and mycoplasma. In some regions, the use of fluoroquinolones is approved for the treatment of colibacillosis of chickens and turkeys, fowl cholera in turkeys, and bovine respiratory disease caused by *Mannheimia haemolytica, Pasteurella multocida, Haemophilus somnus*, and other susceptible organisms. The fluoroquinolones are administered as oral solutions to chickens and turkeys, by injection to cattle, as tablets, and by injection to dogs and cats.

Fluoroquinolone administration during rapid growth has been associated with arthropathies and cartilage erosions in weight-bearing joints in immature cats, dogs, and horses. Retinal degeneration has been associated with the administration of enrofloxacin at high doses in cats. Therefore the use of fluoroquinolones in immature animals and high doses of fluoroquinolones in cats should be avoided.

Literature relating to quinolones in the environment as a result of veterinary use is sparse. Trace amounts of flumequine, oxolinic acid, and sarafloxacin in sediment at fish farms have been detected.[27] Trace amounts of enrofloxacin in soil were detected in a UK monitoring study,[87] while studies into the sorption behavior of danofloxacin and sarafloxacin in soil showed these drugs to be non-mobile and persistent.[100]

On the basis of JECFA evaluations of toxicological and residues depletion data for oxolinic acid,[165] flumequine,[166] enrofloxacin,[167] danofloxacin,[168] and sarafloxacin,[169] CAC MRL recommendations were established for flumequine in cattle, sheep, pigs, chickens, and trout;[43] danofloxacin in cattle, pigs, and chickens;[43] and sarafloxacin in chickens and turkeys[43] but not for oxolinic acid[165] or enrofloxacin.[167] Details of residue studies reviewed by JECFA have been published for oxolinic acid,[170] flumequine,[171–174] enrofloxacin,[175] danofloxacin,[176] and sarafloxacin.[177]

1.3.13 Sulfonamides

The sulfonamides were the first effective chemotherapeutic agents to be employed systemically for the prevention and cure of bacterial infections in humans. Foerster reported the first clinical case study of prontosil in 1933; 2 years later, this compound was shown to be a pro-drug of sulfanilamide. In 1939, Domagk was awarded the Nobel Prize in Physiology or Medicine for discovering the chemotherapeutic value of prontosil. Interestingly, sulfanilamide had been prepared by Gelmo in 1906 while investigating azo dyes.

The sulfonamide class contains a large number of antibacterial drugs, including sulfadiazine, sulfamethazine (sulfadimidine), sulfathiazole, sulfamethoxazole, and many more. Potentiated sulfonamides, in which a sulfonamide and an antibacterial diaminopyrimidine such as trimethoprim are combined, demonstrate improved efficacy compared with sulfonamides alone. Relatively few sulfonamides are currently (as of 2011) approved for use in food-producing species. This is attributed to numerous factors, including toxicological concerns associated with some sulfonamides and the lack of contemporary data to support the historical uses of other sulfonamides.

The sulfonamides are structural analogues of *para*-aminobenzoic acid (PABA) and competitively inhibit dihydropteroate synthetase, the enzyme that catalyzes the synthesis of dihydrofolic acid (folic acid). Organisms susceptible to sulfonamides must synthesize their own folic acid, unlike mammalian cells, which utilize preformed folic acid. The decreased synthesis of dihydrofolic acid, in turn, causes decreased synthesis of tetrahydrofolic acid (folinic acid), which is required for the synthesis of DNA. A variety of effects may result, including suppression of protein synthesis, impairment of metabolic processes, and inhibition of growth and multiplication in susceptible organisms. Sulfonamides, which are not efficacious in the presence of purulent material, are bacteriostatic. Before such activity is exhibited, however, existing stores of folic acid, folinic acid, purines, thymidine, and amino acids are utilized by bacteria. Sulfonamides inhibit both Gram-positive and Gram-negative bacteria, some chlamydia, *Nocardia*, and *Actinomyces* species, and some protozoa including coccidia and *Toxoplasma* species. Organisms

resistant to sulfonamides include *Pseudomonas, Klebsiella, Proteus, Clostridium*, and *Leptospira* species.

Although dihydrofolate reductase catalyzes the synthesis of folic acid in both bacteria and mammals, antibacterial diaminopyrimidines such as trimethoprim and ormetoprim inhibit this enzyme more efficiently in bacteria than in mammalian cells. These drugs are bacteriostatic when used alone; however, when combined with sulfonamides, the sequential blockade of dihydropteroate synthetase and dihydrofolate reductase elicits a bactericidal effect. Sulfonamide–diaminopyrimidine combinations are active against Gram-positive and Gram-negative organisms, including *Actinomyces, Bordetella, Clostridium, Corynebacterium, Fusobacterium, Haemophilus, Klebsiella, Pasteurella, Proteus, Salmonella, Shigella*, and *Campylobacter* species, as well as *E. coli*, streptococci, and staphylococci. *Pseudomonas* and *Mycobacterium* species are resistant to potentiated sulfonamides.

Resistance to sulfonamides is widespread in bacteria isolated from animals, and may involve chromosomal mutations or plasmid-mediated mechanisms. Chromosomal mutations cause impaired drug penetration, production of altered forms of dihydropteroate synthetase for which sulfonamides have a lowered affinity, or production of excessive PABA that overcomes the metabolic block imposed by the inhibition of dihydropteroate synthetase. A more common cause of bacterial resistance to sulfonamides is plasmid-mediated mechanisms, which may result in impaired drug penetration or the synthesis of sulfonamide-resistant dihydropteroate synthetase. There is cross-resistance among sulfonamides.

Resistance to bacterial diaminopyrimidines results from chromosomal mutations or plasmid-mediated mechanisms and develops very rapidly. Resistance conferred by chromosomal mutations allows bacteria to utilize exogenous sources of folinic acid or thymidine, thereby overcoming the drug-imposed blockade. Plasmid-mediated mechanisms result in the synthesis of dihydrofolate reductase characterized by a reduced affinity for antibacterial diaminopyrimidines.

Compared to most classes of antimicrobial drugs, the usage of sulfonamides and potentiated sulfonamides in veterinary medicine is high. Sulfonamides are used to treat or prevent acute systemic or local infections, including actinobacillosis, coccidiosis, mastitis, metritis, colibacillosis, pododermatitis, polyarthritis, respiratory infections, and toxoplasmosis. Sulfonamides are also used in the treatment of American foulbrood disease caused by *Paenibacillus larvae* and European foulbrood disease caused by *Melissococcus pluton* that affect honeybees. Sulfonamides in combination with pyrimethamine are used to treat protozoal diseases such as leishmaniasis and toxoplasmosis. Sulfonamides are most effective in the early stages of acute infections when organisms are rapidly multiplying.

Sulfonamides are administered to food-producing species as additives to feed and drinking water, controlled-release oral boluses, and intrauterine infusions. These drugs are applied to the brood chambers of honeybee hives mixed with confectioners' sugar or in syrup. The insoluble nature of sulfonamides is an important consideration. Highly insoluble sulfonamides such as phthalylsulfathiazole are absorbed from the gastrointestinal tract very slowly and are used to treat enteric infections. With triple sulfas for oral administration, the concentration of individual sulfonamides is limited by the drug's solubility, while efficacy reflects the additive activity of all three components. Sodium salts of sulfonamides, which are readily soluble in water, are available for intravenous administration.

The majority of adverse effects to sulfonamides are mild in nature and reversible, although idiosyncratic drug reactions may occur. Urinary tract disturbances, including sulfonamide crystalluria and hematuria, can be minimized in susceptible animals by maintaining an adequate water intake to maintain a high urine flow. Bone marrow depression and dermatologic reactions have also been associated with sulfonamide therapy in animals.

Literature describing sulfonamides and antibacterial diaminopyrimidines in the environment is sparse. The retransformation of N^4-acetylsulfamethazine to the active sulfamethazine during the storage of manure has been reported by Berger and co-workers[178] and may be an important consideration in species such as rabbits[179] and humans, in which N^4-acetylation represents a major metabolic pathway for sulfonamides. Studies into the transport of sulfonamides in runoff water[180] and the movement of sulfonamides through soil[181] indicate that these substances are not highly sorptive. This is consistent with the finding that sulfamethazine and sulfadimethoxine are non-persistent and highly mobile in soil.[101] A UK study reported that the concentrations of sulfadiazine and trimethoprim in surface water did not represent a risk to the environment.[26]

An ADI for sulfamethazine (sulfadimidine) has been allocated by JECFA.[182] The CAC has established MRLs for sulfamethazine in muscle, liver, kidney, and fat of cattle, sheep, pigs, and chickens.[43] A review of the residue studies considered by JECFA has been published.[183]

Table 1.14 lists the properties of sulfonamides and antibacterial diaminopyrimidines.

1.3.14 Tetracyclines

The tetracyclines (Table 1.15) are a large family of antibiotics, the first members of which were derived from the *Streptomyces* genus of *Actinobacteria*. Chlortetracycline was isolated from *Streptomyces aureofaciens* in 1944, and a few years later, oxytetracycline and demeclocycline were

TABLE 1.14 Sulfonamides and Antibacterial Diaminopyrimidines

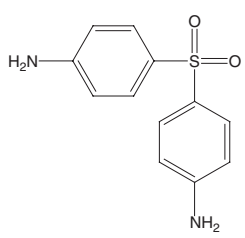
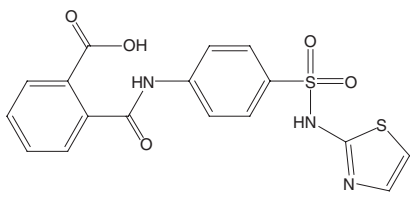
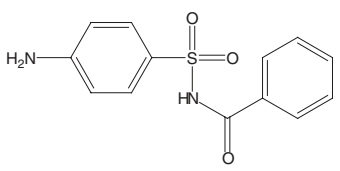
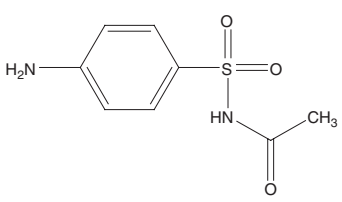
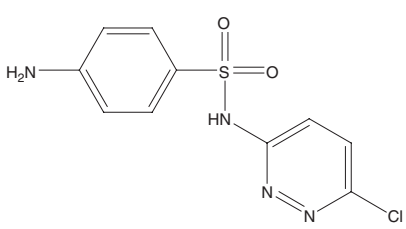
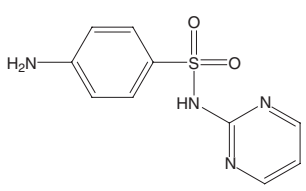
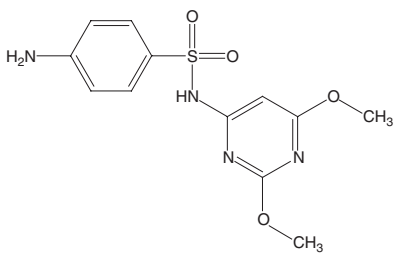
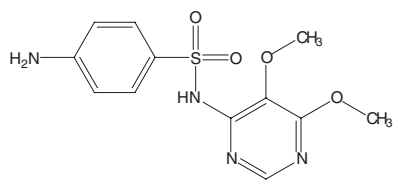
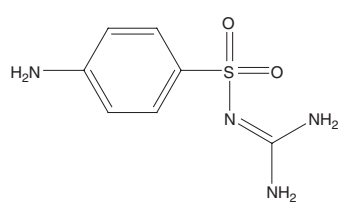
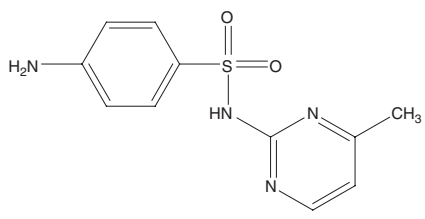
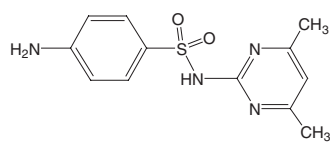
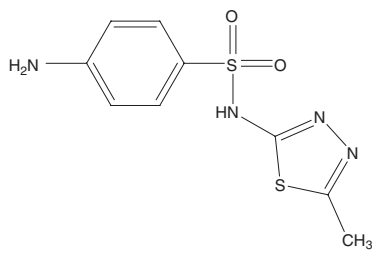
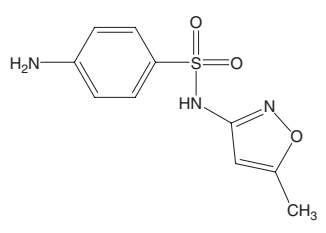
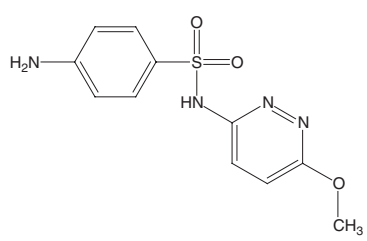
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
	Sulfonamides		
Dapsone	4-[(4-Aminobenzene)sulfonyl]aniline $C_{12}H_{12}N_2O_2S$ 80-08-0		HB^+ 1.3,[56] HB^+ 2.5[56]
Phthalylsulfathiazole	2-[[[4-[(2-Thiazolylamino)sulfonyl]phenyl]amino]carbonyl]benzoic acid $C_{17}H_{13}N_3O_5S_2$ 85-73-4		N/A[a]
Sulfabenzamide	*N*-[(4-Aminophenyl)sulfonyl]benzamide $C_{13}H_{12}N_2O_3S$ 127-71-9		N/A[a]
Sulfacetamide	*N*-((4-Aminophenyl)sulfonyl)acetamide $C_8H_{10}N_2O_3S$ 144-80-9		HA 2.0,[56] HB^+ 5.3[47]
Sulfachloropyridazine	4-Amino-*N*-(6-chloropyridazin-3-yl)benzenesulfonamide $C_{10}H_9ClN_4O_2S$ 80-32-0		HA 6.1[56]
Sulfadiazine	4-Amino-*N*-pyrimidin-2-yl-benzenesulfonamide $C_{10}H_{10}N_4O_2S$ 68-35-9		HA 6.5[56]
Sulfadimethoxine	4-Amino-*N*-(2,6-dimethoxypyrimidin-4-yl)benzenesulfonamide $C_{12}H_{14}N_4O_4S$ 122-11-2		HB^+ 2.0,[56] HA 6.7[56]

TABLE 1.14 (*Continued*)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Sulfadoxin	4-Amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)benzenesulfonamide $C_{12}H_{14}N_4O_4S$ 2447-57-6		N/A[a]
Sulfaguanidine	4-Amino-*N*-[amino(imino)methyl]benzenesulfonamide $C_7H_{10}N_4O_2S$ 57-67-0		HB^+ 2.4[56]
Sulfamerazine	4-Amino-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide $C_{11}H_{12}N_4O_2S$ 127-79-7		HB^+ 2.3,[56] HA 7.0[56]
Sulfamethazine (sulfadimidine)	4-Amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide $C_{12}H_{14}N_4O_2S$ 57-68-1		HB^+ 2.4,[56] HA 7.4[56]
Sulfamethizole	4-Amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)benzenesulfonamide $C_9H_{10}N_4O_2S_2$ 144-82-1		HA 5.4[56]
Sulfamethoxazole	4-Amino-*N*-(5-methyl-3-isoxazolyl)-benzenesulfonamide $C_{10}H_{11}N_3O_3S$ 723-46-6		HA 5.6[56]
Sulfamethoxy-pyridazine	4-Amino-*N*-(6-methoxypyridazin-3-yl)benzenesulfonamide $C_{11}H_{12}N_4O_3S$ 80-35-3		HA 7.2[56]

(*continued*)

TABLE 1.14 (*Continued*)

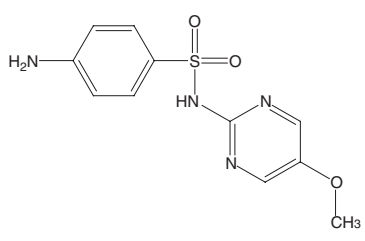
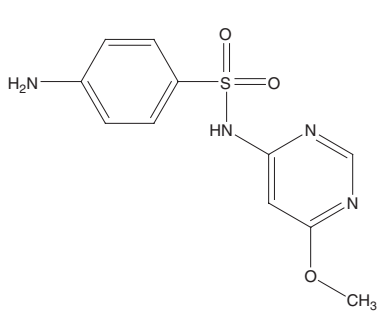
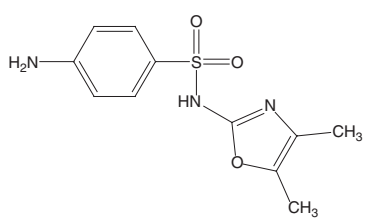
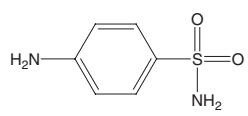
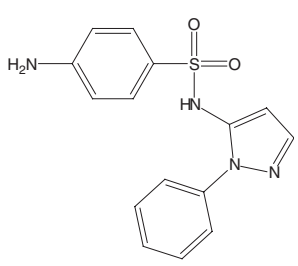
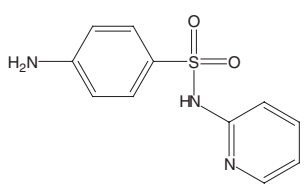
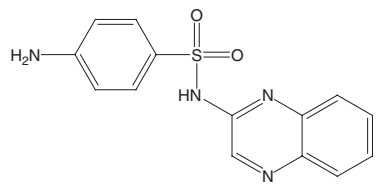
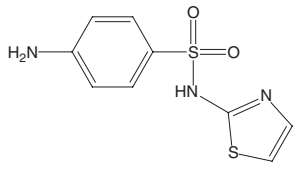
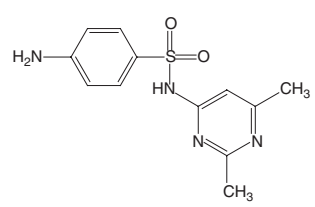
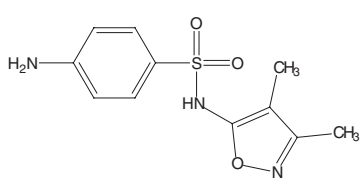
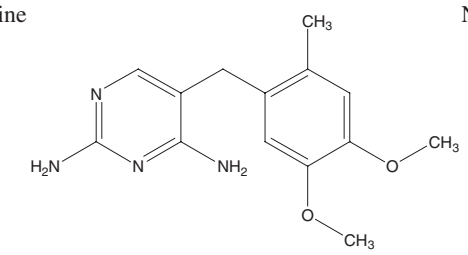
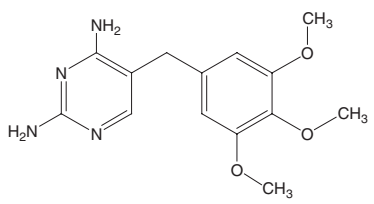
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Sulfamethoxydiazine (sulfameter)	4-Amino-*N*-(5-methoxy-2-pyrimidinyl)benzenesulfonamide $C_{11}H_{12}N_4O_3S$ 651-06-9		HA 6.8[56]
Sulfamonomethoxine	4-Amino-*N*-(6-methoxy-4-pyrimidinyl)benzenesulfonamide $C_{11}H_{12}N_4O_3S$ 1220-83-3		HA 5.9[56]
Sulfamoxole	4-Amino-*N*-(4,5-dimethyl1,3-oxazol-2-yl)benzenesulfonamide $C_{11}H_{13}N_3O_3S$ 729-99-7		N.A.*
Sulfanilamide	4-Aminobenzenesulfonamide $C_6H_8N_2O_2S$ 63-74-1		HB^+ 2.4[56]
Sulfaphenazole	4-Amino-*N*-(1-phenyl-1*H*-pyrazol-5-yl)benzenesulfonamide $C_{15}H_{14}N_4O_2S$ 526-08-9		HA 5.7[56]
Sulfapyridine	4-Amino-*N*-pyridin-2-yl-benzenesulfonamide $C_{11}H_{11}N_3O_2S$ 144-83-2		HB^+ 1.0,[56] HB^+ 2.6,[56] HA 8.4[56]
Sulfaquinoxaline	4-Amino-*N*-2-quinoxalinylbenzenesulfonamide $C_{14}H_{12}N_4O_2S$ 59-40-5		N/A[a]

TABLE 1.14 (***Continued***)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Sulfathiazole	4-Amino-*N*-(1,3-thiazol-2-yl)benzenesulfonamide $C_9H_9N_3O_2S_2$ 72-14-0		HA 7.1[56]
Sulfisomidine	4-Amino-*N*-(2,6-dimethylpyrimidin-4-yl)benzenesulfonamide $C_{12}H_{14}N_4O_2S$ 515-64-0		HA 7.6[56]
Sulfafurazole (sulfisoxazole)	4-Amino-*N*-(3,4-dimethyl-1,2-oxazol-5-yl)benzenesulfonamide $C_{11}H_{13}N_3O_3S$ 127-69-5		HA 5.0[56]
	Antibacterial Diaminopyrimidines		
Ormetoprim	5-(4,5-Dimethoxy-2-methylbenzyl)-2,4-diaminopyrimidine $C_{14}H_{18}N_4O_2$ 6981-18-6		N/A[a]
Trimethoprim	5-[(3,4,5-Trimethoxyphenyl)methyl]pyrimidine-2,4-diamine $C_{14}H_{18}N_4O_3$ 738-70-5		HB^+ 6.6[56]

[a]The author was not able to find a pK_a value for the substance in the public literature.

isolated from *Streptomyces rimosus* and *Streptomyces aureofaciens*, respectively. Tetracycline is sourced from the hydrogenolysis of chlortetracycline, while doxycycline is a semi-synthetic derivative of oxytetracycline that was developed in the early 1960s. The glycylcyclines are a new subgroup of tetracyclines that emerged with the introduction of tigecycline in 2005.

Tetracyclines are broad-spectrum antibiotics that inhibit protein synthesis—a mechanism that involves reversible binding of the drug to receptors of the 30*S* ribosomal subunit of susceptible organisms. This, in turn, blocks binding of the aminoacyl-tRNA to the acceptor site on the mRNA-ribosomal complex and prevents the addition of new amino acids to the peptide chain. Tetracyclines display bacteriostatic activity but can be bactericidal to sensitive organisms at high concentrations. They are more effective against organisms that are rapidly replicating. Tetracyclines exhibit activity against Gram-positive and Gram-negative bacteria, including some anaerobes. Susceptible organisms include *Escherichia coli, Klebsiella* species, *Pasteurella* species, *Salmonella* species, and *Streptococcus* species. Tetracyclines are also active against chlamydia, mycoplasmas, some protozoa, and several rickettsiae.

Resistance to tetracyclines is conferred on bacteria by at least three mechanisms. One mechanism involves the efflux of tetracyclines from bacterial cells and is the

TABLE 1.15 Tetracyclines

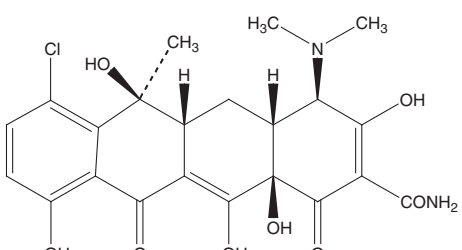
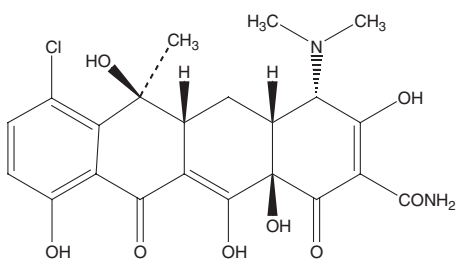
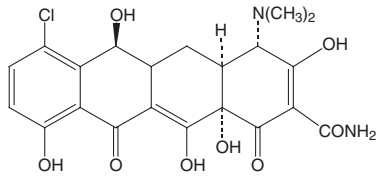
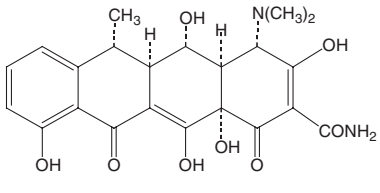
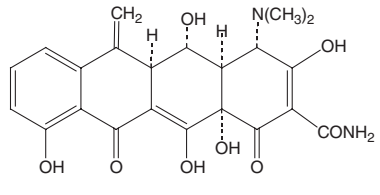
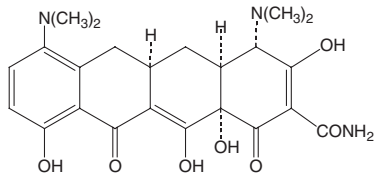
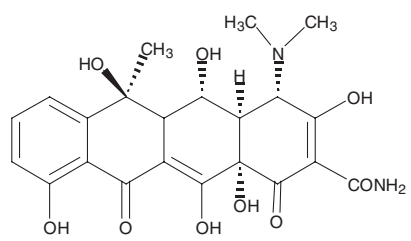
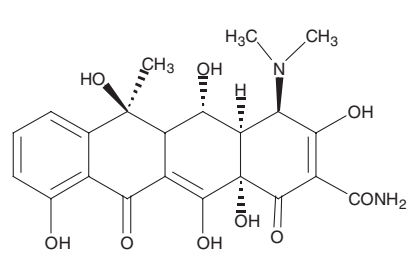
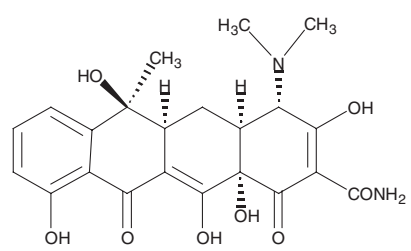
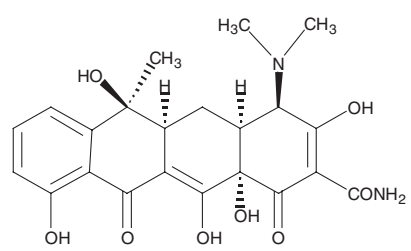
INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Chlortetracycline	(4*S*,4a*S*,5a*S*,6*S*,12a*S*,*Z*)-2-[amino(hydroxy)methylene]-7-chloro-4-(dimethylamino)-6,10,11,12a-tetrahydroxy-6-methyl-4a,5,5a,6-tetrahydrotetracene-1,3,12(2*H*,4*H*,12a*H*)-trione $C_{22}H_{23}ClN_2O_8$ 57-62-5		HA 3.3,[56] HA 7.4,[56] HB^+ 9.3[56]
4-epi-Chlortetracycline	4*R*,4a*S*,5a*S*,6*S*,12a*S*)-7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate $C_{22}H_{23}ClN_2O_8$ 14297-93-9		HA 3.7,[56] HA 7.7,[56] HB^+ 9.2[56]
Demeclocycline	(2*E*,4*S*,4a*S*,5a*S*,6*S*,12a*S*)-2-[Amino(hydroxy)methylidene]-7-chloro-4-(dimethylamino)-6,10,11,12a-tetrahydroxy-1,2,3,4,4a,5,5a,6,12,12a-decahydrotetracene-1,3,12-trione $C_{21}H_{21}ClN_2O_8$ 127-33-3		HA 3.3,[56] HA 7.2,[56] HB^+ 9.3[56]
Doxycycline	4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide $C_{22}H_{24}N_2O_8$ 564-25-0		HA 3.2,[56] HA 7.6,[56] HB^+ 8.9,[56] HA 11.5[56]
Methacycline	(2*Z*,4*S*,4a*R*,5*S*,5a*R*,12a*S*)-2-(Amino-hydroxymethylidene)-4-dimethylamino-5,10,11,12a-tetrahydroxy-6-methylidene-4,4a,5,5a-tetrahydrotetracene-1,3,12-trione $C_{22}H_{22}N_2O_8$ 914-00-1		HA 3.5,[56] HA 7.6,[56] HB^+ 9.5[47]
Minocycline	2*E*,4*S*,4a*R*,5aS,12a*R*)-2-(Amino-hydroxy-methylidene)- 4,7-bis(dimethylamino)-10,11,12a-trihydroxy-4a,5,5a,6-tetrahydro-4H-tetracene- 1,3,12-trione $C_{23}H_{27}N_3O_7$ 10118-90-8		HA 2.8,[56] HA 5.0,[56] HA 7.8,[56] HB^+ 9.5[56]

TABLE 1.15 (***Continued***)

INN	IUPAC Name, Molecular Formula, and CAS Registry No.	Chemical Structure	pK_a
Oxytetracycline	[4S-(4α,4aα,5α,5aα,-6β, 12aα)]-4-(Dimethylamino)-1,4,4a,5,5a, 6,11,12a-octa-hydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarbox-amide $C_{22}H_{24}N_2O_9$ 79-57-2		HA 3.3,[56] HA 7.3,[56] HB^+ 9.1[56]
4-epi-Oxytetracycline	[4*S*-(4α,4aα,5α,5aα,-6β,12aα)]-4-(Dimethylamino)-1,4,4a,5,5a, 6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide $C_{22}H_{24}N_2O_9$ 14206-58-7		N/A[a]
Tetracycline	[4*S*-(4α,4aα,5aα,6β,-12aα)]-4-(Dimethylamino)-1,4,4a,5,5a,6,-11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide $C_{22}H_{24}N_2O_8$ 60-54-8		HA 3.3,[56] HA 7.7,[56] HB^+ 9.7[56]
4-epi-Tetracycline	[4*S*-(4α,4aα,5aα,6β,-12aα)]-4-(Dimethylamino)-1,4,4a,5,5a,6,-11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide $C_{22}H_{24}N_2O_8$ 79-85-6		HA 4.8,[56] HA 8.0,[56] HB^+ 9.3[56]

[a]The author was not able to find a pK_a value for the substance in the public literature.

result of a resistance gene encoding for a membrane protein that actively pumps the drug out of bacterial cells. Another mechanism involves the overexpression of a gene encoding for a protein that prevents tetracyclines from binding to bacterial ribosomes. The rarest form of resistance involves the acetylation of tetracycline, which inactivates the drug.

The tetracycline derivatives are amphoteric substances that can form salts with both acids and bases. Hydrochloride is the most common salt form and is used in a variety of dosage forms, including medicated feeds, soluble powders, tablets and boluses, intrauterine infusions, intramammary infusions, and injections. Because the tetracyclines are relatively inexpensive, they tend to be used as first-line antimicrobials, especially in ruminants and pigs. Uses include those for acute uterine infections; actinobacillosis; anaplasmosis; bacterial enteritis; *Clostridium* diseases; diphtheria; infectious keratoconjunctivits; pneumonia; pododermatitis; skin and soft tissue infections in cattle; bacterial arthritis, bacterial enteritis, and vibrionic abortion in sheep; and atrophic rhinitis, bacterial enteritis, erysipelas, leptospirosis, mastitis, and pneumonia in pigs. Tetracyclines are administered to chickens for bacterial enteritis, fowl cholera, chronic respiratory disease, and infectious sinusitis; to salmon for furunculosis, bacterial haemorrhagic septicaemia, and pseudomonas disease; and to honeybees for American and European foulbrood disease, although strains of *Paenibacillus larvae* spp., the causative organism of American foulbrood disease in honeybees, are becoming increasingly resistant to oxytetracycline. Tetracyclines are

also used for improved feed efficiency in cattle, chickens, pigs, sheep, and turkeys.

The rapid intravenous administration of tetracyclines causes acute toxicity in most animal species. In horses, intravenous doxycycline has caused cardiovascular dysfunction, collapse, and death. Long-acting formulations of oxytetracycline administered intramuscularly may cause local irritation at the site of injection in food-producing species. Tetracycline administration causes overgrowth of nonsusceptible organisms in several species of animals and, in horses, may result in colitis and severe diarrhea. Tooth discoloration in young animals may result when tetracyclines are administered during late pregnancy or during the period of tooth development.

A relatively small number of environmental studies in the literature relate to tetracyclines. The sorption behavior of these drugs is characterized by persistence and low mobility[101] and accounts for their superficial location in soil profiles[184] and their paucity in runoff water.[180] The enviromental fate of oxytetracycline used in aquaculture was extensively researched (cited by Boxall[26]). In these studies, oxytetracycline has been detected in wild fauna and in the sediment around fish farms. Soil samples collected from regions with intensive livestock production in Germany[184] and the UK[88] have been shown to contain tetracyclines, which possibly originate from manure.

A group ADI for tetracycline, oxytetracycline, and chlortetracycline has been allocated by JECFA.[185] The CAC has also established MRLs for tetracycline, oxytetracycline and chlortetracycline applicable to cattle, sheep, pigs, and poultry; and to fish and giant prawn for oxytetracycline only.[43] JECFA has prepared a number of reviews detailing residue studies on tetracyclines that support the development of the MRLs adopted by the CAC.[186–191]

From an analytical perspective, tetracyclines are relatively stable in acids but not in bases, and they can decompose rapidly under the influence of light and atmospheric oxygen.[152] Their decomposition is minimized by maintaining standard solutions in amber bottles and by drying samples under nitrogen in a dark room. Tetracyclines are susceptible to conformational degradation to their 4-epimers in aqueous solutions and during sample preparation. For example, Lindsey and coworkers[192] reported that the use of phosphate buffer solutions cause their degradation during the evaporation step.

1.4 RESTRICTED AND PROHIBITED USES OF ANTIMICROBIAL AGENTS IN FOOD ANIMALS

The therapeutic use of a very small number of antimicrobials in food-producing animals is prohibited because of public health concerns. The drugs involved are found on the websites of the regulatory authorities. In the United States, the provisions of the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994 allow for the extralabel (off-label) use of drugs by veterinarians under certain conditions. However, the extralabel use of chloramphenicol, furazolidone, sulfonamide drugs in lactating dairy cattle (except for approved use of sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine), fluoroquinolones, and glycopeptides in food-producing animals is prohibited. The use of chloramphenicol, dapsone, furazolidone, and nitrofurans (except furazolidone) in food-producing species is banned in the EU, as is the use of antimicrobial drugs for growth promotion. In Canada, the use in food-producing animals of chloramphenicol and its salts and derivatives, and the 5-nitrofuran and nitroimidazole compounds is banned, while the sale of carbadox has been prohibited since 2006. In Australia, the use of carbadox, chloramphenicol, nitrofurans (including furazolidone and nitrofurazone), fluoroquinolones, gentamicin, [dihydro]streptomycin, and several sulfonamides is not permitted in food-producing animals. Currently, the use of carbadox is approved in pigs in the United States, while olaquindox is approved for use in pigs in Australia.

Several countries have acted to reduce the agricultural use of antimicrobial agents, and this has resulted in the discontinuation of some antimicrobial growth promotion uses. The UK banned the use of penicillin and the tetracyclines for growth promotion in the early 1970s; other European countries followed suit shortly thereafter. Sweden banned the use of all antibiotics for growth promotion in 1986. In the EU, the approval of avoparcin was withdrawn in 1998; the productivity claims for bacitracin, spiramycin, tylosin, and virginiamycin were discontinued in 1999; and the productivity claims for avilamycin, flavomycin, monensin, and salinomycin were discontinued in 2006. In the United States, the extralabel (off-label) use of drugs in treating food-producing animals for improving weight gain, feed efficiency, or other production purposes is prohibited under AMDUCA. In Australia, the registration of avoparcin was withdrawn in 2000, and the use of virginiamycin as a growth promoter was discontinued in 2005.

1.5 CONCLUSIONS

Antimicrobial drug use in food animal production is fundamental to animal health and well-being and to the economics of the livestock industry. Therefore the prudent use of antimicrobials is critically important because few new drugs are entering the market, and existing uses need to be preserved for as long as is practicable. Prudent use will minimize the development of antimicrobial resistance and maximize therapeutic effect. When introducing new products onto the market, pharmaceutical companies need to rule out the presence of cross-resistance to old products

in the same class, some of which may no longer be used in animals. From a food safety perspective, responsible use of antimicrobials in food-producing species as reflected by the results of residue-monitoring programs is of paramount importance to reassure the community that the food supply is safe.

In conclusion, this chapter has discussed the major antibiotic classes used in food-producing species and in particular, the PD component depicted in the chemotherapeutic triangle (Fig. 1.1). The antimicrobial potency of antimicrobial drugs to bacterial isolates is characterized using *in vitro* MIC and/or MBC values. The killing kinetics of an antibiotic, which are the basis for determining whether the antibacterial effect of a drug is concentration-dependent, time-dependent, or co-dependent, are also established *in vitro*. While this information is fundamental to antimicrobial therapy, when considered in isolation, it is insufficient to predict effectiveness *in vivo*. Both the dosage regimen and the PK of the drug are important determinants of drug concentration at the infection site (the biophase). These important topics are discussed in Chapter 2.

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