Bacterial Topoisomerase Inhibitors: Quinolone and Pyridone Antibacterial Agents

Lester A. Mitscher*

Departments of Medicinal Chemistry and Molecular Biosciences and The Chemical Methodologies and Library Development Center of Excellence, The University of Kansas, 4010 Malott Hall, 1251 Wescoe Hall Drive, Lawrence, Kansas 66045-7582

Received May 10, 2004

Contents

* Phone: (785) 864-4562. Fax: (785) 864-5326. E-mail: lmitscher@ku.edu.

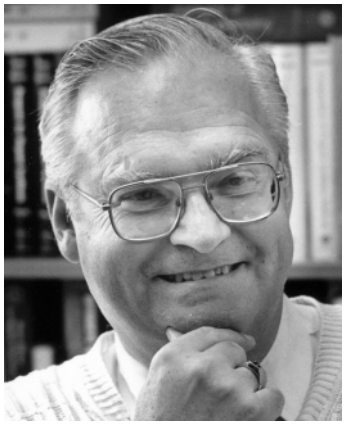
1. Introduction: History and Overview

The quinolone anti-infective agents are of wholly synthetic origin and are not modeled knowingly after any natural antibiotic. Several ring systems are or have been involved. Those of greatest prominence and their numbering systems are illustrated in Figure 1. The reader will note that the numbers assigned to analogous positions frequently change when different quinolone ring systems are considered.

Of all the totally synthetic antimicrobial agents, the (fluoro)quinolones have proven to be the most successful economically and clinically. They are orally and parenterally active, have a broad antimicrobial spectrum that includes many frequently encountered pathogens, are bactericidal in clinically achievable doses, generate comparatively tolerable resistance levels, possess a fascinating molecular mode of action, are comparatively easily synthesized, and with a few notable exceptions are safe. That is not to say that they are perfect drugs and cannot be improved but rather that they are important weapons in the ongoing struggle against morbidity and mortality caused by microbial pathogens. Consequently, from a slow beginning as a modest group of urinary tract disinfectants they have grown to be a group of nearly two dozen institutional and office practice agents of which ciprofloxacin and levofloxacin, most notably, have become billion dollar agents persistently found among the top 200 most frequently prescribed medications in North America and, indeed, worldwide.

This paper presents an overview of this important topic with an emphasis on recent developments. Reflecting their importance and the high interest in quinolones, they have been the subject of numerous books[1–9] and recent reviews.[10–26] These books and reviews can be consulted for further information and differing opinions about them.

10.1021/cr030101q CCC: $53.50
Published on Web 01/22/2005



Lester A. Mitscher received his Ph.D. degree in chemistry in 1958 from Wayne State University, Detroit, where he worked on the structure of coffee oil diterpenes and on optical rotatory dispersion. He continued his work on natural product chemistry at Lederle Laboratories, where he rose to group leader in antibiotic discovery until he accepted an Associate Professorship in Natural Products Chemistry at The Ohio State University (1967), rising soon to the position of Professor. In 1975 he accepted a University Distinguished Professorship and Chairmanship in the Department of Medicinal Chemistry at Kansas University. He returned to the faculty in 1991, where he remains. His academic studies have centered around spectroscopy, synthesis, screening, and structure determination primarily of naturally occurring antimicrobial and antimutagenic agents. He has published actively in the quinolone field since 1965. He consults extensively in the pharmaceutical industry and is a member of the National Institutes of Health Drug Discovery and Antimicrobial Resistance Study Section. His research awards include the Smissman Award in Medicinal Chemistry (American Chemical Society), Volweiler Award (American Association for Pharmaceutical Education), Research Achievement Award in Natural Products Chemistry (American Pharmaceutical Association), and Award in Medicinal Chemistry, Medicinal Chemistry Division, American Chemical Society, and he is an Elected Fellow of the American Association for the Advancement of Science.

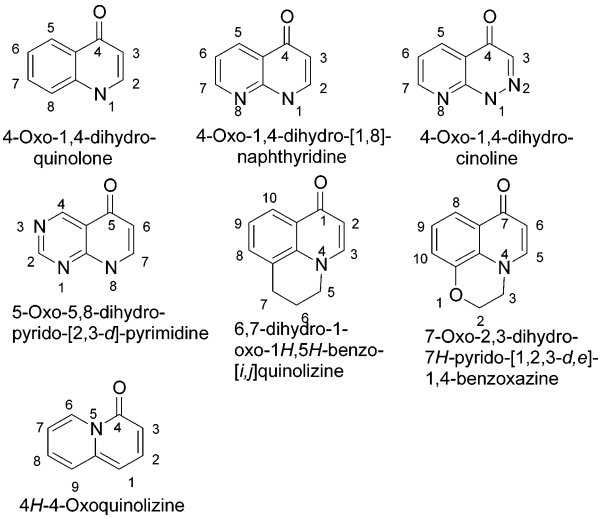


Figure 1. Structures and numbering systems of the most significant ring systems in the antibacterial quinolone family.

The first antimicrobial quinolone was discovered about 50 years ago as an impurity in the chemical manufacture of a batch of the antimalarial agent chloroquine (Figure 2).[27] It demonstrated anti Gram-negative antibacterial activity, but its potency and antimicrobial spectrum were not significant enough to be useful in therapy. Building on this lead, however, subsequently nalidixic acid was commercialized. Nalidixic acid remains on the market today and

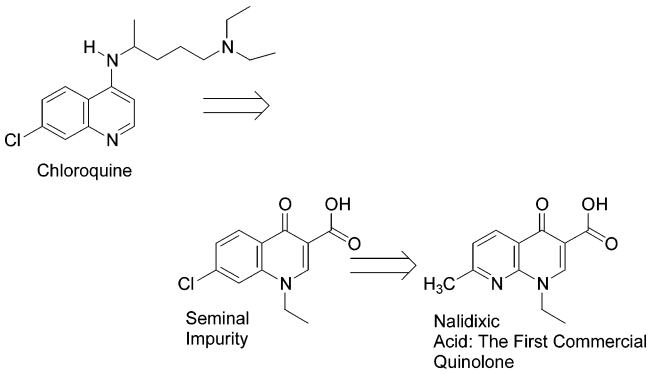


Figure 2. Origin of the quinolones.

represents the so-called first generation quinolones. Despite its convenient oral activity, bactericidal action, and ease of synthesis, its limited antimicrobial spectrum (primarily activity against *Escherichia coli*) and poor pharmacokinetic characteristics limit its use primarily to treatment of sensitive community-acquired urinary tract infections. For a few years research on analogues resulted mainly in the introduction of competing products with enhanced though still moderate activity against Gram-negatives and a sniff of anti Gram-positive activity, but these agents were also used primarily for urinary tract infections of community origin. Sales of this group of agents never became impressive.

This picture changed significantly with the discovery of norfloxacin, the first of the second-generation family of quinolones.[28] This agent had dramatically enhanced and broader spectrum anti Gram-negative activity and possessed significant anti Gram-positive activity as well. The potency of norfloxacin was in the same range as that of many fermentation-derived antibiotics, and its comparative structural simplicity and synthetic accessibility lead to a very significant effort to find even more improved analogues. Norfloxacin and its *N*-methyl analogue pefloxacin ultimately failed to find major use outside of the genitourinary tract because of poor active blood levels and limited potency against Gram-positives.

Shortly thereafter, ciprofloxacin[29−31] and ofloxacin,[31,32] as well as its optically active form levofloxacin,[33] were introduced. The second-generation agents have significant broad-spectrum antimicrobial activity including important Gram-positive pathogens. This is coupled with gratifying safety and pharmacokinetic characteristics. These agents have found excellent acceptance as office practice anti-infective agents worldwide, and ciprofloxacin and levofloxacin are regularly found among the top 100 most frequently prescribed drugs in North America. Gatifloxacin and ofloxacin have also appeared among the top 200 during the past decade but have subsequently fallen in popularity.

A wide variety of clinical indications have been approved for quinolones including many infections commonly encountered in community practice including upper and lower respiratory infections, gastrointestinal infections, gynecologic infections, sexually transmitted diseases, prostatitis, and some skin, bone, and soft tissue infections.[34]

Recently introduced members of the fluoroquinolone family belong to the third generation. These include gatifloxacin[35] and moxifloxacin,[36] which possess further enhanced activity against Gram-positive infections, and anti-anaerobic coverage is now present although at present only trovafloxacin[37] is approved for this indication. Among the agents still in preclinical study, clinifloxacin[38] is the most promising anti-anaerobic agent.

When first introduced, there was no idea of the molecular mode of action of these agents. Indeed, the availability of nalidixic acid was instrumental in assisting the discovery of the targeted bacterial type II topoisomerases.[39] Those of importance to the quinolones are bacterial topoisomerase II,[40] also known as DNA gyrase, and bacterial topoisomerase IV.[41,42] These enzymes are vital for dictating the proper topology of DNA important for protein biosynthesis, DNA replication and repair, and DNA decatenation. Fluoroquinolones form a ternary complex consisting of drug, DNA, and enzyme that interferes with DNA transcription, replication, and repair and promotes its cleavage, leading to rapid bacterial cell death. They are without apparent significant action on individual molecules of DNA or topoisomerases alone, but the interaction of DNA with enzyme creates a binding pocket for the quinolones. The ternary complex is rapidly bactericidal through processes that are not completely understood.

As with the aminoglycoside class of antibiotics, bacterial killing with fluoroquinolones is concentration-dependent rather than dosage-interval-dependent and the fluoroquinolones possess a significant postantibiotic action lasting for 1 or 2 h.[43] Although the distinction is not precise, generally anti Gram-negative activity is more closely associated with DNA gyrase inhibition and anti Gram-positive activity is more closely associated with bacterial topoisomerase IV inhibition.[44] With a number of quinolones activity is attributed to interference with the function of both of these enzymes. For example, a survey of the ability of a collection of quinolones to inhibit the catalytic action of topoisomerases showed that the ratio of DNA gyrase to topoisomerase IV action for *E. coli* was between about 15 and 27, whereas for *Staphylococcus aureus* the ratio was reversed, with topoisomerase IV inhibition over DNA gyrase inhibition being from about 1.7 to 21.[45] In a later study of 15 quinolones, they were divided into three groups on the basis of their relative ability to inhibit *S. aureus* strains with a resistance mutant toward one or the other enzyme. With group I (norfloxacin, enoxacin, fleroxacin, ciprofloxacin, lomefloxacin, trovafloxacin, grepafloxacin, ofloxacin, and levofloxacin) topoisomerase IV was the more sensitive target. With group 2 (sparfloxacin and nadifloxacin) DNA gyrase was the more sensitive target. With group 3 (gatifloxacin, pazufloxacin, moxifloxacin, and clinafloxcin) both were equivalently sensitive. The latter were termed the dual targeting quinolones.[42] This classification holds up better against the intact microorganisms than it does against the purified enzymes. Human topoisomerase II is generally not inhibited by these agents at the doses normally employed since it is often at least 100−1000 times less sensitive to them.[46] Despite significant homologies with the bacterial enzyme, creative analoguing is able to distinguish clearly between them, and safe agents are readily produced. The novel molecular mode of action of the quinolones helps to account for their popularity. Unfortunately, combination of quinolones with other anti-infective agents is not reliably synergistic.

Resistance in the clinic to this class of anti-infective agents was comparatively slow to develop but is now worrisome.[47] There are no clear-cut examples of resistance due to bacterial modification of the chemical structures of fluoroquinolones. Rather, resistance is most commonly associated with genetic-based alterations in the topoisomerases, resulting in decreased drug binding[48] and, particularly, with efflux of these agents from bacterial cells before they reach their intercellular targets.[49] Decreased porin presence also decreases their uptake. Active uptake seems not to be a significant factor in their absorption although it is believed to contribute to distribution and excretion.

Absorption of quinolones following oral administration is usually good, mostly 50% or better, and, in some cases, in excess of 95%.[50] Most of the available literature is consistent with passive absorption. They are well distributed in the body; however, efflux pumps protect the central nervous system to some extent.[51] Comparatively little metabolism takes place with them, and excretion is mostly in active form in the urine. Active excretion into the urine takes place to some extent and is modestly enantioselective.[52]

They are generally safe and well-tolerated drugs. With few exceptions, their side effects are mostly annoying rather than severe although the list is comparatively long. Side effects include GI, CNS, rashes and photosensitivity, arthropathy, arthralgia and joint swelling, and interactions with various drugs. These occur to a greater or lesser extent with all the members of this family but are more pronounced with certain individual agents.[13]

Recently, a number of comparatively rare but severe toxicities have been observed with particular quinolones. For example, trovafloxacin can cause severe enough liver toxicity to require transplant or cause death,[53] temafloxacin has been associated with a collection of severe problems involving kidney failure, hemolysis, thrombocytopenia, and disseminated intravenous coagulation, leading to a number of deaths,[54] sparfloxacin and grepafloxacin cause significant prolongation of the cardiac QT interval,[55] and the C-8 halogenated members have been associated with an increased incidence of phototoxicity.[56]

Discovery research has slowed a bit recently, but many agents are at various stages of clinical development. The field promises to remain active well into the foreseeable future.

2. Synthetic Chemistry

Intense research in this therapeutic area has now resulted in the introduction of nearly two dozen competing agents into the clinic, and these agents return very significant profits to the firms responsible for them. Analogues are relatively accessible in

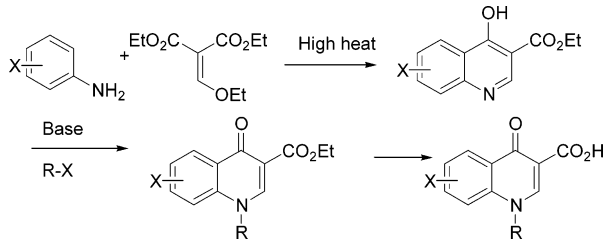


Figure 3. Basic Gould–Jacobs reaction.

substantial variety by short syntheses. As a consequence, tens of thousands of analogues have now been prepared and tested. The deficiencies being addressed by synthetic campaigns include the desire to address worries about drug resistance, the need to avoid toxicities, the ability to administer both orally and parenterally, one-a-day administration, freedom from drug–drug interactions, and the desire to include anaerobic microorganisms and other presently relatively insensitive pathogens in their activity spectrum.

2.1. Gould–Jacobs Reaction

The synthetic chemistry that makes all of this activity possible consists fundamentally of variants on a few pathways. The original method was the well-precedented Gould–Jacobs reaction between suitably substituted anilines and a substituted ethylenemalonate analogue at high temperature. This is illustrated in Figure 3.[57] The initial reaction is an addition–elimination sequence followed by cycloacylation. The specific nature of the product depends on the symmetry properties of the starting aniline, and its facility depends on the degree and position of maximum electron richness of the ring. Alkylation follows. This requires an alkyl halide or its equivalent capable of S_N2 displacement. This largely restricted the products to *N*-alkyl groups from which ethyl or bioisosteric analogues thereof (*O*-methyl and *N*-methyl, for example) proved commercially significant. The synthesis concludes with an ester hydrolysis.

When X = Cl or F at carbon 7, a nuclear aromatic displacement reaction with a secondary amine conveniently introduces an amino substituent at the C-7 position because of the activation by the C-4 carbonyl substituent. It is expedient to perform the hydrolysis first to avoid an ester–amide exchange. This reaction sequence allows a wide number of analogues to be prepared, variously substituted piperazines and pyrrolidines of which are most significant. Substitutions at other points require variants of this overall process.

2.2. Grohe–Heitzer Reaction

Access to novel quinolones was greatly expanded subsequently by the introduction of the Grohe–Heitzer cycloacylation synthesis (Figure 4).[58] In this process, the aromatic ring is acylated to begin with so the positions of substitution are preset in the starting acid and persist to the end. Commonly, a suitable benzoic acid derivative is first elaborated into benzoylmalonate ester. The active methylene function is then condensed under dehydrating condi-

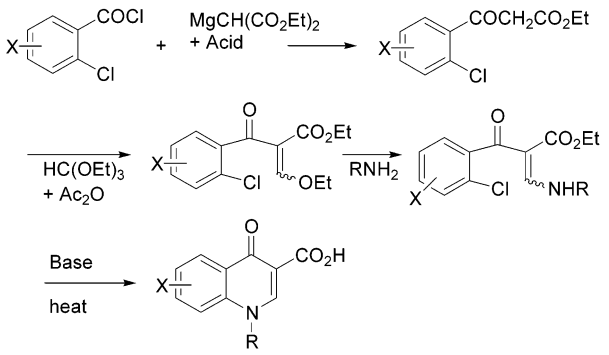


Figure 4. Basic Grohe–Heitzer reaction.

tions with an ortho ester. The resulting enol ether is often subjected to an addition–elimination reaction with a suitable primary amine, and this product can cyclize in a tandom addition–elimination reaction at the *ortho* position. Alternatively, a different primary amine can be used to complete this reaction. These processes establish the pharmacophoric keto acid moiety of the pyridine ring and allow for introduction of a wide variety of *N*-substituents including those possible with the Gould–Jacobs reaction, but now aryl and cycloalkyl substituents that could not be made in that manner are accessible as well. Ciprofloxacin is the most important of this group and bears an *N*-cyclopropyl substituent. Temafloxacin and trovafloxacin have *N*-fluoroaromatic substituents, and their preparation was also made possible by developing this reaction. This important reaction is now very widely employed for the preparation of analogues, including even *N-tert*-butyl-bearing substances.[59]

An adaptation of the Grohe–Heitzer synthesis to synthesis on beads employing traceless linker combinatorial methods has been reported.[60] Use of the Groehe–Heitzer method in a solution-phase multiple parallel synthesis has also been published.[61]

2.3. Gerster–Hayakawa Syntheses

The Gerster–Hayakawa synthesis of the N-1 to C-8 bridged compounds is a variant of the Gould–Jacob reaction (Figure 5). In the Gerster synthesis, a carbocyclic analogue is produced by a Gould–Jacobs reaction using a suitably substituted tetrahydroquinoline synthon.[62] This synthesis was subsequently modified by resolution of the tetrahydroquinolone intermediate using an optically active amino acid ester as a chiral auxiliary. The Gerster chemistry was initially used to produce flumequine, the first fluoroquinolone, the first chiral member of this class, and one of the first rigid analogues involving the N-1 substituent.[62,63] Resolution demonstrated that the absolute configuration of the N-1 substituent was very important for maximal antimicrobial activity. Despite the possession of so many trend-setting characteristics, flumequine has not seen human use.

In the Hayakawa variant, bioisosteric C-1 oxo or C-1 thio analogues became accessible by starting with a nucleophilic aromatic displacement reaction on a fluorinated nitrobenzene synthon (Figure 5).[64]

The rigidification resulting from these two methods led to enhanced potency and greater activity against Gram-positive analogues. These reactions are il-

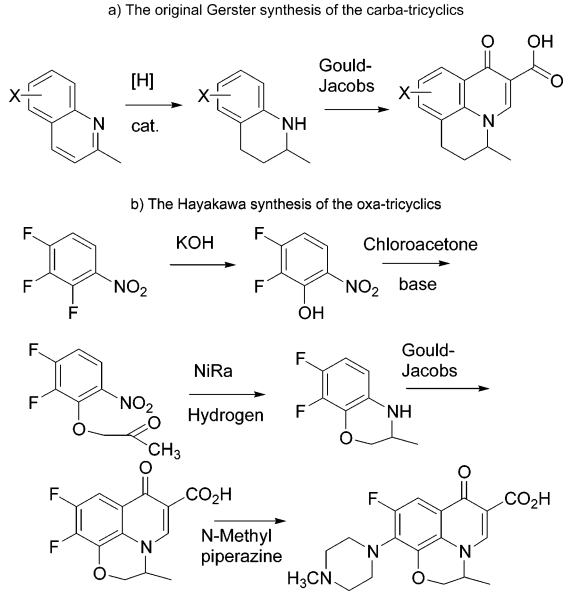


Figure 5. Basic Gerster−Hayakawa syntheses: (a) original Gerster synthesis of the carbatricyclics, (b) Hayakawa synthesis of the oxatricyclics.

lustrated with the synthesis of ofloxacin.[32] Resolution of one of the intermediates led to its more active enantiomer, levofloxacin, one of the market leaders.[33]

2.4. Chu−Mitscher Synthesis

Chu and Mitscher introduced an efficient chiral synthesis of levofloxacin and its analogues (Figure 6).[65] This method uses a chiral α-amino alcohol,

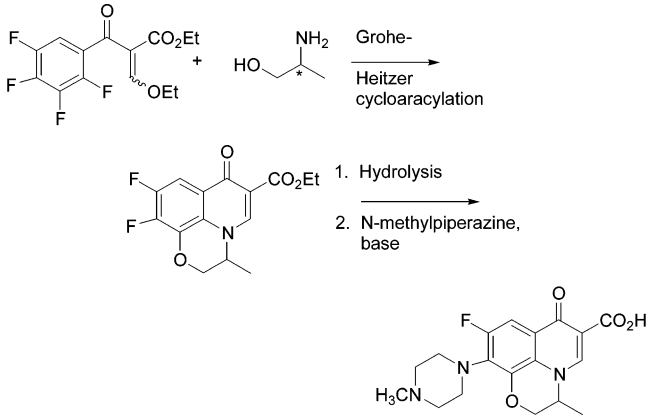


Figure 6. Chu−Mitscher synthesis.

avoiding the necessity of a wasteful resolution step at the end. Starting with optically active alanol and employing a variant of the Grohe−Heitzer cycloarylation, chiral products were produced directly, and the eutomeric configuration was established unambiguously to be *S*. The overall process is short and very efficient. If somewhat forcing conditions are employed in the tandem ring forming reaction, the formation of the benzoxazine ring takes place also and does not require a separate step.

2.5. Chu−Li Syntheses

Synthesis of the investigational 9-cyclopropylpyrimidones required the development of chemistry novel to the quinolone area.[66] First are illustrated

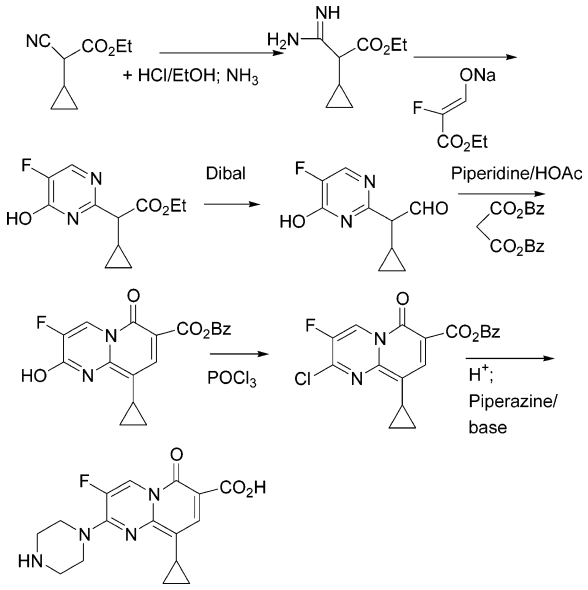


Figure 7. Chu−Li synthesis of *C*-cyclopropylpyridones.

analogues of the naphthyridine series (Figure 7). The pyrimidine ring is assembled on a cyclopropylated cyanoacetyl ester nucleus. Selective reduction to the aldehyde allows formation of an alkylidine malonyl ester. Heating then, analogous to the Gould−Jacobs process, results in aroyl amide formation. Transformation of the hydroxyl moiety to a halogen subsequently enables the necessary nucleophilic aromatic substitution reaction required to complete the synthesis.

Synthesis of 9-difluorophenylpyridopyrimidone analogues follows a closely similar path (Figure 8) but starts with the aryl group in place at the outset.

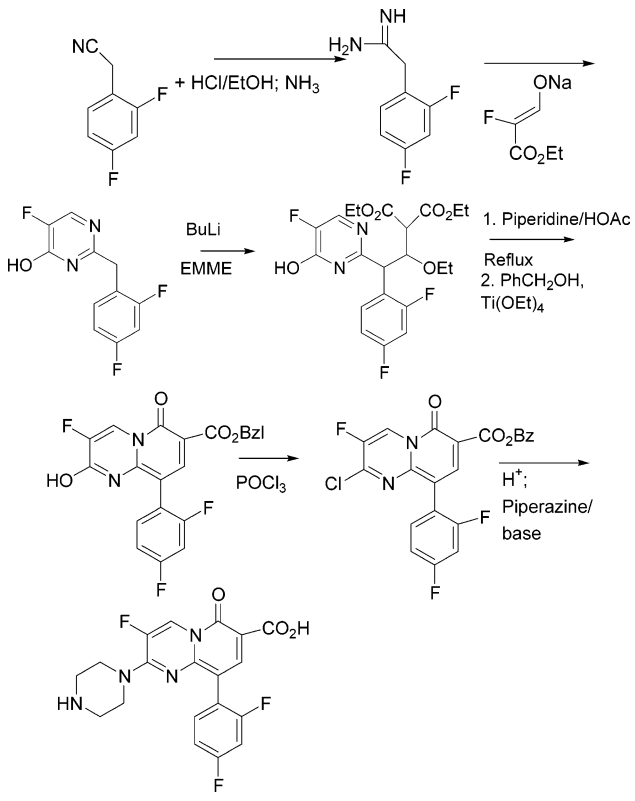


Figure 8. Synthesis of *C*-arylpyridones.

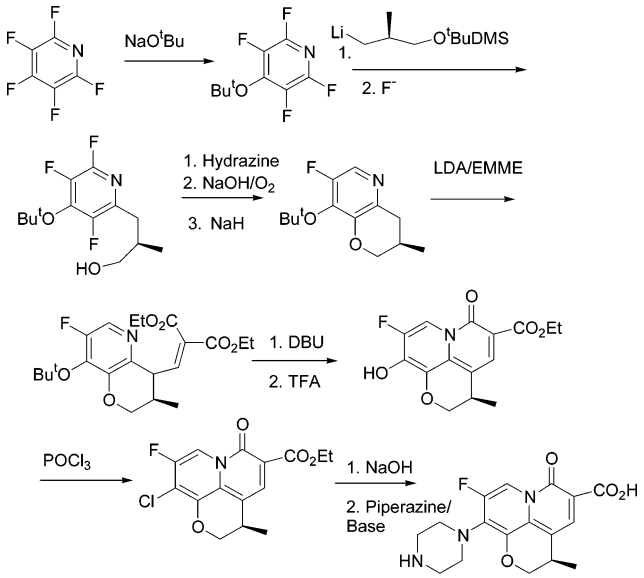


Figure 9. Synthesis of tricyclic pyridone derivatives.

Synthesis of tricyclic 3(*S*)-3-methyl-6-oxo-2,3-dihydro-6*H*-pyrano[2,3,4-*ij*]quinolizinone analogues requires initial replacement of the most reactive fluorine atom of pentafluoropyridine by an oxygen substituent using *tert*-butoxide (Figure 9). This symmetrical product is alkylated and deprotected. Next, reductive removal of the now surplus fluorine on the other side of the pyridine nitrogen is carried out. A nucleophilic aromatic substitution reaction completes the second ring. A carbon version of the usual Gould–Jacobs reaction then follows to produce the tricyclic ring system. Hydrolysis next is followed by a nucleophilic aromatic substitution reaction.

With one or another variation of these flexible syntheses many thousands of pyridone analogues have been prepared and evaluated.

3. Some Quinolone Chemical Reactions of Significance to Their Medicinal Properties

The clinical behavior of the quinolone anti-infectives is strongly affected by a few of their chemical properties.

3.1. Chelation

The carboxylic acid moieties of quinolones form salts with metal ions, particularly in neutral to basic solutions.[67,68] The proximity of the carbonyl group at C-4 leads to electron donation such that strong chelate rings are formed. Chelation with metal ions of higher valence, such as aluminum(III), magnesium(II), calcium(II), iron(II and III), copper(II), and so on, often leads to water-insoluble complexes that can interfere with blood levels following oral coadministration. Quinolones resemble the tetracyclines in this aspect. This is not only inconvenient for formulation but leads to drug–food interactions (especially with dairy products) and to drug–drug interactions, leading to poor blood levels, particularly with co-administration of certain antacids and with hematinics. This problem is alleviated significantly by administration in acidic media. If such co-

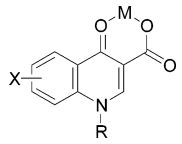


Figure 10. quinolone chelates.

administration cannot be avoided, then a patient who is a good complier with complicated administration regimens can take the ion-rich drug or food 1 h before or 2 h after taking the drug. This should minimize the problem. Some toxic effects of quinolones are exacerbated by this kind of interaction. There are suggestions that photosensitivity is increased under these conditions as is tendon erosion and even mutation to resistance. The chemical nature of the chelates is illustrated in Figure 10.

3.2. Acid–Base Character

Although the first generation of quinolones contains a number of monovalent, acidic examples of largely hydrophobic character, the bulk of the quinolones of present clinical importance are amphoteric substances possessing enhanced hydrophilicity. Consequently, the more recent compounds possess their minimum aqueous solubility in the vicinity of neutral tissue compartments. They are salts at pH extremes and so have better solubility under these generally nonphysiological conditions. Figure 11 illustrates this

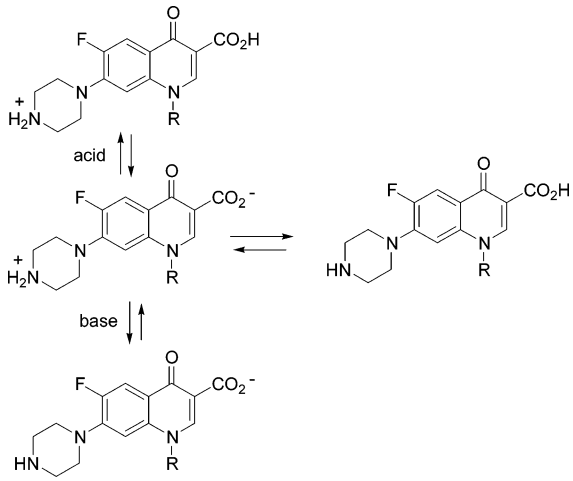


Figure 11. Protonation/deprotonation scheme for quinolones.

with ciprofloxacin and the proton equilibrium it undergoes at various pH levels. At alkaline pH values quinolones form carboxylate salts with reasonable water solubility, and at acidic pH values they form protonated amine salts likewise with reasonable water solubility. At neutral pH values near their isoelectric point they possess two forms in equilibrium. The zwitterion form is primarily responsible for the degree of water solubility that they retain under these conditions. On the other hand, the nonionized form is also populated, and it is this form that is the better absorbed. The particular relevance of these features is that the quinolones are known to enter most mammalian tissues and almost all bacterial cells by a combination of passive uptake or porin

passage.[69,70] Thus, a more accurate prediction of their potential bioavailability following oral administration is obtained by measuring their partition coefficient under physiological conditions. Since they can form insoluble salts with buffer components, particularly when multivalent metal salts are present, the nature of the buffer must be taken into consideration as well.[67,68]

Since it is desired to administer quinolones by injection and this is likely to suffer from the insolubility disadvantage at neutral pH values, it is common to buffer injectable preparations of quinolones at acidic pH values where solubility is much improved, but it is therefore required to infuse them comparatively slowly rather than by push to avoid pain and blood vessel occlusion due to precipitation.

3.3. Photochemistry

Quinolone anti-infectives are often quite photoactive, especially under neutral or acid conditions.[71,72] Product formations involve free radical intermediates, and the nature of the products depends on the structure of the quinolone and the specific conditions employed. Those quinolones substituted with a halogen at C-8 are particularly associated with these reactions, whereas those with C-8 methoxy substitutents are less so.

Many quinolones, particularly those with halogen substituents, absorb light in the 350−425 nm region and are transformed thereby into singlet and triplet states. The triplet state in particular is strongly oxidizing, presumably leading in part to generation of reactive oxygen species, and many of these agents have nucleofugic groups (fluorine and chlorine atoms) and so undergo facile nucleophilic aromatic substitution reactions. When a chlorine atom or a second fluorine atom is present along with additional functional groups able to donate electrons to this site, these tendencies are enhanced.[72] Sparfloxacin, lomefloxacin, and fleroxacin are examples.

Since patients undergoing quinolone therapy may experience photosensitivity, it is believed that this chemistry is relevant to this side effect. One notes in particular that the 350−425 nm wavelength range is that part of visible light associated with suntan and sunburn.

At first glimpse the comparatively ready photochemical defluorination of quinolones is surprising given the normally high stability and strength of the aromatic C−F bond, but the quinolones are substituted in such a manner as to overcome these effects. Once a quinolone radical is formed, the molecule further reacts in one or more of several manners. The *N*-alkyl substituent can be lost entirely or react with the C-8 position to form a new ring, the C-3 carboxyl group can be lost with or without further hydroxylation at either C-2 or C-3, a C-5 or C-6 fluorine can be replaced by a phenolic hydroxyl or a hydrogen, the C-7 aliphatic side chains can undergo a variety of ring cleavages, and C-8 can lose its halogen with or without reaction with the N-1 substituent. These reactions are illustrated in Figures 12−18.

The early quinolones not possessing a C-6 fluorine substituent or a piperazinyl moiety at C-7 underwent

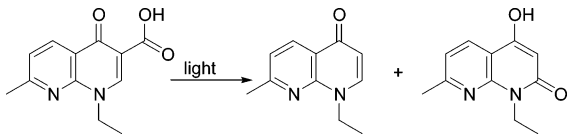


Figure 12. Photochemistry of nalidixic acid, a nonfluorinated quinolone.

decarboxylation, oxidation, and dimerization under photolysis conditions in water.[73] This is illustrated with nalidixic acid, which is transformed primarily to its C-3 H, or to its C-2 keto analogues. These products are antimicrobially inactive (Figure 12). Decarboxylation to a C-3 H analogue can also take place with ciprofloxacin, but this is a minor product. The point of origin of the triggering radical is not very obvious but can be posited to involve chelation with suitable transition-state metals, such as ferrous iron.

With ofloxacin the more significant photolysis product is one where the piperazinyl moiety at C-7 has been oxidatively cleaved.[74] When an electron-donating group is attached to C-8, as with ofloxacin, levofloxacin, and moxifloxacin, the products observed often involve such bond cleavages in the C-7 side-chain moiety.[75] This tendency is exaggerated in acidic solutions. T-3761, however, has a rather different C-7 amino substituent, and on photolysis it produces a C-3 OH analogue instead (Figure 13).[76] The analogy

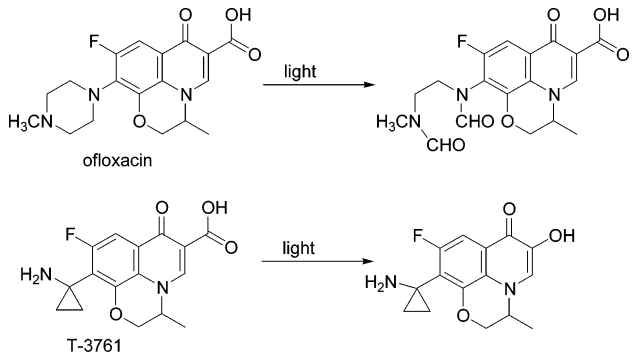


Figure 13. Photochemical reactions of T-3761 and ofloxacin.

between this carbon-linked molecule and nalidixic acid is clear.

Radicals generated at C-8 by loss of halogen atoms can interact with the N-1 substituent, resulting either in its loss, as seen in Figure 14 in the case of *N*-cyclopropyl-substituted sparfloxacin, or in formation of a carbocyclic ring as seen with N-1 ethylated analogues.[72] Alternately, they can be quenched by hydrogen atoms, leading to replacement of F by H. These reactions are also illustrated in Figure 14.

When a second halogen atom is added to C-5 or C-8, photolysis is enhanced and replacement of the halo group by H, OH, or Cl or interaction with the alkyl group at N-1 is observed.[77] Cleavage of a C-7-amine-containing moiety is also observed. Quinolones such as sparfloxacin, lomefloxacin, and fleroxacin also undergo these reactions.

Photolytic loss of C-8 chlorine is more facile than loss of C-8 fluorine, and C-8 fluorine is lost in preference to C-5 fluorine as shown by the photochemistry of orbifloxacin (Figure 15), which possesses molecular features similar to those of sparfloxacin

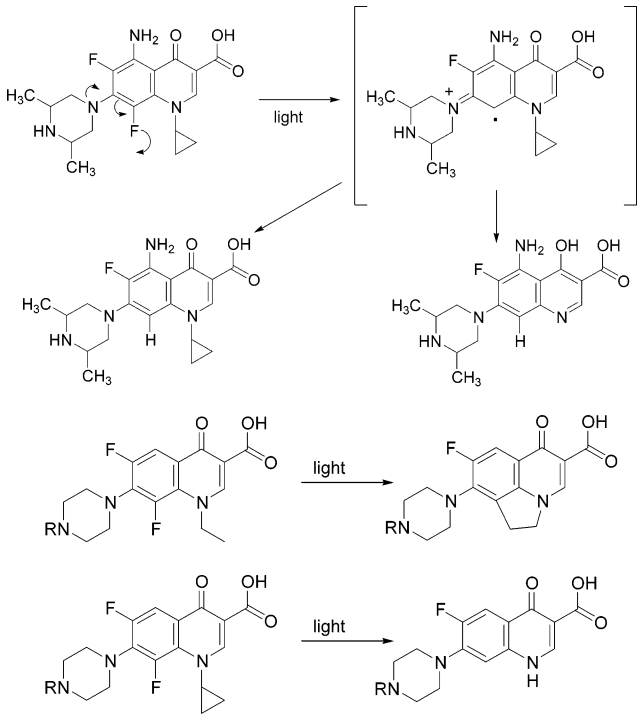


Figure 14. Photolysis of sparfloxacin and selected *N*-ethylated quinolones.

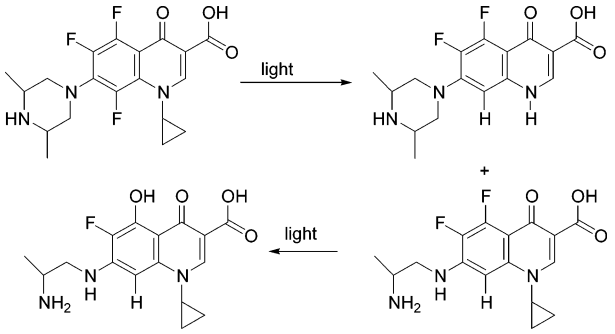


Figure 15. Photolytic reactions of orbifloxacin.

but with the addition of a fluorine moiety at C-5. In addition to losses of N-1 cyclopropyl and C-8 fluoro, it also undergoes C-7 piperazinyl cleavage and replacement of the C-5 fluorine by hydroxyl. One perhaps can infer from this that halogen atoms at C-5 and C-8 are more sensitive to photolysis than halogens at C-6.[72] Nonetheless, when there is only a C-6 fluorine as with norfloxacin, enoxacin, and ciprofloxacin, photolytic replacement of their C-6 fluoro atom by a hydroxyl group can take place as illustrated by a generic formula in Figure 16.[78] Here,

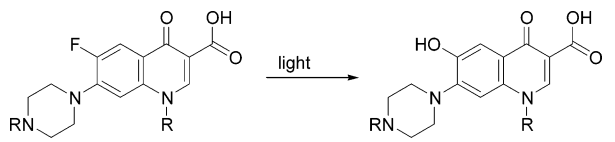


Figure 16. Photochemical replacement of a C-6 fluoro atom by OH.

as elsewhere, analogous chemistry takes place in the quinolone and naphthyridinone ring systems when they are similarly substituted.

It is interesting to note that the presence of certain aromatic and heteroaromatic substituents at N-1 can

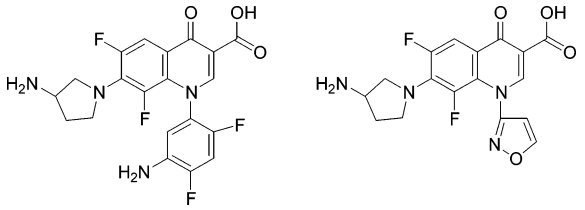


Figure 17. Novel quinolone anti-infectives apparently relatively stable to photolysis.

quench or prevent the formation of radicals at C-8, and therefore, such quinolones promise to possess milder phototoxicity. Such quenching groups are 1-aminodifluorophenyl and 1-isoxazoyl. These compounds possess at the same time quite satisfying antimicrobial potency in vitro.[71] Figure 17 presents the structure of two of these.[71] It will be interesting to see whether exploitation of these findings produces clinical benefits.

In summary, a large number of photodegradation reactions are possessed by quinolones, and these reactions are quite capable of generating reactive oxygen species and may trigger photosensitivity reactions in patients. The recent finding that some members of the quinolone class retain very significant antibacterial activity even though they do not have a C-6 fluorine moiety has excited much interest.[79−81] It is reasonable to suggest that they may have reduced phototoxicity. None of these have yet reached the marketplace, however.

4. In Vitro Antimicrobial Spectra

Overall, the quinolones possess antimicrobial spectra and potency attractive for clinical use. In particular, they are bactericidal in achievable oral doses and possess significant postantibiotic effects. These features are especially useful for treating infections of immune-suppressed patients.

Microorganisms regarded as highly susceptible to quinolones have minimum inhibitory concentration values ranging from 0.01 to 0.2 μg/mL. Examples include *E. coli*, *Klebsiella pneumoniae*, *Enterobacter*, *Salmonella*, *Shigella*, *Vibrio*, *Hemophilus influenzae*, *Neisseria*, and *Legionella*. Less susceptible but still sensitive microorganisms lie in the range of 0.25−2 μg/mL. These organisms include those that become resistant more easily. Especially notable are *Pseudomonas aeruginosa* and *S. aureus* (with particular emphasis on MRSA). Organisms that are regarded as insensitive have MIC values of 2 μg/mL or higher. Examples include *Nocardia*, *Treponemia*, and anaerobes.

Clinicians often classify quinolones as first-, second-, and third-generation agents on the basis of their antimicrobial spectra. The classical first-generation quinolones such as nalidixic acid and pipemidic acid were of interest because of their activity against Gram-negative microorganisms, with particular emphasis on the commonly encountered urinary tract pathogen *E. coli* acquired in the community and therefore less likely to be drug resistant. Their specific potency is not very high, and resistance development can occur even during the course of therapy.

Table 1. Antimicrobial Sensitivities of Selected Quinolones[a]

microorganism	Nor	Cipro	Enox	Oflox	Levo	Gati	Lome	Moxi	Spar	Trov Alatro
				Gram-Positives						
S. aureus	+	+	+	+	+	+	+	+	+	+
MRSA							+			
Staphylococcus epidermidis	+	+	+	+	+	+	+	+	+	+
MRSE						+	+	+		
Staphylococcus hemolyticus		+								
Streptococcus pyogenes		+		+	+	+		+	+	+
Streptococcus viridans					+				+	+
Enterococcus faecalis	+	+			+					+
				Gram-Negatives and Special Microorganisms						
E. coli	+	+	+	+	+	+	+	+	+	+
Chlamydia trachomatis				+						
Chlamydia pneumoniae				+	+	+	+	+	+	+
Enterobacter sp.	+	+	+	+	+	+	+	+	+	
Gardnerella vaginalis				+						+
H. influenzae		+		+	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+	+	+	+	+
Legionella pneumoniae		+		+	+	+	+	+	+	+
Mycoplasma hominis				+						+
Mycoplasma pneumoniae				+	+	+	+	+	+	+
Neisseria gonorrhoeae	+	+	+	+		+				
Proteus mirabilis	+	+	+	+	+	+	+	+	+	+
Proteus vulgaris	+	+	+	+	+	+	+		+	+
Providencia rettgeri	+	+		+	+		+			
Providencia stuartii	+	+	+	+	+					
P. aeruginosa	+	+	+	+	+		+			+
Salmonella typhi		+								
Seratia marcescens	+	+	+	+	+		+			
Shigella sp.		+		+	+	+		+	+	+
Ureaplasma urealyticum	+			+						+
Vibrio chloerae		+								
				Anaerobes						
Bacteroides fragilis										+
Clostridium perfringes	+			+		+				+

[a] "+" means that the culture is normally regarded as sensitive to ordinary concentrations.

A comparison of the susceptibilities of a wide number of pathogenic bacteria with representatives of each generation of quinolones is presented in Table 1. If space allowed, the first generation of quinolones would be represented by nalidixic acid. In that case, only *E. coli* and *Enterobacter* sp. would be checked. Other members of this generation include rosoxacin, oxolinic acid, and cinnoxacin.

In the table the second generation of quinolones is represented by ciprofloxacin, norfloxacin, enoxacin,[82] lomefloxacin,[83] and ofloxacin. The antimicrobial spectra of these agents, ciprofloxacin of which is the sales leader, show a progressive broadening of the antimicrobial spectrum, retaining an emphasis on Gram-negatives and including some significant Gram-positives. Activity against *P. aeruginosa*, *S. aureus*, and *Streptococcus pneumoniae* is often observed, but the effective dose is marginal, and breakthrough to resistance is not uncommon. Anaerobes are only occasionally inhibited. A number of microbes lacking cell walls such as *Legionella*, *Chlamydia*, and *Mycoplasma* are also inhibited.

The third-generation quinolones in the table include levofloxacin, gatifloxacin, lomefloxacin, moxifloxacin, sparfloxacin, and trovafloxacin/alatrofloxacin. These agents are even broader in spectra, retaining the overall spectrum of the second-generation agents but possessing in addition activity against some strains marginally sensitive to the second-generation group and many resistant strains and adding a number of anaerobes as well. They are, however, somewhat more toxic. Of the newer quinolones, WCK-771 (the L-arginine salt of nadifloxacin) has one of the most impressive in vitro anti-anaerobe spectra.[84]

At present the search is on for quinolones that retain the attractive features of the present members but lack the constellation of side effects, most particularly the severe toxicities occasionally encountered with the most potent members.

Drlica and Hooper classify the microbes sensitive to quinolones in a genetic/microbiological sense rather than in a clinical sense.[5] This is based upon the genes encoding the target enzymes and the relative sensitivity they possess to inhibition by quinolones.

Type 1 includes a group of pathogens that are comparatively insensitive to quinolones. These have genes predominantly producing a DNA gyrase that has a nonpolar alanine residue at a position normally occupied by a polar serine or threonine residue in the A subunits. This decreases the sensitivity of this gyrase to quinolone action. This type also appears not to have a significant content of topoisomerase IV and so depends on its gyrase to decatenate, etc. Microorganisms belonging to this class include *Mycobacterium tuberculosis* and related mycobacteria

Treponema pallidum and *Helicobacter pylori*.[85,86]

Types 2 and 3 are less precisely delineated. In type 2, both DNA gyrase and topoisomerase IV are present, but the gyrase is more sensitive to quinolone inhibition. These organisms are mainly Gram-negatives. Mutations of topoisomerase IV have comparatively less effect on sensitivity unless accompanied by mutations in gyrase.[85,86] Type 3 microorganisms also have both DNA gyrase and topoisomerase IV, but the gyrase is less sensitive to quinolones so that resistance mutations in the genes for topoisomerase IV are more significant for resistance. These microorganisms are mainly Gram-positives.[85,86]

5. Structure–Activity Relationships

It is convenient to discuss quinolone structure–activity relationships position by position with the caution that apparently distant constituents modulate each other's properties because they are all connected to the same electronic system and cross-talk is possible. The modulation normally involves the intensity of specific properties rather than the kind but should be kept in mind in making predictions and comparisons. Cross-talk is diminished somewhat in the benzoxazines and quinolizines where aliphatic atoms intervene.

With the structures that follow, those that have names are those of particular prominence, especially those that have been marketed. The marketed agents are further identified by the year of their introduction. Each figure also contains a selection of microbes and their sensitivities (μg/mL) to the agent in question.

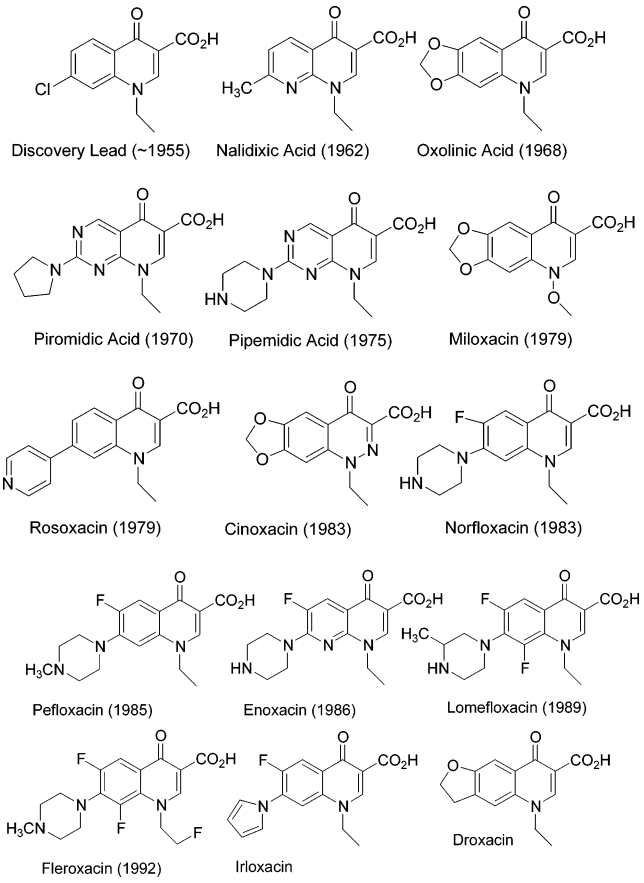
5.1. N-1 Ethyl Family

With the early, classic agents, small, nonpolar, unbranched aliphatic agents proved best, with *N*-ethyl being most useful.[87] Polar substituents at N-1 have generally been disappointing. This relationship has generally held up with later agents.

From Figure 18 it can be seen that the N-1 ethyl substituent, sometimes halogenated, is still found in many contemporarily important quinolone anti-infective agents. As time passed, there was a continual enhancement in anti Gram-negative potency and breadth of the spectrum when the various other positions were explored. Norfloxacin was the breakthrough molecule as its potency and spectrum approximated those of the fermentation-derived antibiotics. Norfloxacin retains the classical *N*-ethyl moiety. In recent years there has been an increased emphasis on anti Gram-positive activity with these agents, and this is reflected also in Figure 18. Activity against anaerobes is generally lacking in this group.

5.2. N-1 Cyclopropyl Family

The N-1 cyclopropyl moiety first materialized with ciprofloxacin.[30] This substituent could not be introduced with the original Gould–Jacobs chemistry, but the Groehe–Heitzer chemistry proved enabling for it. As compared with norfloxacin, this change enhanced anti Gram-negative potency (Figure 19). This moiety became standard for a long time thereafter



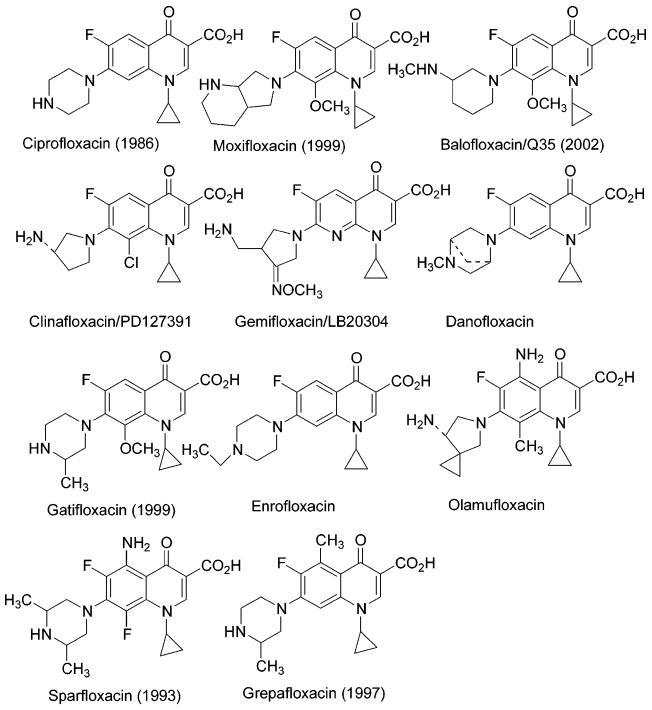
Quinolone	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pneumoniae*	*B. fragilis*
Discovery Lead	126	-	-	-	-
Nalidixic Acid	6.3	-	-	-	-
Oxolinic Acid	0.2	6.3	-	-	-
Piromidic Acid	25	-	50	-	-
Pipemidic Acid	1.6	6.3	50	-	-
Miloxacin	0.4	12.5	6.3	100	-
Rosoxacin	0.2	6.3	0.4	12.5	-
Cinoxacin	3.6	-	-	-	-
Norfloxacin	0.25	0.2	0.8	1.6	-
Pefloxacin	0.25	0.4	0.2	0.8	-
Enoxacin	0.05	0.39	0.39	4-8	-
Lomefloxacin	0.12	1.0	1.0	8.0	-
Fleroxacin	1.0	2.0	0.5	0.8	-
Irloxacin	1.0	>128	0.5	8	-
Droxacin	0.05	3.1	3.1	-	-

Figure 18. Structures and abbreviated antimicrobial spectra of significant members of the N-1 ethyl family of quinolones.

in quinolone analoguing, with potency being modulated by substituent changes at C-5, C-7, and C-8, but ciprofloxacin has largely withstood these challenges and remains a market leader to this day. With progressively later entries, anti Gram-negative activity intensified and anaerobic activity became more frequent.

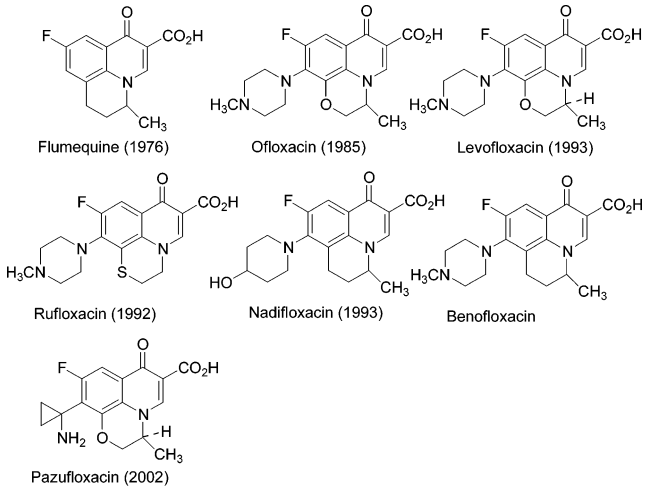
5.3. N-1 to C-8 Bridged (Tricyclic) Family

In this group a C-8 substituent is linked to an *N*-ethyl moiety at N-1, resulting in the tricyclic family of quinolones (Figure 20). This results in restricted rotation of the ethyl group and introduces a chiral atom. This feature was first present in flumequine,[62,63] which incidentally also was the first fluoroquinoline anti-infective. Flumequine is used today primarily for agricultural purposes, but its unmarketed *S*-analogue was shown to be the eutomer.[63] This stereochemistry persists in all of the resolved marketed analogues to this day.



Quinolone	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pneumoniae*	*B. fragilis*
Ciprofloxacin	0.012	0.05	0.1	0.39	6.25
Moxifloxacin	0.015	>32	0.3	0.25	0.125-4
Balofloxacin	0.2	12.5	0.2	0.39	-
Clinafloxacin	0.008	0.5	0.06	0.12	0.5
Gemifloxacin	0.13	16	0.063	0.031	-
Danofloxacin	0.05	-	0.39	-	-
Gatifloxacin	0.02	0.72	0.05	0.016	-
Enrofloxacin	[Veterinary use only]				
Sparfloxacin	0.0125	0.39	0.05	0.39	0.78
Grepafloxacin	0.1	0.78	0.1	0.39	-
Fleroxacin	1.0	2.0	0.5	0.8	-

Figure 19. Structure−activity relationships in the N-1 cyclopropyl family.



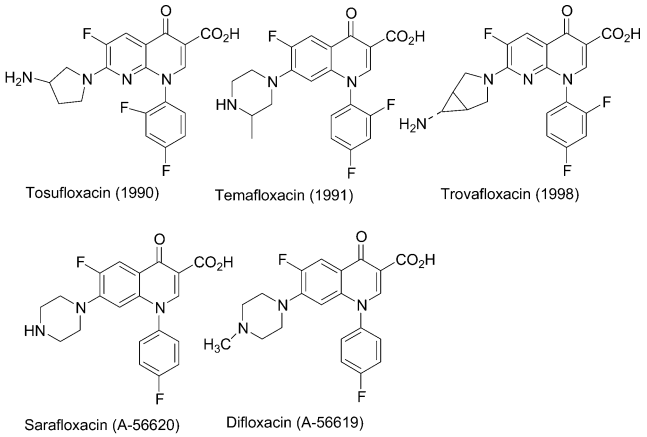
Quinolone	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pneumoniae*	*B. fragilis*
Flumequine					
Ofloxacin	0.05	2.0	0.25	2.0	-
Levofloxacin	0.008	1.2	0.12	0.5	-
Rufloxacin	0.78	12.5	0.78	2-8	-
Nadifloxacin	0.39	3.13	0.024	-	-
Pazufloxacin	0.025	3.13	0.39	3.13	-

Figure 20. Structure−activity relationships of the tricyclic quinolone family.

Racemic ofloxacin was the next member of this group to be marketed, and it quickly became popular. Subsequently, it was largely replaced by its resolved *S*-analogue levofloxacin,[64] which is one of the present market leaders. Rigidification of the N-1 substituent in this way resulted in a significant enhancement in anti Gram-positive activity although some anti Gram-negative activity was concomitantly lost. Resolution of ofloxacin leads to a doubling of potency, and this is apparently helpful against some otherwise marginally sensitive microbes such as *S. pneumoniae*. The potency enhancement agrees with the finding that the distomer has very little biopotency against bacteria. The nature of the atom attached to C-8 seems comparatively unimportant as C, O, and S bioisosteres possess similarly significant activity and the products show the same chiral dependence.

5.4. N-1 Aryl Family

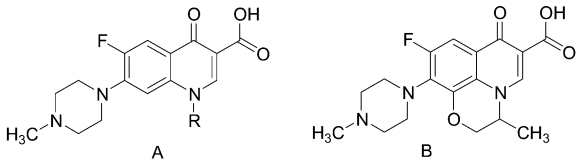
The introduction of an *N*-aryl substituent was made possible by the Groehe−Heitzler methodology, and this substituent change has proven to be quite successful in specific instances (Figure 21). Difloxacin[88] and sarafloxacin[89] represent early entries that did not see commercial use. The presence of a second fluorine atom in the aromatic ring, as with tosufloxacin[90] and trovafloxacin,[91] however, further enhanced both potency against Gram-positives and pharmacokinetics, and they were marketed. Unfortunately, as will be noted in greater detail later, the addition of the difluorobenzene substituent was accompanied by uncommon but severe toxicities that would be difficult to detect in clinical trials unless a mammoth number of patients were to be enlisted. The connection between this substituent and these unusual toxicities is logical but remains to be firmly established. It seems that the connection will not be easy to establish as the toxicities are rather different from each other and so do not present a common pattern. They appear to be class effects, but the specific molecules in question possess them to an unusual degree.



Quinolone	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pneumoniae*	*B. fragilis*
Tosufloxacin	0.02	0.2	0.05	0.25	-
Temafloxacin	0.06	1.0	0.06	1.0	3.13
Trovafloxacin	0.02	0.5	0.03	0.12	-
Sarafloxacin	0.25	1.0	0.5	-	-
Difloxacin	0.03	1.0	0.5	-	-

Figure 21. Structure−activity relationships in the *N*-aryl family.

One supposes that a useful future role for genomics might be in the detection of the genetic differences between these unfortunates and the bulk of the



R	*E. coli*	*Ps. Aeruginosa*	*S. aureus*	DNA gyrase Cleavage	DNA gyrase inhibition
A=Et	0.025	0.40	0.20	1.0	5.5
A=cPr	0.05	0.40	3.10	-	-
A=2,4-Di-F-Ph	0.02	1.56	0.10	-	-
B	<0.05	0.78	0.78	-	-

Figure 22. A comparison of the effect of important N-1 substituents on in vitro activity.

general population so those who should not receive these agents could be reliably predicted. Not only would this enhance the likelihood of success in clinical evaluations but could also prevent devastating results encountered by particular patients and their loved ones following general use. It would also be welcomed by firms that must put forth very large sums in research and development of quinolones at the risk of suffering economic disaster at the latest stages.

Of the various noncyclic N-1 substituents investigated, the *N-tert*-butyl analogues were found to be unexpectedly potent in vitro, especially against Gram-positives.[59] This substituent is not very stable in acidic solutions, and no example of this class ultimately reached the clinic.

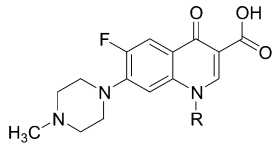
Thus, it is clear that several important series of antimicrobial agents are associated with particular N-1 substituents. The properties of these agents are clearly modulated by the nature of the C-7-amine-containing substituent, and often the addition of a suitable C-5 and C-8 substituent produces added potency.

In each of these individual series the structures depicted have emerged as best in show. Mostly, when the other constituents are held constant, the potency of *N*-1 analogues against Gram-positives follows the order aryl > ethyl > 1,8-fused > c-propyl. Against Gram-negatives the order is slightly different: ethyl ≈ c-propyl > aryl > 1,8-fused (Figure 22). In Figure 22 the compounds chosen for comparison are identically substituted away from N-1.

In Figure 23 a selection of analogously substituted earlier quinolones is presented, demonstrating that ethyl is ordinarily the best of the aliphatic group. Branching, increased chain length, and polar groups are all usually detrimental. Vinyl and *tert*-butyl are however close to ethyl in allowing potency. These findings strongly influenced subsequent work.

5.5. Positions C-2, C-3, and C-4

Compared to the other positions, carbons 2, 3, and 4 have not been much represented in analoguing studies, and no analogue modified at C-2 remains on the market bearing other than a CH substituent. This comparative lack of apparent exploration stems from the early findings that alterations here are usually unsatisfactory[87] (Figure 24).[92] It is possible that the basis for this phenomenon is that the carboxyl group at C-3 needs to be coplanar with the C-4 carbonyl so that it can effectively hydrogen bond



R	*E. coli*	*P. aeruginosa*	*S. aureus*
H	i	i	i
CH_3	0.39	1.56	6.25
C_2H_5	0.20	0.78	0.78
$CH=CH_2$	0.10	0.20	1.56
C_2H_4F	0.10	0.78	1.56
$CH(CH_3)_2$	0.50	1.00	1.00
$C(CH_3)_3$	0.06	0.50	0.06
n-C_3H_7	0.20	3.13	1.56
CH_2CH_2OH	0.39	3.13	1.56
$NHCH_3$	1.00	1.00	1.95
$CH_2CH=CH_2$	0.20	1.56	3.13
CH_2c-Pr	0.50	4.00	1.00
CH_2Ph	0.78	1.56	1.56

Figure 23. An abbreviated list of quinolone substituent potencies at N-1.

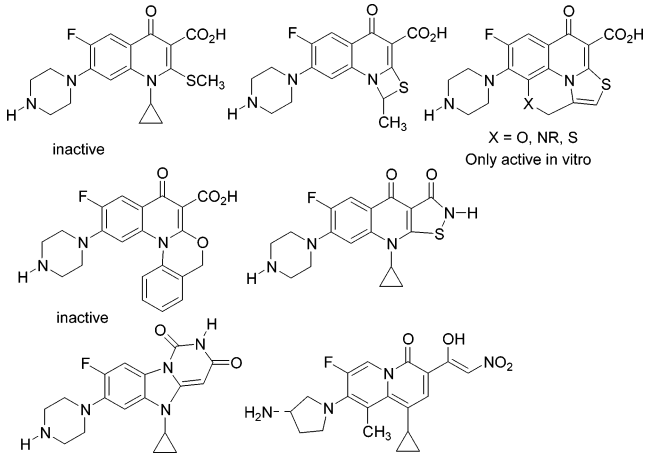
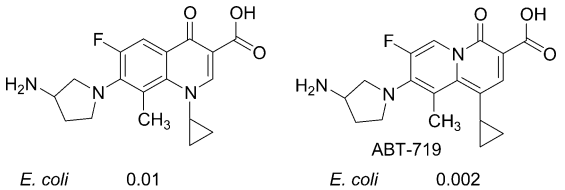


Figure 24. Some substituent changes explored involving carbons 2−4.

with DNA bases made available by strand separation catalyzed by DNA gyrase or topoisomerase IV. The steric deficit that substitution at C-2 presumably causes can be partly overcome by rigidifying a C-2 substituent in a ring connecting it with N-1, especially when the group attached to C-2 can accept a hydrogen bond and form a virtual ring with the C-3 substituent.[93] In support of this idea is the outstanding potency seen with formation of a thiazolone ring fused to the carbonyl ring.[94] When this is done, the NH moiety is strongly acidified by resonance stemming from the aromaticity of the ring when enolization takes place. This produces a coplanar carboxyl surrogate and leads to a striking enhancement of antimicrobial potency. Unfortunately, this attractive feature is negated by the propensity of these molecules to kill mammalian cells also by damaging their DNA through intercalation. The flat fused three-ring aromatic system is no doubt responsible. These compounds might have a future as antitumor agents.

Aside from the thiazolone modification just noted, replacement of the C-3 carboxyl by other moieties has not led to economically valuable agents. Those that retain significant acidity show some potency.

Some prodrugs have been made by esterification of the C-3 carboxyl moiety or synthesis of the aldehyde (which is oxidized in the body to the acid).[95−98]



E. coli	0.01	*E. coli*	0.002
S. aureus	0.05	*S. aureus*	0.01
S. pyogenes	0.1	*S. pyogenes*	0.02

Figure 25. Substituent changes at the C-4a position: the 2-pyridones.

A commercially significant prodrug is alatrofloxacin, an injectable form of trovafloxacin which is otherwise too lipophilic for ordinary parenteral administration.[99] The generally excellent absorption characteristics of the quinolones diminish motivation for prodrugging.

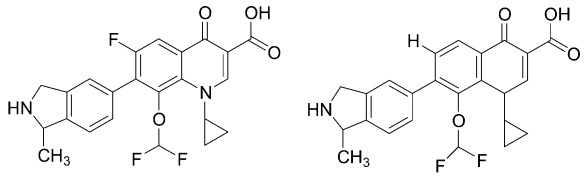
The C-4 carbonyl is essential for bioactivity. This finding provides one of the primary motivations for alkylation of N-1. Unless this is done, the 4-pyridone ring enolizes primarily to its 4-hydroxypyridine form, and analogues of this type are inactive.

5.6. C-4a Substituted Analogues

The C-4a position in quinolones has no valences left for substitution. It can, however, be exchanged for the nitrogen atom normally found at N-1.[66] This allows for a redistribution of the electrons in the rings so as to preserve aromaticity. These analogues have been named 2-pyridones so as to differentiate them clearly from the quinolones. Interestingly, these double bioisosteric analogues possess substantially increased in vitro antibacterial potency as shown by a comparison of ABT 719 and the quinolone possessing the same substitution pattern at the other positions (Figure 25).[100] Notably, potency against otherwise resistant microorganisms is significant among the pyridones. A selected number of 2-pyridone analogues have progressed into development, and at least one has received significant clinical examination. Despite the passage of significant time, commercialization has not occurred yet. Although details are sketchy, it is believed that they possess some toxicities requiring caution. Interestingly, ABT 719 is also significantly lower melting and more water-soluble than the corresponding quinolone. X-ray studies indicate that the *C*-methyl group twists the ring system out of plane so that it does not stack as compactly as the corresponding quinolone. This provides a probable rationale for these observations.

5.7. C-5 Substituents

Substituents at C-5 are tolerated, especially if they are small, and it helps potency if they are polar as well. Groups that have been studied include N, COH, CNH_2, CNHMe, $CNMe_2$, CNHAc, CCH_3, CEt, CCl, and CF. Sparfloxacin[101] and grepafloxacin[102] (illustrated under the N-1 cyclopropyl molecules) are the most successful of these. As noted elsewhere in this paper, the 5-halo substituents contribute significantly to phototoxicity, so there is a tradeoff involved in using them. The other substituents have been unsatisfying and have not progressed.



E. coli MIC	0.015	0.015
S. aureus MIC	0.007	0.007
E. coli Gyrase, IC-50	0.16	0.17
S. aureus Topo IV	4.60	2.19
Human Topo II	128	510

Figure 26. Comparison of the potencies of a C-6-fluorinated and -desfluorinated quinolone.

5.8. C-6 Substituents

Substituents at C-6 in the early investigations were generally limited to the terminus of a methylenedioxy bridge to C-7 (oxolinic acid,[103] miloxacin,[104] and cinoxacin[92]), CH (nalidixic acid[27] and rosoxacin[105]), and N (pipemidic acid[106] and piromidic acid[107]). Those that were marketed have been largely supplanted with analogues possessing a CF moiety after the attractive properties of norfloxacin were revealed. It is generally believed that the C-6 fluorine substituent conveys enhanced DNA gyrase potency and enhances cell penetration. These favorable features operate in conjunction with compatible C-7 and C-8 substituents, and the need for a C-6 fluoro became dogma until fairly recently.

It has now been shown in a number of cases that the positive effect of a C-6 substituent is diminished when the molecule contains other helpful substituents. A present vogue for investigation of nonfluorinated analogues has developed enhanced by the suggestion that C-6 F may play a role in the potential mammalian genotoxicity and central nervous system side effects of quinolones.[108,109] The examples in Figure 26 reveal no difference in potency against *E. coli* and *S. aureus* or the *E. coli* derived gyrase whether the C-6 substituent is fluorine or hydrogen. There is a slight advantage against topoisomerase IV and human topoisomerase II, however.

Other C-6 substituents that have been investigated, with less satisfactory results, are Cl, Br, methylketo, CN, nitro, methyl, and amino.

5.9. C-7 Substituents

The most versatile position for substitution of quinolones has been C-7 and its analogous position in other ring systems. Many thousands of analogues have been prepared employing various substituents at this forgiving position, leading to the conclusion that a cyclic system containing a secondary or tertiary amino moiety is usually best. The beneficial effects are usually believed to be enhanced potency and favorable pharmacokinetics. The nature of the C-7 substituent (along with C-8) also strongly affects the target preferences (DNA gyrase and/or DNA topoisomerase IV) of quinolones. Larger substituents on the distal nitrogen generally decrease potency. The earliest quinolones possessed a chlorine, a methyl (nalidixic acid), the terminus of a methylenedioxy bridge to C-6 (oxolinic acid, miloxacin, or cinnoxacin), a 4-pyridyl (rosoxacin), a pyrrolidinyl (piromidic acid), or a piperazinyl moiety (pipemidic acid). On the basis

of these early findings, a consensus formed that cyclized amino moieties were superior. One notes, on the other hand, that a proximal electron-releasing group at C-7 can stabilize a radical generated at C-8 and so could contribute to phototoxicity and genotoxicity.

After the discovery of norfloxacin[28] the piperazinyl moiety or its *N*-methyl analogue became a standard feature among quinolones. Later it was found that a pyrrolidinyl substituent with a pendant amine was a suitable moiety also.[24] The piperazinyl analogues usually have enhanced potency, emphasizing Gram-negatives, whereas the pyrrolidinyl analogues have enhanced activity against Gram-positives. Subsequently, bicyclic moieties of a variety of types were also introduced. These had the virtue of retaining potency but also diminishing metabolic liability. The amino function that is proximal to the aromatic ring appears primarily to be a synthetic convenience.[61] Clearly, it is at the same time much less basic than the distal nitrogen atom. The potency effect of the stereochemistry of carbons near the distal nitrogen is variable but often weak. This leads to the reasonable belief that this is not a point of close approach to the edges of the binding pocket. On the other hand, chiral substituents closer to the aromatic ring have a more significant effect on potency.[90] It is speculated that this may influence the rotamer distribution of the C-7 substituent.

The structures in the following few figures include many of the groups that have been attached to C-7. In these structures "Q" represents one of the quinolone, naphthyridine, or pyridone ring systems. The looseness of the SAR associated with this position is demonstrated by the wide variety of such moieties that are associated with roughly similar potencies.

5.9.1. Piperazinyl and Related Moieties

Figure 27 depicts a selection of the many simple and fused piperazines and piperidines that have been attached to C-7 of quinolones. The attention of the interested reader is drawn back to the properties of lomefloxacin (Figure 18), balofloxacin (Figure 19), danofloxacin, gatifloxacin, sparfloxacin, grepafloxacin, and nadifloxacin (Figure 20), and trovafloxacin (Figure 21), important quinolones decorated with these groups.

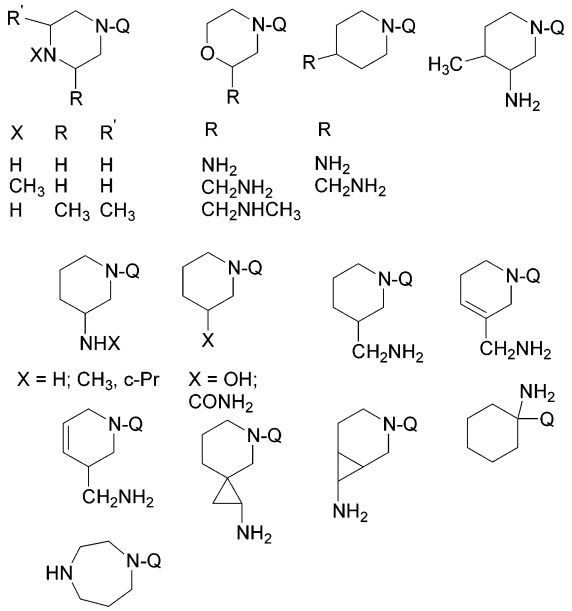


Figure 27. A selection of piperazinyl and related moieties attached to quinolones.

5.9.2. Pyrrolidinyl and Related Moieties

Figure 28 depicts a number of five-membered heterocyclic rings that have been attached to C-7 of various quinolones. Of these, isloxacin (Figure 18), moxifloxacin, clinifloxacin, gemifloxacin, and olamufloxacin (Figure 19), and tosufloxacin (Figure 20) stand out.

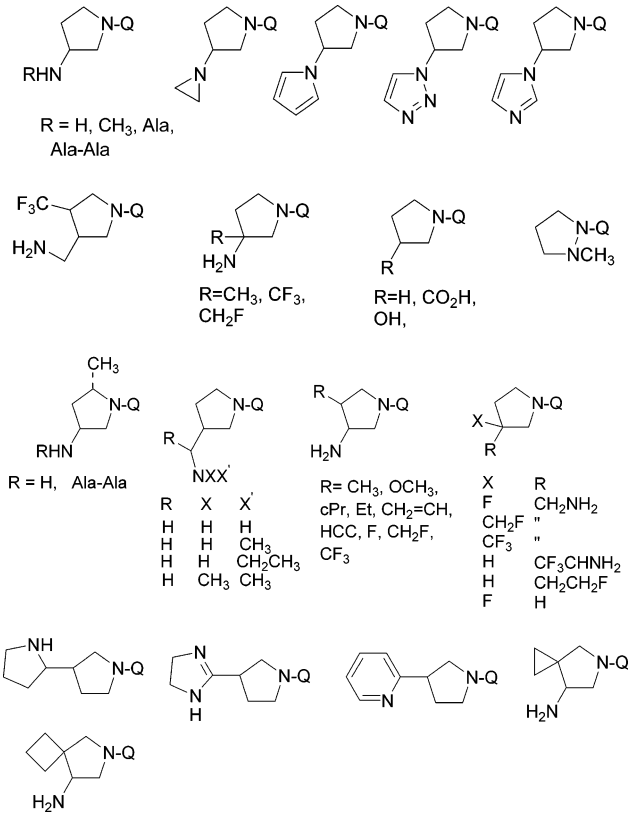


Figure 28. Some pyrrolidines and related moieties attached to quinolones.

5.9.3. Cyclobutylaminyl and Related Moieties

No prominent quinolones incorporate this moiety as yet. Figure 29 shows some examples.

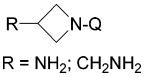


Figure 29. Some cyclobutylamines attached to quinolones.

5.9.4. Bicycloaminyl Moieties

Prominent quinolones with this structural feature include moxifloxacin (Figure 19), danofloxacin, olamufloxacin, and trovafloxacin (Figure 21). Figure 30 depicts some additional examples.

5.9.5. Carbon-Linked Substituents

There has only been a limited exploration of substituents attached to C-7 through carbon in large

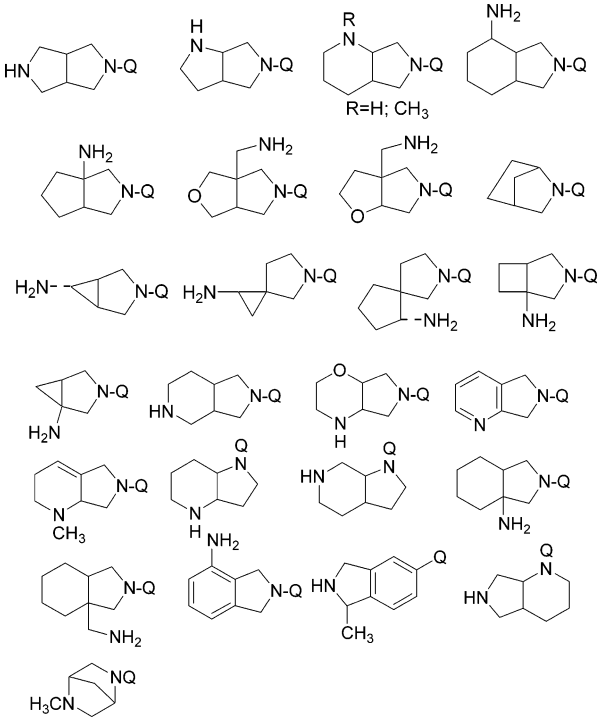
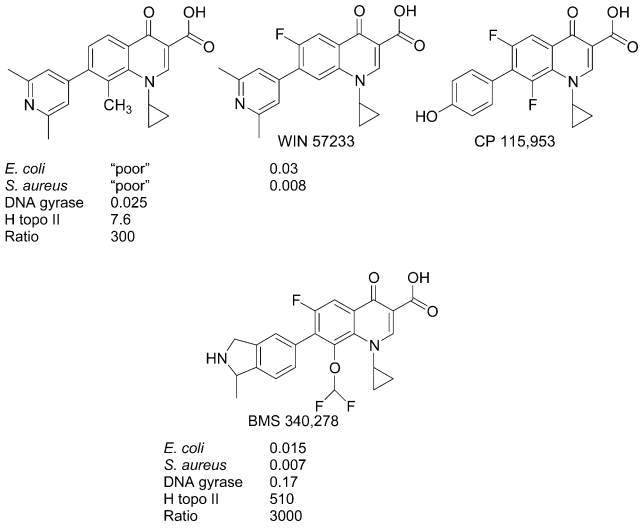


Figure 30. Bicycloamines attached to quinolones.

measure because they are more cumbersome to synthesize. Most of these possess aromatic rings, and a significant number possess enhanced activity against Gram-positive microorganisms. They are often more active against human topoisomerase II and so are potentially toxic. A selection of these is shown in Figure 31.



E. coli	"poor"	0.03
S. aureus	"poor"	0.008
DNA gyrase	0.025	
H topo II	7.6	
Ratio	300	

E. coli	0.015
S. aureus	0.007
DNA gyrase	0.17
H topo II	510
Ratio	3000

Figure 31. Some moieties attached to quinolones through C−C bonds.

5.10. Substituents at C-8

C-8 substituents have an important effect on in vivo efficacy and the antimicrobial spectrum of quinolones. In particular, the C-8 substituents appear to play a significant role in determining the comparative affinity of quinolones for DNA gyrase or topoisomerase IV. This effect is modulated significantly by the nature of the C-5 substituent, especially if it is a fluorine atom. Unfortunately, the favorable potency effect of a C-8 halogen atom is counterbalanced by an enhanced likelihood of phototoxicity and mammalian clastogenicity. Prominent C-8-F-bearing quinolones include lomefloxacin and sparfloxacin. An *O*-methyl group, on the other hand, often increases potency without increasing photoxic liability (see gatifloxacin and moxifloxacin). Oxygen constrained in a ring as in ofloxacin/levofloxacin, or sulfur as with rufloxacin, seems to carry over these benefits. Bridging to N-1 with a carbon moiety such as with flumequine and nadifloxacin may or may not also convey these properties. The oxo and thio series can be considered as rigid analogues of methoxyl- or thiomethyl-bearing quinolones.

The first successful variant at C-8 was the bioisosteric replacement of CH by N (nalidixic acid). This sort of replacement reoccurs regularly in the quinolone field and often leads to enhanced pharmacokinetic characteristics. Other groups at C-8 that have been investigated with less salubrious effects include *O*-ethyl, OH, OCH_2F, $OCHF_2$, OCF_3, and SMe.

5.11. Resume of Structure–Activity Relationships of Quinolones

Figure 32 contains a pictorial summary of these findings with reference to the parts of the molecule that are presumed to be in contact with the enzyme, with DNA, and with other quinolone moieties (thus forming the ternary complex). At the left is a cartoon viewed from the top. In this view, north and south represent DNA-binding sites wherein the keto and carboxyl groups hydrogen bond to single-stranded segments made available to them by enzymic action. West and east represent binding sites to DNA gyrase. In the interior of the complex, the quinolone molecules are associated with each other through hy-

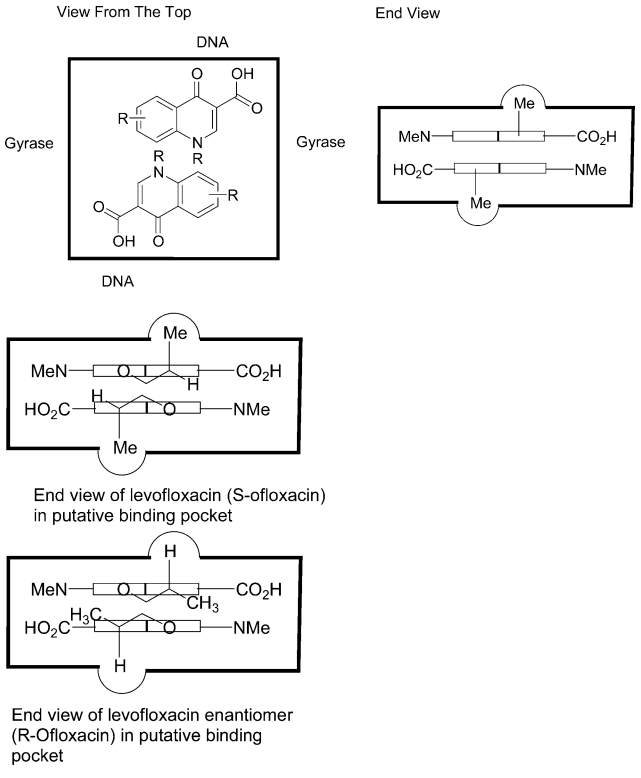


Figure 32. Proposed ternary complex drug-binding pocket.

drophobic contacts. Virtually every portion of the quinolone molecule is employed in one or more of these interactions. The requirement for the enzyme to open a saturable drug-binding pocket is implicit. The important role of having a basic moiety attached to C-7 is also apparent. The need for the groups attached to N-1 and C-8 to be small and hydrophobic is also rationalized. The two molecules are aligned "head to tail" with respect to each other by electrostatic interactions involving the carboxyl and the amino substituents. A second pair of interacting quinolone molecules can also be aligned in a vertical stack by the $\pi-\pi$ interactions and the same electrostatic interactions.

An end view with the N-1, C-8 edge closest to the viewer is shown on the right side of the figure. The significance of the chiral preference conveyed by the methyl group of flumequine and levofloxacin and their analogues is rationalized by invoking the presence of a lipophilic drug-binding site above and below the pocket. The methyl portion of the *N*-ethyl moieties is illustrated as fitting into this pocket and providing a favorable interaction. Aligned in this way, the *S*-enantiomer of ofloxacin (levofloxacin) has its methyl groups aligned in a "Boston−San Diego" manner and, when axially oriented, fits this putative pocket quite well. The *R*-enantiomer would have its methyl groups aligned in a "Seattle−Miami" manner and clearly would not benefit from this interaction. This is discussed in greater detail in the next section.

5.12. Absolute Configuration

Chiral substitution of quinolones has largely been associated with particular substituents bridging from C-8 to N-1 and to groups attached to C-7. Of these, by far the most successful have been the bridged examples. The molecular rigidification of an N-1 ethyl substituent brought about in this way produces a chiral center. The first examples using this idea were the enantiomers of flumequine and ibaquine.[62,110] From this work it was found that a very substantial preference was displayed for the *S*-enantiomer with respect to antibacterial and enzyme inhibitory potency. The 1-*R*-antipodes were very weakly active.

Later the antipodes of commercially successful racemic ofloxacin were prepared with analogous results. Levofloxacin, the *S*-antipode, is a market leader among the quinolones today. Subsequently, analogous findings have been made repeatedly, and benofloxacin,[111] nadifloxacin,[112] pazufloxacin,[113] S-12684, GRB-23790, WIN-58161,[114] and DV-7751a[115] have all shown the same enantiopreference. The single exception to this otherwise general rule is the assertion in a meeting abstract, not subsequently published, that with the 6-methyl-6,7-dihydro-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylic acids, such as Ro-14-5319 and Ro-14-4299, the eutomer has the *R* absolute stereochemistry (Figure 33).[116]

If it is fair to set aside this exception because it has a different ring system, it is attractive to rationalize the absolute configurational findings with the rest of the quinolones along with a number of other observations by proposing the existence of a small lipophilic cavity in DNA gyrase above the plane of

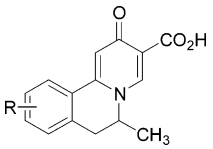


Figure 33. 6-Methyl-6,7-dihydro-2*H*-benzo[*a*]quinolizin-2-one-3-carboxylic acids.

the quinolones whose occupation results in significant binding energy enhancing enzyme inhibitory power and subsequent antibiosis. The methyl group of the enantiomeric *R*-antipodes could not make this contribution (Figure 32).

The requirement for a small, unbranched, lipophilic moiety attached to N-1, such as *N*-ethyl, is consistent with this hypothesis. *N*-Cyclopropyl can be accommodated in a similar fashion although not as well. The N-1 aryl series does not fit quite as well either, but this substituent is not too bulky in the important dimension to fit. One notes that the aromatic N-1 substituents are orthogonal to the major plane of the molecule to the extent of at least 30°. Most interestingly, the *N*-*tert*-butyl analogue's surprising potency can also be accommodated as it has three ways to fill this putative cavity with each rotation. These proposed interactions are illustrated in Figure 34.

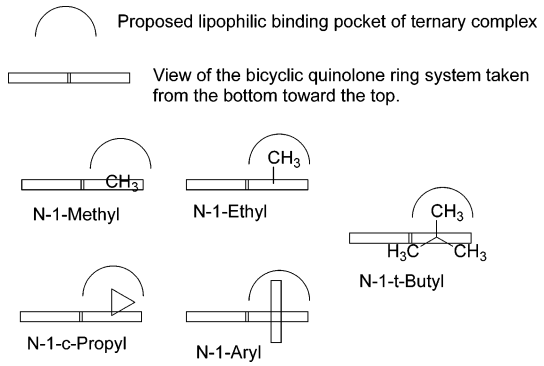


Figure 34. Schematic view of interactions of various quinolone analogues with the putative DNA gyrase lipophilic binding pocket.

In contrast, when asymmetric substituents are present in the cyclic amino moieties attached to C-7, the biological consequences are by and large minimal if the asymmetry is distal to the aromatic ring (Figure 35). Ariens notes that exceptions to the classical Pfeiffer rule correlating the intensity of chiral recognition as a function of high potency breaks down if the chirality is not near a point of close approach of a ligand to a receptor.[117] This would suggest that the majority of quinolones only loosely fill the sides of the binding pocket proposed for the C-7 substituent. On the other hand, significant eudesmic ratios are seen when asymmetry is proximal to the aromatic ring.[90] This is likely to inhibit free rotation selectively so as to produce a lateral but not a linear directional bias. It is also possible that the binding pocket is small in this region, resulting in chiral contacts.

The work of Shen et al. demonstrates that the quinolones serially associate (show cooperativity in binding) rather like liquid crystals assemble in the

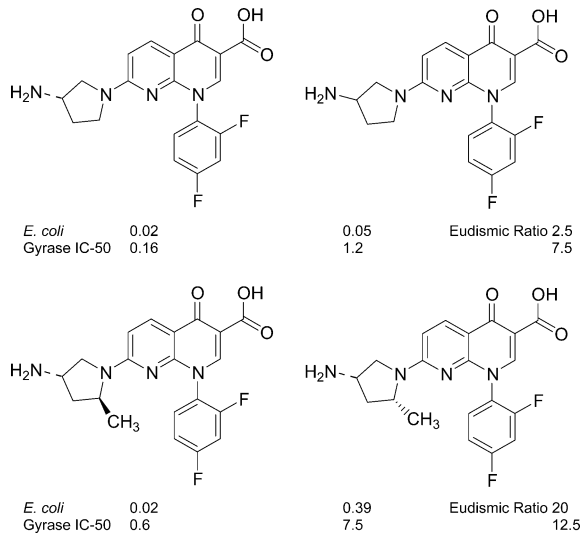


Figure 35. Enantiomers and diasteriomers at C-7.

drug-binding zone created by the interaction of DNA gyrase and topoisomerase IV with DNA.[118] In this view, the first molecule enters the melt zone and conditions it to accept the second more readily possibly through induced conformational alterations and water displacement. The third and fourth would each bind more easily still. Morressey et al. have extended this idea with a stacking model accommodating chirality.[119] The lipophilic cavity idea illustrated in Figures 32 and 34 fits this proposal well and extends it somewhat. There are various ways in principle in which quinolones could assemble in the pocket. Energetically stacking that involved acidic moieties in proximity to basic moieties (i.e., head to tail) would be favored. The oxazine ring can possess two conformers—one in which the methyl group is axial and the other in which it is equatorial. The two conformers are approximately equal in energy, but a conformer in which the methyl group is axial would agree better with the prominent role that it plays in interfering with the enzyme. A stack in which the methyl groups are external would allow much closer approach of the ring systems and therefore be energetically favored. If the methyl groups were internal to a stack, then closeness of approach would be difficult. Finally, the chirality would make the stack itself chiral. That is, the stacked supermolecules would be chiral themselves. A chiral stack such as that illustrated would clearly rationalize enantiopreference and show how chiral association of quinolones induced by the enzyme would enable both single-stranded segments of the DNA bubble and/or both arms of the cut gate to interact with drug, stopping further movement and thereby inhibiting enzyme action. Other enzymes involved in DNA replication and function, such as helicase, bumping into the frozen bubble cannot function because of this frozen blockade. This may well be the sort of interaction that leads to apoptosis. In this view, mammalian topoisomerase II would not have the same lipophilic cavity, providing a molecular rationale for the difference in toxicity of marketed quinolones against DNA gyrase over mammalian topoisomerase II.[120]

6. Structure–Toxicity Relationships

Although a keenly important topic, an understanding of structure–toxicity relationships of quinolones is not well developed yet. In general, certain substituents at C-5 and C-8 are sometimes associated with genotoxicity and phototoxicity. Substituents at C-7 are associated with genetic toxicity, GABA binding, and P-450-related drug–drug interactions.[24,68] Particular substituents at N-1 are associated with genetic toxicity and P-450-related interactions. Since there is cross-talk between substituents, some substituents modulate the effects of substituents at more distant centers. Those quinolone molecules that have had to be removed from the market or have been restricted in use due to severe toxicities (temafloxacin, trovafloxacin, sparfloxacin, and grepafloxacin) bear C-5 substituents or *N*-difluorobenzene moieties, but this group of molecules is too small to support convincing generalizations.

6.1. Chemotype Toxicities and Side Effects

There are a number of annoying but not life-threatening toxicities that are regarded as chemotype side effects and toxicities in that they are common among quinolones but are not shared equally. They are more or less prominent depending upon the structure of the particular agent. They are listed in all the package insert precautions.[34] Since these adverse effects are general, they contribute little to an understanding of individual structure–toxicity relationships among quinolones.

Gastrointestinal complaints, including diarrhea, dyspepsia, and nausea, are comparatively common among antibacterials and often are primarily due to disturbances of the normal gut flora. Thus, these are commonly mechanism-based problems rather than structure-based problems. These often resolve in a few days without requiring discontinuance of the drug.

Neuropsychiatric complaints include headache, dizziness, sleep disturbances, and occasionally even seizures. The seizure tendency is often attributed to $GABA_A$ receptor binding and is commonly potentiated by co-administration of certain nonsteroidal anti-inflammatory agents. Fenbufen is particularly singled out as being involved as a potentiator of this effect.[24,29] The problem is attributed to the biphenyl acetic acid moiety formed from fenbufen upon mammalian metabolism.[121]

Allergic reactions to quinolones include rash, urticaria, skin rashes, serum sickness such as reactions, anaphylactoid reactions, and photosensitivity. Photosensitivity has been discussed briefly in a previous section and is associated particularly with the presence of halogens at C-5 and C-8 and a participating amino function at C-7. The free radical generated is quite capable of producing reactive oxygen species, leading to inflammatory responses to doses of sunlight that would normally not lead to a significant response. The relationship between this and other allergic reactions is unclear.

On intravenous dosing, irritation at the site of injection is not uncommon. This manifests itself as

erythrema and phlebitis. These effects are ameliorated when a suitable substituent is present at N-1 or C-8. Ofloxacin and levofloxacin must be given slowly by drip rather than by push to prevent serious side effects.[122] The chelating capability of quinolones discussed in an earlier section may contribute to irritation on injection. Injectable quinolones are often administered at acidic pH levels to help with this problem as well as to enhance their water solubility.

Tendinitis up to and including tendon rupture has been seen in a few cases.[10,123−125] This side effect is particularly prominent in juvenile beagle dogs but appears to be uncommon in humans. It has been suggested that this might be associated with inhibition of collaginase, leading to interference with remodeling of collagen-dependent structures. Worries about this have led to restrictions in the use of quinolones in sexually active females and in adolescent children. Nonetheless, many courses of treatment have been given to children with severe infections with little apparent resulting tendon damage so the import of this phenomenon is unclear.

There are no reports of teratogenicity in humans taking quinolones even in the first trimester of pregnancy.[126] It is always advisable, however, to administer drugs to pregnant females with caution, and this caution certainly applies to quinolones. In particular, high doses may lead to decreased weight gain of fetuses, so caution is advised.[127]

6.2. Severe Toxicities Associated with Particular Quinolones

Temafloxacin was withdrawn a few months after marketing when severe hemolytic reactions, occasionally including clotting abnormalities and renal failure, caused significant distress to a small number of patients, several of whom died. These effects were not seen in preclinical animal studies or in the various phases of clinical study before marketing took place.[54]

Trovafloxacin postmarketing surveillance revealed a number of cases of hepatotoxicity, some of which were severe enough to require liver transplantation, and some patients died. Because of these problems, trovafloxacin use is now restricted to those cases where the potential benefits from its use outweigh the potential risks.[53] The high lipophilicity of trovafloxacin makes the liver one of the organs in which drug might well accumulate, and this may contribute to the observed problem.

Grepafloxacin was withdrawn following episodes of postmarketing cardiotoxicity.[128] Prolongation of the QT interval is observed with most quinolones but may well be more severe in particular cases. In this regard, clinicians are cautious with sparfloxacin in that a small percentage of patients demonstrate this phenomenon, and a few cases of torsades-de-pointe syndrome have been associated with its use.[129] [Torsades-de-pointe is a French term used to describe a specific ventricular rhythm/tachycardia most frequently seen in connection with a prolonged QT interval. This phenomenon is associated mostly with cardiovascular drugs but occurs with some quinolones in a few cases per million prescriptions.] Moxifloxacin use has also led to some reports of QT interval prolongation, but these appear as yet to be minor.[130]

Thus, the trend to enhanced breadth of the spectrum has been accompanied in some cases by severe toxicities that limit or prevent the use of some of the newer agents. These were rare enough and unusual enough that they were not detected in detailed preclinical and clinical studies. Only administration to a large number of patients following marketing revealed these problems. Several of these agents possess an *N*-1 2,4-difluorophenyl substituent. Whether this is coincidental or ominous remains to be established. These instances were seen in the one-a-day administration days, and it is not clear whether they could have been avoided or minimized through use of divided dosing schedules.

The underlying mechanisms leading to these severe toxicities have yet to be disclosed.

The rest of the quinolones have demonstrated reasonable freedom from such severe side effects. Millions of doses of quinolones have been administered over a period of several decades, demonstrating a side effect incidence of 2−10%. Most of these are discomforting but not severe enough to require discontinuation of administration. No commonality of toxic response is seen among the idiosyncratically severely toxic quinolones. Drug developers cannot help but be nervous in the face of unexpected problems of this type in a class of drugs previously regarded as comparatively safe.

6.3. Drug−Drug Interactions

Co-administration of quinolones with a number of other classes of drugs can lead to interferences complicating their best use.[34]

The interaction of quinolones with multivalent ion-rich substances has been discussed above. Sucralfate also interferes, and didanosine contains such ions in its formulation and so can interfere with absorption.[67,131]

Co-administration of theophylline and drinking significant amounts of caffeinated beverages (including some popular soft drinks) can lead to unusually high blood levels of theophylline by competition for P-450-based metabolic transformation.[132,133] P-450-catalyzed metabolic transformation is an important means of metabolism for purines. Quinolones compete for the action of the same enzymes. Quinolones that undergo significant metabolic transformation by this means are thus more likely to cause negative drug−drug interactions. Ciprofloxacin, trovafloxacin, and enoxacin are such agents to watch.[68]

7. Molecular Mode of Action

The manner in which quinolones killed bacteria was unknown when they were first discovered. Some years later, it was discovered that DNA gyrase, a previously unknown topoisomerase II essential for altering the topology of DNA, was an important target of these agents.[39] Subsequent research has shown that DNA gyrase is commonly the primary target in most Gram-negative bacteria. Much later,

a second target enzyme was discovered to be DNA topoisomerase IV.[41] Topoisomerase IV is a primary target for quinolones in certain Gram-positive bacteria, whereas in a number of bacterial species activity is due to interference with a blend of both of these enzymes.[42] In mycobacteria, on the other hand, DNA gyrase appears to be essentially the only target.[134] Thus, the question of the specific target enzyme for a particular microbe is complex. Both enzymes are vital for bacterial life and are very widely distributed, readily rationalizing the broad spectrum of the fluoroquinolones. Inhibition of either enzyme by quinolones can be lethal to bacteria. The one that is more sensitive is the primary determinant of the minimum inhibitory concentration, but a mutation in either enzyme can be influential. Thus, a particular resistance mutation can be very or only slightly important. Further, high-level resistance can require the acquisition and consolidation of more than one mutation.

Both of these enzymes are type II topoisomerases since they temporarily cleave both strands of duplex DNA and then reseal them following passage of an uncut portion of the molecule through the temporary gap. The net result is a change in topology of the DNA molecule, hence their names. Since most of the functional features of DNA are topologically dependent, these enzymes are essential for transcription, translation, repair, and storage processes. The two enzymes function in rather similar ways and possess substantial amino acid sequence homology, so it is believed appropriate to some degree to extrapolate knowledge from one to the other. Less aptly, the drug−enzyme−DNA complex bears similarities to similar eukaryotic complexes of importance to anticancer chemotherapy as with, for example, adriamycin treatment.[94] The quinolones of antibacterial prominence are carefully crafted not to have significant inhibitory power against the human topoisomerase II. Some extrapolations of data can nonetheless be made to human topoisomerase II, but it must be kept in mind that there are quite significant drug structures involved.[42] Other significant topoisomerases exist. These, however, belong to the topoisomerse I type and operate rather differently in that they produce transient single-strand cuts in double-stranded DNA. Inhibitors of bacterial topoisomerases I and III have not become significant in antimicrobial chemotherapy as yet. Although named topoisomerase III, this is a class I topoisomerase.

By way of review, DNA can occur in circular form in which the ends of the double helices are joined covalently (Figure 36). For convenience this is more often shown schematically as an untwisted ribbon or tube (Figure 37). The natural situation is even more complex in that the circular DNA molecules can be twisted further around themselves, rather like one does with a rubber band, to form a supercoiled molecule. The further twisting can be done in either a right-handed or a left-handed manner so as to produce positively or negatively supercoiled molecules. The action of DNA gyrase is to produce negative supercoils. Each time the molecules are twisted over themselves, a node results. The linking number is the sum of the positive and negative nodes

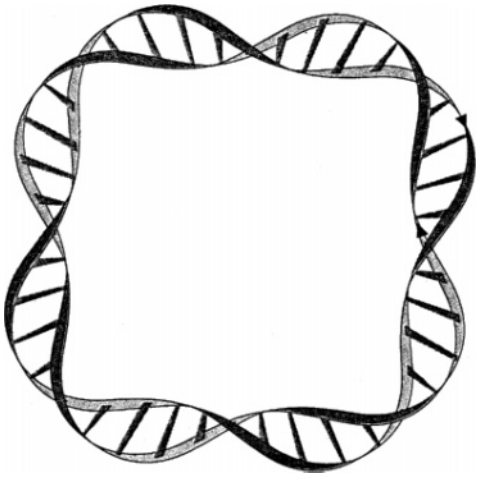


Figure 36. Circular DNA. Circular DNA is a double helical molecule. It is most often illustrated, however, as a ribbon as this is much easier to draw and to understand. Reprinted with permission from ref 135, p 18, Figure 2.1 (originally in color). Copyright 1993 Oxford University Press.

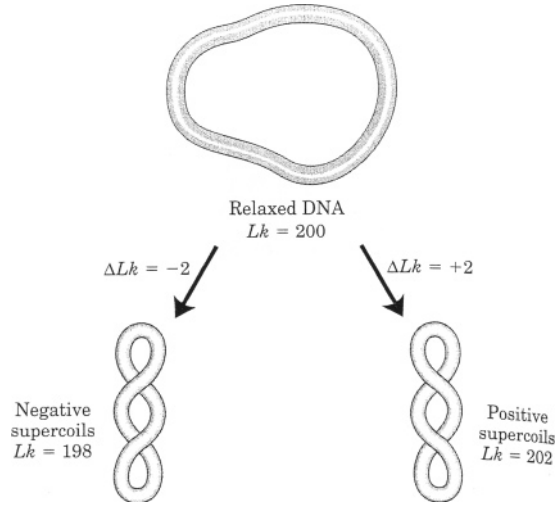


Figure 37. Supercoiling of relaxed DNA. The DNA is depicted in ribbon form. Supercoiling can result in positive or negative supercoils. DNA is intrinsically coiled in a right-hand helix so positive supercoils result in increased torsional stress whereas negative supercoils unwind the molecule. Each point of crossing over is called a node. If the upper strand crosses in a clockwise manner, this is assigned to be negative and vice versa. The linking number (Lk) is the sum of the positive and negative nodes divided by 2. Reprinted with permission from ref 136, p 934, Figure 24-17. Copyright 2005 W. H. Freeman and Co.

divided by 2 and is a measure of the degree of twisting. Excellent treatments of this topic are available for the interested nonspecialist.[135−137]

Unless further processed, supercoiled DNA would be tortionally unstable and spontaneously revert to the relaxed circular state. Topoisomerases II stabilize supercoiled DNA molecules by cutting both strands, holding onto the cut ends, passing an uncut double helical segment through this gap, and resealing the cut ends. This process can take place once or several times, thus producing a family of topoisomers differing from one another in the degree of twisting.

The resulting topoisomers differ in compactness and so can be separated by gel electrophoresis.

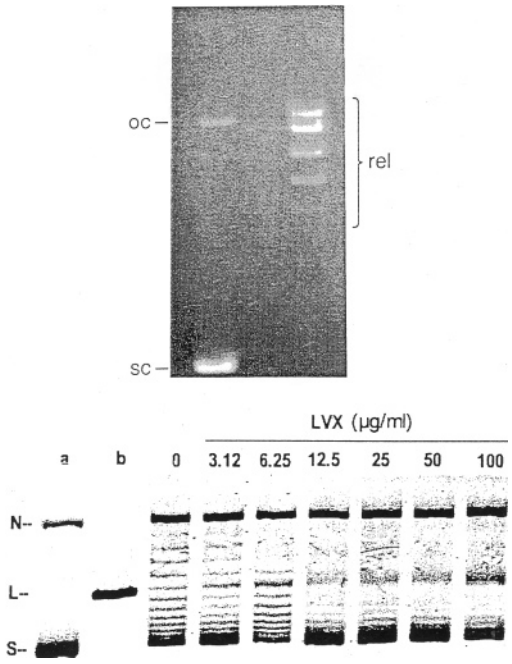


Figure 38. Agarose gel electrophoretic examination of the action of DNA gyrase to produce topoisomers (A, upper gel) and strand breaks (B, lower gel) in DNA. (A) is reprinted with permission from ref 135, p 32, Figure 2.9. Copyright 1993 Oxford University Press. (B) is reprinted with permission from ref 138, p 1285, Figure 5, p 1285. Copyright 2004 American Society for Microbiology.

Under normal circumstances isolated supercoiled DNA of bacteria produces two kinds of electrophoretic bands. The upper band in the left-hand track of Figure 38, top, consists of open circular DNA, and the lower band consists of negatively supercoiled DNA. When acted upon by a topoisomerase, a series of topoisomers are produced. These are visible as a ladder in the right lane of the figure, top, where the various degrees of supercoiling can be detected. Visualization of the bands is greatly emphasized by fluorescence following intercalation with the dye ethidium bromide.[135]

When quinolones are present, the transitions are inhibited in a concentration-dependent manner, providing the basis for an assay. The IC_{50} value is that concentration of drug that inhibits supercoiling by 50%.[138]

The ability to relax supercoiled DNA is also crucial in allowing transcription. Transcription requires temporary separation of the duplex strands so that each can be duplicated faithfully. Since the enzyme that copies the base sequence moves only in one direction, the bubble containing the single-strand segments must move with it. As the bubble goes forward it meets increasing resistance from supertwisting of the strands ahead and from the undertwisting of the strands behind as a consequence of the whole molecule being intertwined and stabilized by extensive hydrogen-bonding networks. Replication of small segments in this manner is much more efficient than would be involved if each strand of duplex DNA had to be completely separated into single strands for transcription. This activity of DNA is highly conserved, and interference with it serves as a ready rationale for the broad-spectrum activity

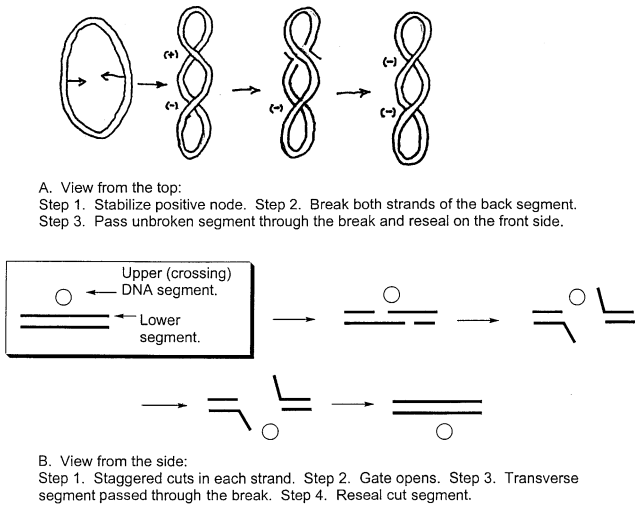


Figure 39. Cartoon illustrating cleavage, strand passage, and resealing of DNA.

of quinolones.[136] Quinolones freeze the bubble, leading to rapid cell death.[136] Assay of this effect must be done in a different manner.

Another central feature of the activity cycle of topoisomerases is to produce strand cuts in DNA. The cut molecules can be released by detergent denaturation and protein digestion. The amount of cleavage is a function of quinolone concentration. The CC_{50} value is the amount of drug that will trap half of the maximal amount of linear DNA formed. This is illustrated in the lower portion of Figure 38. In this particular example, supercoiled circular DNA was incubated with the DNA gyrase component proteins (GyrA and GyrB) from *M. tuberculosis* without added ATP but with increasing concentrations of levofloxacin (abbreviated as LVX) added. Without the enzyme, the left lane shows nicked and supercoiled substrate DNA. The next lane shows linearized DNA, and the remaining lanes show increasing amounts of linear DNA produced at the expense of supercoiled DNA molecules.

It is believed that release of cut DNA strands results in lethal consequences.[139,140] Those particular consequences are not well understood at present. It appears that a lethal protein is biosynthesized when cut ends are produced. The identity of this putative protein is as yet unknown. The presumption that such a cell poison is involved stems largely from the observation that certain protein biosynthesis inhibitors, such as chloramphenicol, are partially antagonistic to the lethal action of quinolones with some bacteria.[141,142]

The cleavage-passing process is illustrated in Figure 39. In the upper view (A) one views the process from the top. A relaxed circular DNA molecule is acted upon by DNA gyrase. First, the molecule is distorted so that the segments overlap, producing a positive and a negative node. Next, the phenolic OH moiety of tyrosine 723 attacks the deoxyribose backbone of each of the sessile strands, producing four-base-pair staggered cut ends with the ends covalently attached to the phenolic oxygen of the enzyme. These separate into two short single-stranded regions, and then an uncut segment is passed through the gate.

The molecule is resealed behind, thereby changing the linking number and producing two negative nodes.

An alternate view of the process is presented in the bottom part of the figure (B), illustrating the process from the side. First, one sees an intact molecule with one portion lying in the plane of the paper and another segment of the same molecule coming out directly at the reader and lying above the first. These segments are attached to each other. Next, each strand of the transverse segment is cut by the enzyme to produce a "gate". This opens with the energy provided by ATP hydrolysis so that the forward projecting segment of the molecule can pass through to the bottom. Closing the gate and resealing behind complete the catalytic cycle. This can repeat a number of times, or the ADP can dissociate, returning the enzyme to its ground state and releasing the DNA segment now possessing an altered topology. The result is highly negatively twisted (superhelical) but stable DNA.

Topoisomerase IV can also resolve the catenated (knotted or intertwined) DNA that is a natural consequence of replication of circular DNA. The intertwined catenanes or knots can be converted into individual closed circular or unknotted DNA molecules by double-strand cleavage, passage, and resealing. Topoisomerase IV therefore must possess many similarities to DNA gyrase, but it also differs in its substrate preferences and the outcome of its action. It must differentiate between the two strands in catenanes and knots and pass the segments in the proper order and direction.

Structurally, DNA gyrase is a heterotetramer composed of two copies each of an A and a B subunit connected to each other by hinge strands. Unfortunately, there are no X-ray structures of the enzyme either with or without substrate or inhibitor on board. The present understanding of its structure is illustrated schematically in Figure 40, which is a composite construction made from the amino-terminal ATP-binding domain of *E. coli* topoisomerase II and the carboxyl-terminal fragment from yeast topoisomerase II.[137] The smaller B subunits are on the top, and the binding site for ATP/ADP resides there as indicated. The A subunits are encoded by a *gyrA* gene and the B subunits by a *gyrB* gene. The combined A units are responsible for the breakage and reunion functions and the B subunits for providing the ATPase activity that generates the energy for the substantial cyclic movements that the enzyme undergoes in functioning. Apparently, binding of ATP to the B subunits is the signaling event that starts the cycle of events resulting in topoisomeric change. The negative supercoiling activity of DNA gyrase is balanced by the relaxing action of topoisomerases I and topoisomerases IV. In some bacteria DNA gyrase apparently also performs some of the decatenations that are normally the function of topoisomerases IV. In mycobacteria, treponema, and helicobacter, for example, topoisomerase IV is apparently entirely lacking.[86,134,143] The degree of dependence of decatenation on types of DNA gyrase is not yet entirely clear but obviously is relevant to antimicrobial action.

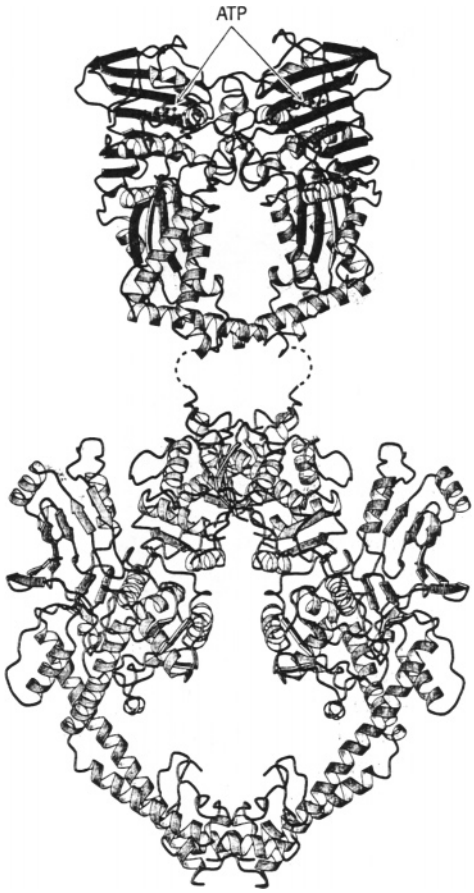


Figure 40. A schematic view of DNA gyrase. Reprinted with permission from ref 137, p 78, Figure 27.23. Copyright 2002 W. H. Freeman and Co.

The A subunits of DNA gyrase are 97 kDa molecules (in *E. coli*) containing the tyrosine moiety whose phenolic OH group is the nucleophile that cleaves the phosphodiester bonds of DNA and covalently holds the cut ends. Another region contains those amino acids whose mutation is most influential in leading to quinolone resistance. The latter is called the quinolone resistance-determining region (QRDR).[144] The N-terminal two-thirds of the A subunits is where the cleavage-resealing activity is found whereas the C-terminal one-third is where the DNA is wrapped around the enzyme.[145]

The B subunits are slightly smaller (90 kDa) molecules that contain an ATP-binding site and ATPase catalytic activity. The ATPase activity is found in the N-terminal half of the B subunits,[146] while the C-terminal portion is involved in DNA binding and attachment to the A subunits.[147−150]

A variety of molecules bind to the B units and interfere with the function of DNA gyrase. The B region is sensitive to inhibition by the coumermycin and cyclothialidine classes of antimicrobials following binding to the ATP site.[151,152] Novobiocin is best known of the coumermycins and was once marketed for antibiotic use. It interferes with the energy transduction required for the substantial molecular movements involved during the enzymatic action of these key enzymes.[153] Unfortunately, the coumermycins are not well tolerated in humans, and in addition, resistance develops readily. Cyclothialidine

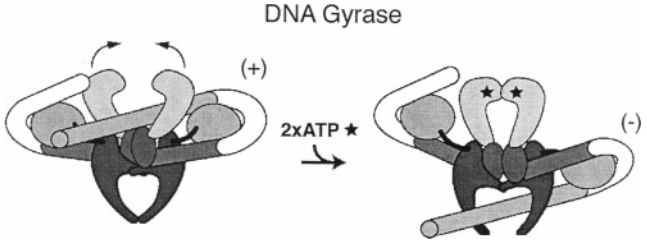


Figure 41. A cartoon view of the catalytic cycle of DNA gyrase cleavage of DNA. The DNA molecule (drawn as a tube) wraps around DNA gyrase such that the crossing segment of DNA enters the upper chamber consisting of the two B segments of the enzyme. In the next step the enzyme binds two molecules of ATP, closes the opening, and cuts the transverse segment of DNA to produce a gate through which the molecule can pass into the lower chamber consisting of the two A segments of the enzyme. The cut in the DNA molecule is resealed behind. Finally, the molecule is ejected from the lower chamber, which opens to allow this. The stage of the cycle that is sensitive to quinolone binding is yet to be completely characterized. Reprinted with permission from Corbett, K. D.; Schultz-aberger, R. K.; Berger, J. M. The C-terminal domain of DNA gyrase A adopts a DNA-bending beta-pinwheel fold. *Proc. Natl. Acad. Sci. U.S.A.* **2004** *101* (19), pp 7293−7298 Copyright 2004 National Academy of Sciences.

inhibits DNA gyrase efficiently in cell-free systems, but the substance penetrates poorly into intact cells. Creative analoguing has partially overcome this, leading to molecules that possess a better hydrophilic/lipophilic balance.[154] The clinical future of the improved agents is not yet clear. Microcin B-17 is a peptide-based natural product that binds to the B region at a site apparently containing Trp751, and this inhibits DNA gyrase.[155] The microcin B-17 story is in its infancy.[155,156] Screening of a chemical library for DNA gyrase inhibitors using an assay dependent upon production of bacterial anucleate cells due to failure of partitioning led to the discovery of pyrazole analogues. Analoguing produced a substance with significant inhibitory potency against DNA gyrase and DNA topoisomerase IV that, at the same time, did not significantly inhibit DNA topoisomerase II.[157−159]

It will be interesting to see how many B-unit-binding agents appear in the future and whether any of these will survive into clinical utilization. These various agents are outside the scope of this review and will not be treated further herein.

The various interactions postulated between DNA and DNA gyrase in the catalytic cycle are illustrated in Figure 41. The DNA molecule wraps around the enzyme more or less at the AA/BB interface such that the segment to be cut is in the plane of the paper and the segment to be passed enters the BB chamber, which opens to allow this and then closes to prevent premature escape. Next, the portion of the DNA molecule in the plane of the paper is cut to produce a gate. The enzyme holds on to the cut ends to keep the process under control. The enzyme then separates the cut strands, and the uncut strand of the molecule is passed through this gate into the lower chamber (BB). The cut strand is resealed. Expulsion of the uncut strand from the lower chamber and disassembly completes the catalytic cycle. Drug binding resulting in freezing of the tertiary complex takes place somewhere at or between the second or third phases of the cycle, producing either a cleavable complex or a cut segment in which the DNA becomes trapped, neither able to progress nor to revert.[118] The ternary "cleavable" complex becomes or induces a cellular poison so that the bacterial cell rapidly dies.[160] An important bit of evidence leading to this conclusion is that the MIC values of quinolones are frequently much lower than the IC_{50} or CC_{50} values. It appears possible that the permanent gaps in the DNA strands induce the biosynthesis of exonucleases as repair enzymes, leading to poorly controlled repair processes. Often this results in apoptosis. Interference with movement of replication bubbles has been mentioned earlier. It is posited that an as yet unidentified toxic protein is released or formed as part of the interference by quinolones with these processes. This rationalizes the finding that some protein biosynthesis is essential for quinolone toxicity as chloramphenicol, for example, can decrease sensitivity of bacteria to quinolones when employed simultaneously.[141] Much present speculation surrounds this issue, but the details are sketchy at best.

Although the primary sequence and X-ray structures are known for fragments of the gyrase A and B subunits, no molecular level picture of the complete enzyme or of the ternary complex is available.[153,161−163] Indeed, given the various movements required for the functioning of the enzyme and its substrate, it is not clear that success in getting an X-ray picture will clarify the precise mechanism of action. One suspects that a single "freeze-frame" picture might well be misleading and that a sequence of pictures might be required. Thus, the present picture requires inferences from the structures of the agents and data from resistance studies, and understanding lies still at the cartoon level.

The quinolone-binding site on the A subunits is not clearly identified, but amino acids 67−106 are referred to as the QRDR (*E. coli* numbering), with major resistance mutations occurring at S83W, D87A, D87G, D87H, D87N, and D87Y. Interestingly, the serine at position 83 and the aspartate at position 87 are both polar, and the resistance mutations mostly involve replacement *by* hydrophobic amino acids. It is thought by many that the C-7 amino substituents of quinolones interact with gyrase in the ternary complex binding pocket. Replacement with nonpolar side chains would lessen this interaction considerably, and gyrases with hydrophobic amino acids at this position are much less sensitive to quinolones.

Other changes occur in the same general area but are less significant from the standpoint of resistance development. Interestingly, as might be expected from susceptibility studies with intact bacteria, quinolones bearing halogens or alkoxy groups at C-8 are less interfered with by these mutations.

Humans must also be able to alter the shape of their DNA in a controlled manner, and the related enzyme that carries out this function has considerable homology to the prokaryotic enzyme. This enzyme is called human topoisomerase II. Quinolones bind much less avidly to the human counterpart

enzyme (100−1000× less) and so do not cause DNA poisoning to humans at doses normally achievable.[164] For example, ciprofloxacin inhibits DNA gyrase at about 0.3 μM, but it requires a concentration in excess of 300 μM to inhibit mammalian topoisomerase II.[165,166] Etoposide inhibits mammalian topoisomerase II at about 0.81 mM concentration, but it takes more than 850 mmol to inhibit DNA gyrase.[165,166] This explains not only the selective toxicity of these agents but also how a drug that damages DNA could escape being significantly genotoxic or transforming to humans at normal doses. In this regard it is important in passing to consider that potent inhibition of mammalian topoisomerase II by anthracyclines and etoposides, e.g., provides useful antitumor chemotherapy.[167] These drugs are too toxic to be used as antibiotics.

Bacterial topoisomerase IV[41,168] has functions that partly duplicate functions of DNA gyrase and vice versa. It is also composed of two sets of pairs of subunits. *parC* genetically encodes the two ParC subunits, and the *parE* gene encodes the two ParE subunits. The C subunits of topoisomerase IV in *E. coli* are about 36% homologous to the A units of DNA gyrase. The E subunits are about 40% homologous to the B units of DNA gyrase. As noted previously, this enzyme catalyzes decatenation without wrapping a segment around itself, so it produces relaxed DNA. The intimate details of how this is done are still being worked out.[41]

Mammalian topoisomerase I is sensitive to camptothecin analogues, and its inhibition plays a significant role in cancer chemotherapy.[165,169] Mammalian topoisomerase III is as yet not involved in chemotherapy.[170]

Antibiotic activity concentrations of a wide variety of quinolones parallel those that inhibit the enzymes.[42,119] Interestingly, quinolones have little affinity for purified DNA gyrase alone.[171] They do have affinity for DNA, particularly for single-stranded DNA, but this affinity is not readily saturated at meaningful concentrations.[118] On the other hand, when DNA gyrase and double-stranded DNA are both present, a specific binding site for quinolones is created that is saturable at antimicrobially relevant concentrations.[172] The binding is reversible and cooperative and saturates with up to four quinolone molecules.[118] It is also sensitive to the absolute configuration of the quinolones.[119] The cooperativity is seen from the kinetics, which show the first molecule to enter the active site fairly sluggishly, the second more readily (perhaps as a consequence of a compatible distortion of the active site, or perhaps the first molecule provides additional binding interactions for the second), and a third and fourth molecule to add sequentially to complete the saturation of the enzyme-induced binding pocket.[118] Synthetic covalently bound model norfloxacin dimers linked between the pyridine nitrogen atoms by methylene spacer groups bind well but fail to show an anticipated entropic advantage, providing evidence not only for the size and character of the binding pocket but strongly in agreement with the cooperativity phenomena. Interestingly, a four-methylene linker is optimal for inhibition of *E. coli* DNA gyrase activity, whereas five are best for the enzyme from *Micrococcus luteus*. This suggests that the quinolone-binding pocket is larger in Gram-positive microorganisms than in Gram-negatives. These tethered probe molecules have fewer degrees of freedom than the monomers have. They are also inactive in whole cells presumably due to poor pharmacokinetic properties.[118] More recently, a series of dimers of ciprofloxacin linked between the distal nitrogens of their C-7 piperazinyl moieties also failed to show an entropic advantage in potency over the monomers. These compounds were, however, surprisingly active in whole cell cultures of *S. pneumoniae*, approximating the MIC values for ciprofloxacin alone.[173]

The quinolones can also bind to supercoiled DNA in the absence of the enzyme. This is believed to be a consequence of the presence of small segments of single-stranded regions created by the supercoiling.[88] This probably is unimportant for their mode of action. The possibility that enhanced drug binding was due to the conversion of relaxed substrate DNA to the supercoiled form was ruled out by the use of a nonhydrolyzable triphosphate nucleotide in the binding mixture.[174]

The keto−carboxyl system at C-3 and C-4 is proposed to hydrogen bond strongly to the single-stranded sides of the melt zone induced into the DNA by the gyrase. This would dictate the primary orientation of the first drug molecule to enter the complex. The bonding energy lost when the water associated with the ketoacid moiety is displaced would be regained upon hydrogen bonding to the DNA single strands. The enzyme is also nearby, and genetic studies with resistant mutants indicate that an aspartate is important. This probably interacts electrostatically with the protonated distal nitrogen of the piperazine ring or the distal nitrogen attached as a side chain to a C-7 pendant pyrrolidinyl ring. This would provide directionality to the drug binding. The second quinolone molecule would align itself such that the keto−carboxyl group interacts with the other single strand of DNA in the melt bubble. The carboxyl group and piperazinyl groups would line themselves up with the first molecule acid to base and base to acid. The interior regions of the two quinolone molecules thus brought together would be lipophilic and compact, providing a grease−grease surface associating the two molecules. This requires the remainder of the water present in the pocket to be squeezed out as hydrogen bonding is much stronger than lipophilic bonding. This dimer is oriented in reverse to the bases in double-stranded DNA in that the hydrogen-bonding surfaces are on the outside and the lipophilic portions are on the inside. This is perfectly suited to exploit the melt zone. Two more molecules of quinolone can assemble themselves to the original pair, forming a cube of four. This apparently saturates the binding pocket. This view would rationalize satisfactorily the functional roles of the known structure−activity features of the quinolones. The experiments of Critchlow and Maxwell[176] later demonstrate a 2:1 ratio of quinolones in the complex. They were cautious, however, to point out that their

methods of measurement were sufficiently vigorous that they could have disrupted weaker interactions in the complex so that a 4:1 ratio might still be possible.

These and a number of other considerations have been rationalized in a well-received model discussed in the next section along with various other models.[118,119]

7.1. Models of the Ternary Complex

Obviously, the particular value of hypothetical bioactivity models is their utility in rationalizing a mass of observations in structural terms. Furthermore, the best models not only posit a credible role for the functional contributions of individual portions of quinolone molecules but also can provide the basis for prediction of the potential contribution of new chemical features in advance of synthesis.

The first and most successful of the models of the ternary complex was advanced by Shen.[118] It has been modified over the years in light of further information but remains substantially intact. Its initial expression was based upon a series of important observations. Quinolones do not bind to DNA gyrase at their inhibitory concentration although they do bind to various types of DNA to various degrees. They bind poorly to relaxed double-stranded DNA. This binding is weak and nonsaturable. They bind preferentially to single-stranded DNA but in a nonspecific and a noncooperative manner. This form of binding correlates with hydrogen-bonding capability. Indeed, homopolymer work showed that there was a preference of quinolones for poly(dG), which has more hydrogen-bonding functionality. Ring stacking could not be demonstrated and, indeed, appeared unlikely to play a dominant role as the intensity of quinolone binding did not correlate with the type of DNA base present.[174,175] They bind specifically to a saturable site on supercoiled DNA in a highly cooperative manner at a concentration near their K_i value. DNA gyrase enhances quinolone binding to relaxed forms of DNA.[171] The cooperative binding observed saturated at about four molecules of quinolone per DNA site.[174] These findings indicated a significant role for the substrate DNA but did not explicate the specific interactions with the enzyme. It was, however, asserted that bound DNA gyrase induces a binding site for the drug in the relaxed DNA substrate and that this required the action of ATP.[118] Specificity apparently arose through the specific action of this class of enzyme on its substrate. This satisfactorily rationalized the general lack of mutagenicity of these agents.

In its initial iteration, the model suggested that the strong preference for single-stranded DNA regions was satisfied when the enzyme cut the two DNA strands to create short (four-residue) regions that separated to allow passage of an uncut region and passage behind. This model is illustrated in Figure 42. The upper portion of the figure illustrates in cartoon form the DNA bubble attached to DNA gyrase, saturation of the binding site by four quinolone molecules, and the putative cleavage sites. The lower portion of the figure shows four head-to-tail stacked norfloxacin molecules hydrogen bonding to the GCA and T bases of a DNA segment. Note that a preference for guanine would occur because of the presence of two hydrogen-bond-donating NH groups whereas CAT bases have only one such group. Self-association of the hydrogen-bonded quinolone molecules is envisioned as involving π stacking in the vertical dimension and hydrophobic interactions involving the portions of the drugs opposite those hydrogen-bonded to the edges of the DNA bubble.

Later work showed that inhibition of the catalytic cycle by topoisomerases could occur without strand cleavage.[176] The most convincing demonstration of this was made when the tyrosine residue in the active site was exchanged for a phenylalanine residue. This, of course, removed the essential nucleophilic phenolic hydroxyl moiety from the enzyme, and yet quinolone binding still could be measured. To accommodate these data, the model was altered once again in that the binding was attributed to a melt bubble being formed. To visualize this, the reader can examine Figure 42 again but imagine that the cut ends of the DNA strands are still joined and therefore continuous. Localized separation of the strands by the enzyme exposes single-stranded regions to which the quinolones can bind in the manner previously postulated. Since two single-stranded regions are available in both the gate and the bubble forms, efficiency and enhanced power would result from bonding to each of them. The initial form of the model posited four quinolone molecules binding to each binding site. Later work suggested that two might be sufficient. This disparity may be attributed to different experimental conditions being used. Association of two molecules instead of four does not alter the model in any fundamental way. In either case, the quinolone molecules would have their polar edges available on the outside of their aggregate and the internal nonpolar regions would stabilize the complex by lipophilic association. This is consistent with the structure−activity relationships known to date, and the original iteration of the model remains essentially intact. The showing that cleavage was not a necessary precondition for formation of a ternary complex does not rule out complexation with both the bubble and the gate stages and does not indicate which of these might be the most powerful or influential.

The binding cooperativity could be rationalized by positing that the first quinolone molecule displaced water present in the opening single-stranded region and provided a template for the next quinolone molecule as the gap produced by the enzyme widened. The enzyme-induced binding site would saturate with drug and freeze the complex so that it could neither advance nor recede.

Later work suggested that the role of magnesium ion had not been given sufficient prominence.[177−179] It is not immediately clear whether the magnesium ion influence is due to its well-known stabilizing effect on DNA topology or to its equally well-known ability to chelate with the keto and acid moieties of quinolones or to both of these phenomena. If magnesium binding to quinolones is directly involved, then instead of hydrogen bonding directly to DNA

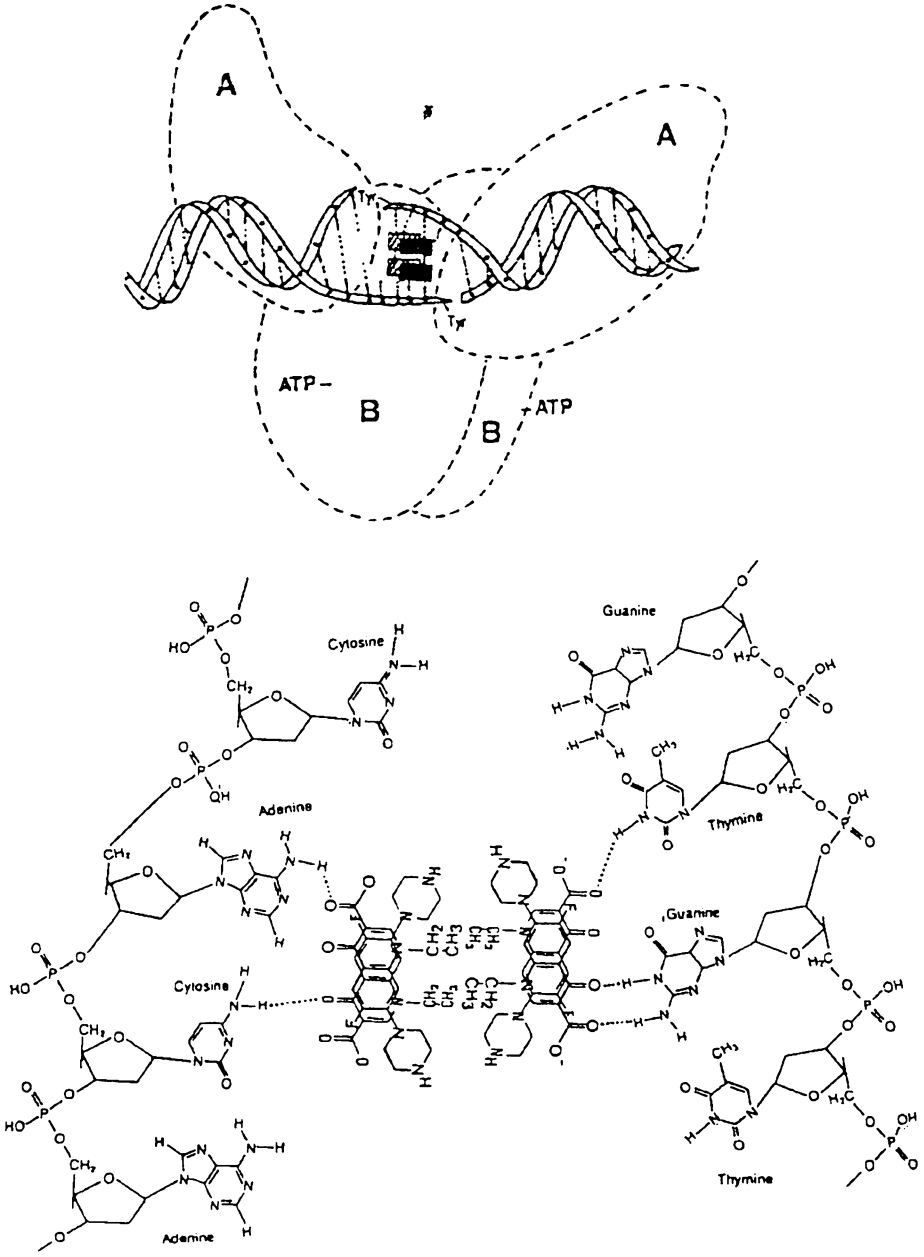


Figure 42. Illustrations of the putative quinolone binding mode to DNA gyrase. The upper portion depicts the binding of four molecules of quinolone (rectangles) to a cleaved bubble in DNA attached to DNA gyrase. The gyrase resembles a Viking helmet. The lower portion depticts a computer-drawn cluster of four quinolone molecules hydrogen-bound to the inner edges of a DNA bubble. Reprinted from ref 118. Copyright 1989 American Chemical Society.

bases, a chelated magnesium ion could serve as an adapter. In Figure 42 this would place a magnesium ion between each quinolone and its hydrogen-bonding DNA base partner. This would require a bigger bubble but is not precluded. The putative magnesium ion effect is less attractive structurally as the binding could alternatively be associated with linkage of chelated quinolone to the phosphate backbone of DNA instead of the bases. A preference for single-stranded regions is not readily accommodated by this idea.

Despite its evolution as newer data become available, the model has served very well in rationalizing structure−activity relationships in the quinolone field. No other model has yet been brought forward that has successfully supplanted it.

A particularly attractive feature of the modified Shen model is its ability to rationalize the role of the N-1 substituent (the C-3 position of the oxazine ring of ofloxacin) and, most particularly, when it is chiral. It is the only model that explicitly rationalizes this. The Morrissey et al. modification of the original Shen model has this as a central feature.[119] The observation that (*S*)-ofloxacin (levofloxacin) binds more firmly to the enzyme-induced pocket than its enantiomer indicates that the methyl groups exploit the binding site differently depending on their orientation into three-dimensional space. Clearly, since racemic ofloxacin binds roughly half as well into the pocket as levofloxacin, a methyl group with the wrong (*R*) orientation fails to take complete advantage of the pocket. This is confirmed by noting that (*R*)-ofloxacin

binds approximately 12-fold less intensely to the enzyme−substrate complex. Furthermore, (*R*)-ofloxacin saturates the binding bubble when only two molecules are added using conditions wherein (*S*)-ofloxacin saturates it with four molecules. Thus, in a competition, the *S*-enantiomer will clearly win out, and the binding model proposed satisfactorily rationalizes these various observations. The relative rates and final amounts of accumulation of these agents into *E. coli* are closely similar, removing differential uptake as an alternate explanation. Additional supportive data include the finding that des-*C*-methylofloxacin binds less well than ofloxacin itself and the *gem*-dimethyl analogue *also* binds less well. When the *C*-methyl group of ofloxacin is exchanged for an olefinic linkage, once again binding decreases. These data are most consistent with a preferred β and axial orientation of the methyl group in levofloxacin being optimal for the enzyme. The interested reader should refer back to Figure 32 for a structural view of this.

The Morrissey refinement of the Shen model is also compatible with many other features of the antibacterial quinolones. For example, it successfully rationalizes the utility of the *N*-methyl compounds of the first series. A methyl group would be too small to take advantage of the positive binding features of the methyl group of levofloxacin but could fit as well as the less active des-*C*-methyl analogue. Possession of an ethyl group at N-1 would allow, in one of the rotamers, the distal methyl group to satisfy the needs of the enzyme expressed in its preference for an axial, β, chiral methyl. It, not being rigid, however, would not be as effective. The finding that bigger, branched *N*-alkylquinolones are deleterious and polar substituents at N-1 are unsatisfactory would agree as the pocket exploited by a suitable methyl could be posited to be limited in size and to be hydrophobic in nature. *N*-Cyclopropylquinolones are also compatible with this hypothesis as are the *N*-vinyl- and *N*-aryl-substituted quinolones since X-ray studies indicate that the aryl groups are about 30° orthogonal to the ring system itself. The otherwise surprising antimicrobial activity of the *N*-*tert*-butylquinolones can also be rationalized with this model. Rotation provides a compatible methyl group every 120°. This substance could be viewed as being related to the *gem*-dimethyl analogue of ofloxacin.[180] The latter would be a molecule possessing simultaneously the virtues of the β-methyl group of levofloxacin and the defects of the α-methyl group of its enantiomer. These concepts are captured in Figure 34.

One of the unusual features of these drugs is that they bind to a pocket that is not present in the ground state of the substrate DNA. Rather the binding site is created by action of the enzyme. This satisfactorily rationalizes their lack of significant genotoxicity despite DNA being their target. They interfere with the functions of DNA, not with the nature of its bases. Another unusual feature is the number of drug molecules required to complete inhibition of DNA gyrase and the cooperative nature of this interference.[118] The much more common arrangement, well-known to medicinal chemists, is one molecule to a single active site in the molecular target. This satisfactorily rationalizes the puzzle that such a small molecule (taken as a monomer) could have such a profound effect on two such large targets.

Covalent tethering of two molecules that linked quinolones at the N-1 position has provided significant support for at least the dimeric interaction. Four methylenes proved optimal against the Gram-negative enzyme, and this coincided closely with the relationship and orientation of neighboring nalidixic acid molecules in the unit cell of its crystals.[118] Tethering with a smaller or larger number of methylenes proved significantly less active. Interestingly, five methylenes proved optimal against the Gram-positive enzyme, providing evidence that homology of the active site of different topoisomerases is not absolute and suggesting that the difficulty in finding quinolones with equal potency against Gram-positives and Gram-negatives may involve features additional to the comparative targeting of DNA gyrase and topoisomerase IV.

The initial cartoons illustrating the complex were silent about the specific role of the enzyme. This could have been interpreted that the role of the enzyme was to produce the binding site. Subsequently, it has come to be believed that the C-7 amino function would provide an orienting binding interaction also with the enzyme.[181−183] This would provide directionality to the bound drug. Conveniently, this orientation is the same as that chosen empirically for drawing the structures on paper. The Morrissey modification of the Shen model includes specific interactions between quinolones and DNA gyrase as well as with DNA.

Thus, the particular value of the evolved version of the Shen model is that it imputes not only a role for each of the chemical features of the quinolones but also for each of the components interacting in the ternary complex. It is not only consistent with the majority of experimental evidence but also predictive about the possible role that novel quinolone structural features might play in the future.

In an alternative model advanced by Nakamura as illustrated by Heddle and Maxwell (see Figure 43), the interaction between the quinolones and the DNA bases involves stacking and confirms that single-stranded regions are important.[179,184,185] In this view, the first step is intercalation in the bubble region created by the enzyme. The bases in the bubble swing outward next and are replaced by intercalating quinolone molecules. The quinolone molecules would be widely separated from each other and would not self-associate. There is no literature support for classical intercalation.[171,177] If this occurs, it must be transient. The model also pictures a binding site for a single quinolone in a pocket near the juncture of the GyrA and GyrB units of DNA gyrase. In this model binding is enhanced by interactions with certain amino acids of the enzyme and the DNA phosphate backbone phosphates.[182,185]

Noble and Llorento advance a model that agrees with many features of that of Nakamura.[179,183] Significant features illustrated in Figure 44 include interspersion of bound magnesium ion between a quinolone molecule and DNA bases and phosphate as well as intercalation/stacking and hydrogen bonding between serine 83 OH and the C-6 fluorine.

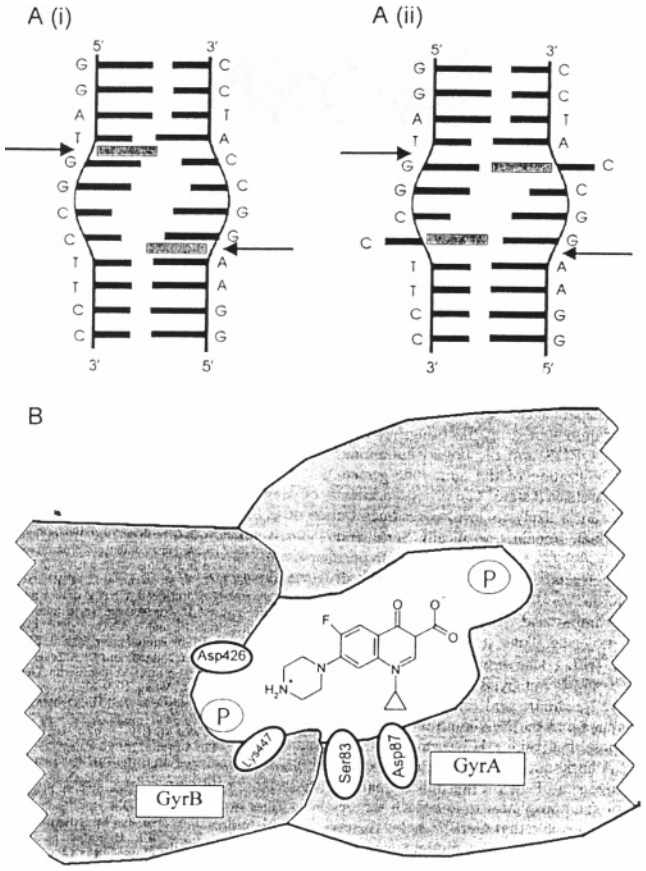


Figure 43. Nakamura model as illustrated by Heddle and Maxwell. (A(i)) and (A(ii)) illustrate intercalation of quinolones in the bubble region of DNA followed by outward rotation of the DNA bases, which are then replaced by the quinolone molecules. (B) illustrates the putative quinolone-binding pocket at or near the junction of the GyrA and GyrB interface. The incorrect double bond position in the original of Figure 43B is trivial and has not been altered. Reprinted with permission from ref 182, p 1813, Figure 8. Copyright 2002 American Society for Microbiology.

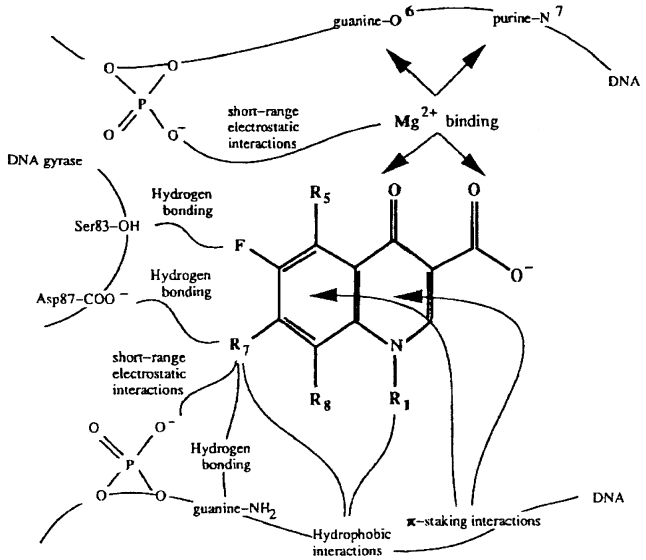


Figure 44. Llorente model. Reprinted with permission from ref 183. Copyright 1996 Elsevier.

Subsequent finding of intense activity possessed by some nonfluorinated quinolones weakens the last point.

Intercalation is also an important element of the hypotheses of Hurley's group for the inhibition of mammalian topoisomerase II by the quinobenzoxazines.[186–188] The problem with the Hurley model for this purpose is that the intercalating quinolones involved in his work are cell poisons and not selective antimicrobials. The antimicrobial quinolones that are invoked as participating stack on the outside of the DNA molecules. The Hurley model is more likely to be applicable to the antitumor effects of certain fused tricyclic quinolones than to the very different phenomenology associated with the antibacterial effects of antibacterial chemotherapy.

Recently, Tuma proposed that quinolones form a molecular cap, stabilizing the DNA duplex by forming a covalent bond with the cut DNA C-5′ segments.[189] Unfortunately, for this view, there is no evidence for a covalent bond between quinolones and DNA with or without the intermediacy of DNA gyrase or the presence of cut ends.

It is important to bear in mind that topoisomerase IV has not been studied in the same detail and that the Shen model may not apply as well to it. The homology is only 40% between the two enzymes, and they function somewhat differently. Also it has been shown that the binding pocket of topoisomerase IV is apparently larger than that of DNA gyrase.[118] Furthermore, the lack of parallelism in structure–activity and potency between these enzymes and human topoisomerase II makes it dubious whether these considerations apply to the human enzyme either. In particular, there is strong evidence that intercalation is a significant feature of several drugs inhibiting the human enzyme, and there is no evidence that intercalation is involved in inhibition of the bacterial enzymes.

8. Assay Methods

To recapitulate briefly, two different sequillae are measurable following tertiary complex formation as illustrated in Figure 38 above. In the catalytic assay (a, top), one measures the relative amounts of negatively supercoiled DNA and equilibrating relaxed DNA topoisomers. In the absence of drug, bacterial DNA normally equilibrates between closed negatively supercoiled DNA and open nicked circular DNA. Increasing concentrations of drug lead to progressive conversion of negatively supercoiled DNA to topoisomers as seen by gel electrophoresis. The IC_{50} values are determined by measuring the concentration of drug required to inhibit DNA gyrase catalyzed relaxation to topoisomers by 50%.[138,190,191] A relaxation assay can also be used to measure inhibition of topoisomerase IV.[190] In the cleavage assay (b, bottom), supercoiled DNA in the absence of ATP is incubated in the presence of increasing amounts of drug (in this example, levofloxacin). The mixture is denatured with detergent and treated with a proteinase followed by electrophoresis to separate the various states of DNA, and these are quantitated following ethidium bromide staining. One notes the concentration-dependent decrease in topoisomers and the progressive appearance of linear DNA. The CC_{50} value is the concentration of drug that achieves 50%

of the maximal amount of cleavage as compared to standard norfloxacin.[192] For most quinolones, the IC_{50} and CC_{50} values correlate reasonably well.[193] The cleavage assay is easier to run and is less sensitive to assay conditions than the catalytic assay.

9. Molecular Modes of Resistance

The optimistic early days of chemotherapy when identification of a pathogen was often sufficient for making a suitable choice of drug have lamentably passed away. Widespread resistance now requires much more diagnostic work if rational chemotherapy is to be instituted. Compared to those of many other classes of anti-infective agents, the resistance levels to quinolones are as yet relatively low but are steadily increasing. This phenomenon is attributed to a variety of causes.[194] Primary, of course, is the recognition that this is a natural evolutionary response mediated by bacterial fecundity and genetic versatility. This war between man and microbes will be punctuated by episodes in which one side or the other gains a temporary advantage but neither will ever achieve a total victory. Today's challenge is to find means to keep the equilibrium point as much in humanity's favor as is technically possible.

Resistance levels in developing countries are notably higher than those in advanced nations. One wonders if this is significantly affected by a greater dependence on older, cheaper, less potent quinolones such as nalidixic acid, to which resistance develops more easily. Certainly, even in advanced nations one can point a finger toward widespread use of quinolones for agricultural purposes as contributing to the resistance problem.[195,196] To the extent that one buys into any of these propositions, what is to be done? One could ban the use of older, less effective quinolones that are more prone to resistance, but this is probably not economically feasible. Easier to effect would be withdrawal of quinolones from agricultural uses that are not essential. Historically, this has not proven easy to accomplish with other antimicrobials either.

Significant clinical resistance was first encountered in microorganisms that were not very sensitive to quinolones and those at the same time requiring only a single mutational step to engender high levels of insensitivity. Such bacteria are *S. aureus* (especially methicillin-resistant strains) and *P. aeruginosa*. For most bacteria, levels of resistance that are troublesome in the clinic often involve more than one mutational step. After a primary resistance step, additional mutations occur that solidify and intensify the level of resistance to quinolones.

Two main resistance mechanisms are clearly established. The most common and influential of these is decreased cellular uptake and/or active expulsion of quinolones, thereby denying them access to their cellular targets. The second involves mutations in the target enzymes, leading to decreased quinolone binding.[197] A third and as yet minor resistance mode involves a plasmid-mediated mechanism about which comparatively little is known at present. Quinolone-resistant bacteria carrying plasmids are comparatively rare as yet but spreading rapidly. An interesting feature of the bacteria carrying this plasmid is that cells carrying it often also are resistant to extended-spectrum β-lactam antibiotics as well. This makes this development potentially worrisome. The gene product for this means of resistance is thought to be a gyrase-protecting protein of unknown present clinical relevance.[198,199]

The molecular mode of action of quinolones and resistance to them are intimately interconnected, so resistance has been touched upon briefly already in several places in this review.

9.1. Uptake Inhibition

In wild Gram-negative strains access of quinolones to bacterial cells is mediated through passive uptake that can involve, in addition, comparatively nondiscriminating porin passage through the outer membrane.[69] The classical hydrophobic quinolones such as nalidixic acid are primarily taken up by passive transport. The newer hydrophilic quinolones are taken up partly through the water-lined porins and partly by passive uptake through the membranes. The peptidoglycan itself is thought to present almost no passage difficulties to quinolones. Passive membrane passage is believed to involve the percentage of a quinolone that is not ionized at physiological conditions (see Figure 11). In some bacteria, mutations leading to a decrease in the number of porins has been noted.[200] In these cases, penetration of hydrophilic quinolones decreases by about half, suggesting something of the degree to which these two phenomena are involved in cellular uptake.

In other cases it has been demonstrated that some of the ubiquitous ATP-coupled export proteins function to expel quinolones. At least 11 quinolone expulsion pumps have been identified to date. These are not quinolone specific but play other roles as well.[201] As yet, there is no definitive information about the normal materials with which these exporters busy themselves.

Clearly reduced availability of porins coupled with derepression of energy-requiring substrate pleotropic efflux proteins can lead to significantly decreased quantities of quinolones reaching the target enzymes in the cytoplasm. Overexpression of these expulsion proteins has been reported in a number of bacterial strains.[17] The efflux proteins span the inner membrane, periplasm, and outer membrane. When these pumps are also modified by mutation, it can be seen that multiple interlocking modes of resistance to quinolones can occur.

From the standpoint of effecting chemical stratagems for overcoming this mechanism, it is possible that providing a more avid substrate for the pumps would keep them busy and allow the quinolones to leak through into the cellular interior. This will be complicated by the variety of such pumps and present lack of knowledge of what the normal function of these pumps and the identity of their substrates is. Work along these lines has begun but has yet to reach clinical significance.[202] Off the shelf chemicals that interfere with the nor A pump of Gram-positives include reserpine[199] and verapamil.[144,203] These, of course, are useless for practical purposes because

they would exert significant pharmacological effects on the patient when given in the quantities required. It is also important to recognize that for this approach to work at all the pharmacokinetic characteristics of the two companion drugs must closely match. This is not easily accomplished.

9.2. Plasmid-Mediated Resistance

Recently, a plasmid has been isolated from a resistant clinical strain that encodes for a gyrase-protecting protein.[190,204,205] Very little is at present known about the molecular details of its actions.

9.3. Enzymatic Alteration of Quinolone Structures

No enzymes have yet been identified that are involved in quinolone structure alterations. This mode of resistance is common among other antimicrobial classes but is not seen here.

9.4. Mutations of DNA Gyrase

Mutations that render the DNA gyrase and/or topoisomerase IV less sensitive to their action are common. Most commonly, chemists believe logically that the more sensitive of the two enzymes controls bacterial response, but there are suggestions that this is not the whole story. One complicating feature, for example, is that the two enzymes are not present in equivalent amounts in all species.[206] It is well established that the principal target of the quinolones in Gram-negative microorganisms such as *E. coli* is usually DNA gyrase, with topoisomerase IV playing a lesser but enhancing role. Resistance involves mutations in both genes. Mutations in the A unit alone are sufficient to cause considerable resistance, and further mutations in the B unit amplify this effect.[197] One notes, however, that there are mutations in other regions of the B subunit that are independent of GyrA.[181,207,208] Clearly, mutations in either subunit that affect the supercoiling process or alter the shape of the drug-binding pocket will influence potency and could be involved in quinolone resistance. The region in subunit A stretching from amino acids 67−106 (*E. coli* numbering) constitutes the QRDR, and serine 83 and aspartate 87 are the most influential residues although a number of amino acid exchanges occur elsewhere in clinical strains.[163,209−212] The QRDR lies comparatively near the catalytic tyrosine residue 122.

A very useful summary of the specific resistance-associated mutations involving amino acid changes in quinolone target enzymes has been published recently by Hooper.[5]

The bulk of the resistance-associated mutations occurring in the GyrA subunit of DNA gyrase in Gram-negatives occur between amino acids 51 and 119. Particularly prominent among these are mutations involving amino acids 83 (normally serine or threonine) and 87 (normally aspartic acid). The changes are many in that 83 becomes leucine, tryptophane, alanine, isoleucine, phenylalanine, or even argenine, depending upon the species involved. Amino acid 87 likewise undergoes many possible substitutions including exchange for asparagine, valine, glycine, tyrosine, alanine, histidine, valine, lysine, or histidine. By and large, but not always, these changes involve substitution of a nonpolar residue for a polar residue. Whether these substitutions affect more prominently the shape of the binding site or its ability to interact with quinolones is not yet clear. This information would, of course, be invaluable in assisting future drug design. It is interesting to note that the ability of these mutations to interfere with quinolone binding is strongly influenced by quinolone structure. This is encouraging from the standpoint of drug design. It is obvious that alterations in amino acid 122, the tyrosine residue that cleaves the phosphate backbone to create an enzyme gate for strand passage, is not involved in these resistance mutations. If it were, it would simultaneously inactivate the enzyme and result in cell death.

An analogous region is found in the B subparticles. Among Gram-negative microorganisms, mutations to resistance in GyrB stretch between amino acids 406 and 495. These do not seem to be associated especially with specific amino acid residues but are singly distributed in this region. This region is posited to be near the quinolone-binding portion of the A subunits and influential regarding the binding of substrate DNA to the enzyme.[179]

Mutations in the B subunit of DNA gyrase are less important unless the A unit has been previously altered. Some structural inferences come from study of the effect of drugs on the B subunit. In this regard, the presence of a basic nitrogen in the form of a C-7 piperazine moiety is much more important than is seen with drugs such as nalidixic acid that has a neutral *C*-methyl moiety at C-7. It has been found that the lys447glu mutation leads to enhanced binding of piperazinylquinolones but has little to no influence on nalidixic acid.[181] This is posited to involve an electrostatic interaction between the glutamate carboxylate and the protonated piperazinyl nitrogen in the B subunit. This interaction would not take place with basic lysine. Being neutral (in that region), nalidixic acid should be indifferent to this change. Supporting this idea is the finding that the asp426asn mutation decreases binding of piperazinylquinolones.[213] Here the change from a putatively significant aspartoyl carboxylate to a neutral asparagine residue would fit this developing picture.

This comparatively vague picture is all that is available to guide synthesis at this moment.

These exchanges take place in the QRDR of the B subunits. This region is distant from the analogous QRDR region of the A subunits, so a mutual interaction would have to take place allosterically over a considerable distance.[144]

9.5. Mutations of Topoisomerase IV

One recalls that the ParC subunits of topoisomerase IV are roughly equivalent to GyrA as is ParE to GyrB. Furthermore, the QRDR is highly conserved, so it seems safe to presume that there should be significant parallels between quinolone activity and the mechanisms of resistance to the two enzymes. There are certainly some significant overlaps. For example, in *E. coli* mutations in *oarC* and

oarE genes convey significant quinolone resistance only if *gyrA* mutations have taken place already. In Gram-positives, significant resistance is seen following amino acid changes in ParC and ParE, with ParC single amino acid changes being more common. Many other mutational changes have also been observed.

In many Gram-positive pathogens the principal quinolone-sensitive target is topoisomerase IV although mutations in the DNA gyrase further enhance resistance. The sequence of resistance mutations here is reversed compared with that in the gyrase occurring later. These gyrase mutations are, however, quite similar to those occurring with Gram-negatives. ParC, the subunit of topoisomerase IV comparable to GyrA of DNA gyrase, undergoes resistance-associated mutational changes in Gram-negatives between amino acids 78 and 116. Most commonly encountered among these are changes in amino acids 80 and 84. These are analogous in position to amino acids 83 and 87 of DNA gyrase, suggesting that they may function similarly. Amino acid 80 is normally serine and can become leucine, isoleucine, argenine, or tryptophan depending upon the species involved. Amino acid 84 is normally glutamic acid and is exchanged for lysine, glycine, valine, or isoleucine. The parallel with GyrA is striking.[197]

With some quinolones (notably sparfloxacin, nadifloxacin, and garenoxacin) DNA gyrase is the primary target at least with *S. aureus.* The fairly substantial homology between DNA gyrase and topoisomerase IV suggests that similarities in the resistance pattern might be found, so one is not surprised that this is the case. In particular, the analogous QRDR segment is highly conserved. Here mutations in ParC are more influential than mutations in ParE, and there is cooperativity between them in determining the overall level of resistance. The genetics of resistance have been studied most thoroughly with *S. aureus* and *S. pneumoniae* than with other Gram-positive microorganisms. In Gram-positives, the picture is less clear and less data are yet available. Frequent mutational changes in the GyrA subunit involve amino acids in a narrower range stretching between 81 and 106. Amino acid 83 is still among the frequently changed, but amino acid 87 is replaced by amino acids at other nearby positions. Amino acid 81, normally serine, becomes phenylalanine, tyrosine, and cysteine. Amino acid 83, still normally serine, becomes phenylalanine, argenine, isoleucine, asparagine, or tyrosine. Amino acid 84, normally serine, becomes leucine, alanine, valine, lysine, tyrosine, or phenylalanine. Amino acid 85, normally serine, becomes proline, lysine, glutamine, or glycine.

The ParC mutational changes in Gram-positives most frequently involve substitutions at amino acids ranging from 23 to 176. Most often serines 79 and 80 and glutamic acid 84 are involved. Amino acid 79 correlates with amino acid 81 of GyrA and 80 correlates with amino acid 84, again suggesting some parallel of effect. Serine 79 becomes phenylalanine, tyrosine, isoleucine, leucine, or alanine. Serine 80 becomes phenylalanine, tyrosine, leucine, isoleucine, or argenine. Glutamic acid 84 becomes lysine, leucine, valine, alanine, glycine, tyrosine, or asparagine.

Mutational changes in the GyrB and ParE subunits of DNA gyrase and topoisomerase IV, respectively, play a modulating role in quinolone resistance. It is thought that changes in this subunit alter its topology and that this is transmitted to the A and C units. In this way they are believed to degrade the fit of quinolones to this part of the enzyme. As noted elsewhere, mutations in both subunits are associated with maximum resistance. Interestingly, mutations in these subunits are significantly less frequently associated with resistance than those in GyrA and ParC.

Likewise in ParE they are singly distributed between residues 25 and 478.

9.6. Effect of Mutations on Microbial Vitality

It is significant to note that while mutations to lesser sensitivity to quinolones have definite survival value to pathogens, in a number of cases the microorganisms carrying these mutations are less vigorous than wild strains. This suggests that drug rotations or "holidays" might have a favorable effect on resistance levels.

9.7. Possible Approaches to Dealing with the Resistance Problem

The prevailing belief is that quinolones bind to GyrA, and possibly ParC, near amino acids 83 and 87. When these amino acids are modified, resistance is common, especially when the mutations result in less hydrophilic character. This is overcome to some extent when C-8 of quinolones bears a chlorine, bromine, or methoxy group.[214,215] It can be speculated that normally hydrogen-bond-donating and -receiving enzyme side chains in this region are influential in maintaining structure, and the structure is disordered when this effect is lost. Perhaps suitable quinolone C-8 structural features compensate for this. Alternatively, it is possible that the polar side chains at positions 83 and 87 participate in hydrogen bonding to the C-7 quinolone positions and that this presents a steric hindrance to substrate processing. In this view, loss of the hydrogen bond character would make the drugs less effective as they would no longer fit as well. In the mutants one would then propose that the C-8 substituents provide an alternate binding capability, compensating for loss of bonding due to C-7.

In partial support of these ideas, mutation to a cysteine residue results in an enzyme that is less tolerant of the size of a substituent at the distal nitrogen of a C-7 moiety. This effect is significantly lost when the substituent is attached to an adjacent carbon compared to the nitrogen. The implication of this is that the enzyme has only a little space available in this region for the drug to fit into. If so, this is not very helpful in terms of analogue production.

It is technically extremely difficult to reactivate a defective enzyme through chemical means. Mostly therapeutic manipulations affecting enzymes are inhibitory. The best of current stratagems that might

Table 2. Officially Approved Clinical Indications for Common Quinolone Anti-Infectives[a]

disease	Nal	Nor	Cipro	Ofl	Levo	Trova	Moxi	Gati
urinary tract infection	X	X	X	X	X			X
sexually transmitted diseases		X	X	X				X
GI infections			X			X		
upper respiratory tract			X	X	X	X	X	X
lower respiratory tract			X					
skin and soft tissue			X	X	X	X	X	X
opthalmic			X	X	X		X	X

[a] The data were assembled from the *Physicians' Desk Reference*.[34]

Table 3. Approximate Pharmacokinetic Values for Commonly Used Quinolones[a]

drug	F	UE	PPB	Cl	VD	$T_{1/2}$	PT	PC
nalidixic acid		22	84	2.9	0.55	11.5		1.57
norfloxacin		30	15			5	1.45	1.44
ciprofloxacin	60	50	40	7.6	2.2	3.3	0.6	2.5
ofloxacin	95	64	25	3.5	1.8	5.7	1.7	1.6
levofloxacin	99	74	31	2.5	1.36	7	1.6	4.5
trovafloxacin	91	9	70	14	1.29	11.3	0.95	2.09
moxifloxacin	86	22	40	2.3	2.05	15.4	2.0	2.5
gatifloxacin		77				6.52	1.49	1.71
grepafloxacin		9.36				12.1	2.77	1.98

[a] Abbreviations: F = bioavailability (%); UE = percent active drug excreted in the urine; PPB = plasma protein binding (%); Cl = clearance ((mL/min)/kg); VD = volume of dilution (L/kg); $T_{1/2}$ = plasma half-life (h); PT = time to peak concentration in blood (h); PC = peak blood concentration (μg/mL).

ameliorate resistance due to enzyme mutations would be to decrease the resistance development rate. The most effective means of accomplishing this is to make sure that sufficient drug is always present to kill the organisms. Dead bacteria do not mutate. Alternatively, apparently under some circumstances in the laboratory mutations causing DNA strand breaks in the presence of reactive oxygen species can be protected against by use of certain antioxidant catechins. These agents can also be shown to delay or prevent resistance emergence at no other effect doses. This suggests the possibility that antimutagenic agents might be useful or, better yet, that quinolones possessing not only antimicrobial activity but also antimutagenic activity might be usefully developed.[138,216]

Resistance is often not an all or nothing phenomenon, so development of ever more potent quinolones will undoubtedly be pursued. Pessimistically, one notes that with widespread use resistance always develops—either easily or after a delay.

10. Clinical Indications

The widespread utilization of the quinolones is a consequence of their usefulness in treating a wide variety of common infections, particularly those encountered frequently in the community. For example, uncomplicated urinary tract, normal upper respiratory tract, and skin and soft tissue infections are quite common, and almost all of the quinolones show utility for these conditions. The special frequency of prescription of ciprofloxacin, ofloxacin, and levofloxacin is understandable from the table of approved indications wherein a great many uses are listed (Table 2).

11. Pharmacokinetics

The precise figures for the pharmacokinetic features of quinolone anti-infectives vary significantly from source to source and are strongly dependent upon the dosages given, but the following data are representative of those published and are reasonably near the consensus values. Figures are averaged, and error bars, which are sometimes substantial, have been omitted for clarity. Thus, the data in Table 3 are suitable for rough comparisons only.

From the data in the table it can be seen that the various agents are significantly to outstandingly bioavailable after oral administration, peak in the blood soon thereafter, and vary widely in the percentage of administered drug appearing in the urine in active form. Urinary excretion of quinolones involves a blend of glomerular filtration and tubular secretion. Tubular secretion is modestly enantioselective.[52] Transintestinal and biliary excretion routes are only significant for highly lipophilic quinolones such as trovafloxacin.[217] The more recently introduced agents have significantly longer half-lives that often permit one-a-day dosing.

A variety of in vitro and in vivo investigations show that many quinolones kill bacteria in a concentration-dependent manner.[218] This contrasts with the classical pattern that is still followed in many other classes of anti-infectives in which one strives to maintain continuously reasonable multiples of the minimum inhibitory or 'cidial concentrations in the blood. In contrast, it is believed that with the quinolones efficacy is more closely related to the concentrations achievable than the dosage interval. Animal infection models indicate that the area under the curve to minimum inhibitory concentration ratio is a significantly useful predictor of efficacy. The ratio of the peak concentration achievable to the minimum inhibitory concentration is believed more important to prevent selection for resistance during the course of therapy than to keep the concentration above a given value. It is suggested that a 24 h AUC/MIC ratio of 25−100, depending on the microorganism, is

satisfactory and is comparatively independent of the dosage interval, the specific drug, the animal species, and the site of infection.[219] It is difficult, however, to establish this proposition in patients. One also has some concern that pushing peak drug levels ever higher can lead to increased toxicity.

12. Future Prospects

The clinical impact of the quinolone anti-infectives makes it a certainty that they will serve for many years to come as mainstays in the unending struggle of mankind against morbidity and mortality due to infectious diseases. Whereas it is clear that future analoguing studies will result in incremental improvements in their useful properties, recent findings demonstrating potential impact in the areas of fungal[220] and viral[221] infections and in cancer chemotherapy[94] suggest a role for carefully crafted analogues in these areas as well. On the other hand, concerns about cross-resistance, particularly stemming from agricultural applications of quinolones, suggest conservatism in application will be appropriate.

The interaction of quinolones with DNA-processing enzymes is fascinating, and this story is still unfolding. Study of the quinolones has revealed much interesting molecular biology, and the future will reveal much more.

13. Conclusions

After nearly 40 years of intensive exploration, approximately 20 quinolone anti-infectives have been marketed, and two of these are present market leaders. Utopiafloxacin remains elusive. Structure−activity relationships of the major present chemotypes are now reasonably clear although surprises pop up from time to time. The molecular details of their interaction with DNA gyrase, bacterial topoisomerase IV, and human topoisomerase II remain largely conjectural and require more attention. Structure−toxicity relationships are not yet well understood although this is increasingly coming into focus. Resistance emergence, as with every other family of antibacterials, is increasing at a disturbing rate. Whereas much detail is now available outlining the alterations in bacteria that lead to resistance, practical means of minimizing this phenomenon have yet to be found.

14. References

(1) Andriole, V. T. *The Quinolones*; Academic Press: San Diego, 2000.
(2) Beermann, D.; Kuhlmann, J.; Dalhoff, A.; Zeiler, H. J. *Quinolone antibacterials*; Springer: Berlin, New York, 1998.
(3) Crumplin, G. C. *The 4-Quinolones: Antibacterial Agents in vitro*; Springer-Verlag: New York, 1990.
(4) Hooper, D. C.; Wolfson, J. S. *Quinolone antimicrobial agents*, 2nd ed.; American Society for Microbiology: Washington, DC, 1993.
(5) Hooper, D. C.; Rubinstein, E. *Quinolone antimicrobial agents*, 3rd ed.; ASM Press: Washington, DC, 2003.
(6) Kuhlmann, J.; Dalhoff, A.; Zeiler, J.-J. *Quinolone Antibacterials*; Springer-Verlag: New York, 1998.
(7) Ronald, A. R.; Low, D. E. *Fluoroquinolone Antibiotics*; Birkhauser: Boston, 2003.
(8) Siporin, C.; Heifetz, C. L.; Domagala, J. M. *The New Generation of Quinolones*; Marcel Dekker: New York, 1990.
(9) Wolfson, J. S.; Hooper, D. C. *Quinolone antimicrobial agents*; American Society for Microbiology: Washington, DC, 1989.
(10) Alghasham, A. A.; Nahata, M. C. *Ann. Pharmacother.* **2000**, *34*, 347, Quiz 413.
(11) Appelbaum, P. C.; Hunter, P. A. *Int. J. Antimicrob. Agents* **2000**, *16*, 5.
(12) Ball, P. *J. Antimicrob. Chemother.* **2000**, *46* (Suppl. T1), 17.
(13) Bertino, J., Jr.; Fish, D. *Clin. Ther.* **2000**, *22*, 798−817, Discussion 797.
(14) Bush, K.; Goldschmidt, R. *Curr. Opin. Invest. Drugs* **2000**, *1*, 22.
(15) Casparian, J. M.; Luchi, M.; Moffat, R. E.; Hinthorn, D. *South Med. J.* **2000**, *93*, 488.
(16) Gootz, T. D.; Brighty, K. E. *Med. Res. Rev.* **1996**, *16*, 433.
(17) Hooper, D. C. *Clin. Infect. Dis.* **2000**, *31* (Suppl. 2), S24.
(18) O'Donnell, J. A.; Gelone, S. P. *Infect. Dis. Clin. North Am.* **2000**, *14*, 489.
(19) Pickerill, K. E.; Paladino, J. A.; Schentag, J. J. *Pharmacotherapy* **2000**, *20*, 417.
(20) Varon, E.; Gutmann, L. *Res. Microbiol.* **2000**, *151*, 471.
(21) Maple, P. B.; Brumfitt, W.; Hamilton-Miller, J. M. *J. Chemother.* **1990**, *2*, 280.
(22) Walker, R. W.; Wright, A. J. *Mayo Clin. Proc.* **1991**, *66*, 1249.
(23) Gootz, T. D.; McGuirk, P. R. *Expert Opin. Invest. Drugs* **1994**, *3*, 93.
(24) Domagala, J. M. *J. Antimicrob. Chemother.* **1994**, *33*, 685.
(25) Chu, D. T. W.; Fernandes, P. B. *Adv. Drug Res.* **1991**, *21*, 39.
(26) Rosen, T. *Prog. Med. Chem.* **1990**, 235.
(27) Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, R. P. *J Med. Pharm. Chem.* **1962**, *91*, 1063.
(28) Koga, H.; Ito, A.; Murayama, S.; Suzue, S.; Irikura, T *J. Med. Chem.* **1980**, *23*, 1358.
(29) Jaber, L. A.; Bailey, E. M.; Rybak, M. J. *Clin. Pharm.* **1989**, *8*, 97.
(30) Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 559.
(31) Sato, K.; Matsuura, Y.; Inoue, M.; Une, T.; Osada, Y.; Ogawa, H.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1982**, *22*, 548.
(32) Hayakawa, I.; Hiramitsu, T.; Tanaka, Y. *Chem. Pharm. Bull.* **1984**, *32*, 4907.
(33) Atarashi, S.; Yokahama, S.; Yamazaki, K.; Sakano, K.; Imamura, M.; Hayakawa, I. *Chem. Pharm. Bull.* **1987**, *35*, 1896.
(34) *Physicians' Desk Reference*; Thompson PDR: Montvale, NJ, 2004.
(35) Blondeau, J. M. *Expert Opin. Invest. Drugs* **2000**, *9*, 1877.
(36) Nightingale, C. H. *Pharmacotherapy* **2000**, *20*, 245.
(37) Ling, T. K.; Liu, E. Y.; Cheng, A. F. *Chemotherapy* **1999**, *45*, 22.
(38) Bron, N. J.; Dorr, M. B.; Mant, T. G.; Webb, C. L.; Vassos, A. B. *J. Antimicrob. Chemother.* **1996**, *38*, 1023.
(39) Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Nash, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3872.
(40) Cozzarelli, N. R. *Science* **1980**, *207*, 953.
(41) Kato, J.; Nishimura, Y.; Imamura, R.; Niki, H.; Hiraga, S.; Suzuki, H. *Cell* **1990**, *63*, 393.
(42) Takei, M.; Fukuda, H.; Kishii, R.; Hosaka, M. *Antimicrob. Agents Chemother.* **2001**, *45*, 3544.
(43) Neuman, M. *Int. J. Clin. Pharmacol. Res.* **1987**, *7*, 173.
(44) Pan, X. S.; Fisher, L. M. *Antimicrob. Agents Chemother.* **1998**, *42*, 2810.
(45) Tanaka, M.; Onodera, Y.; Uchida, Y.; Sato, K.; Hayakawa, I. *Antimicrob. Agents Chemother.* **1997**, *41*, 2362.
(46) Hoshino, K.; Sato, K.; Une, T.; Osada, Y. *Antimicrob. Agents Chemother.* **1989**, *33*, 1816.
(47) Scheld, W. M. *Emerging Infect. Dis.* **2003**, *9*, 1.
(48) Tanaka, M.; Wang, T.; Onodera, Y.; Uchida, Y.; Sato, K. *J. Infect. Chemother.* **2000**, *6*, 131.
(49) Jalal, S.; Ciofu, O.; Hoiby, N.; Gotoh, N.; Wretlind, B. *Antimicrob. Agents Chemother.* **2000**, *44*, 710.
(50) Bazin, M.; Bosca, F.; Marin, M. L.; Miranda, M. A.; Patterson, L. K.; Santus, R. *Photochem. Photobiol.* **2000**, *72*, 451.
(51) Ooie, T.; Terasaki, T.; Suzuki, H.; Sugiyama, Y. *Drug Metab. Dispos.* **1997**, *25*, 784.
(52) Sorgel, F.; Kinzig, M. *Am. J. Med.* **1993**, *94*, 56S.
(53) Lazarczyk, D. A.; Goldstein, N. S.; Gordon, S. C. *Dig. Dis. Sci.* **2001**, *46*, 925.
(54) Blum, M. D.; Graham, D. J.; McCloskey, C. A. *Clin. Infect. Dis.* **1994**, *18*, 946.
(55) Satoh, Y.; Sugiyama, A.; Chiba, K.; Tamura, K.; Hashimoto, K. *J. Cardiovasc. Pharmacol.* **2000**, *36*, 510.
(56) Jeffrey, A. M.; Shao, L.; Brendler-Schwaab, S. Y.; Schluter, G.; Williams, G. M. *Arch. Toxicol.* **2000**, *74*, 555.
(57) Gould, R., Jr.; Jacobs, W. A. *J. Am. Chem. Soc.* **1939**, *61*, 2890.
(58) Grohe, K.; Heitzer, H. *Liebigs Ann. Chem.* **1987**, 29.
(59) Bouzard, D.; Di Cesare, P.; Essiz, M.; Jacquet, J. P.; Remuzon, P.; Weber, A.; Oki, T.; Masuyoshi, M. *J. Med. Chem.* **1989**, *32*, 537.
(60) MacDonald, A. A.; DeWitt, S. H.; Ramage, R. *Chemia* **1996**, *50*, 266.
(61) Frank, K. E.; Jung, M.; Mitscher, L. A. *Comb. Chem. High Throughput Screening* **1998**, *1*, 89.

(62) Gerster, J. E.; Rohlfing; S. R.; Pecore, S. E.; Winandy, R. M.; Stern, R. M.; Landmesser, J. E.; Olsen, R. A.; Gleason, W. B. *J. Med. Chem.* **1987**, *30*, 839.
(63) Gerster, J. E.; Rohlfing, S. R.; Winandy, R. M. *Proc. North Am. Med. Chem. Symp., Toronto, Canada* **1982**, 153.
(64) Hayakawa, I.; Atarashi, S.; Yokohama, S.; Imamura, M.; Sakano, K.; Furukawa, M. *Antimicrob. Agents Chemother.* **1986**, *29*, 163.
(65) Mitscher, L. A.; Sharma, P. N.; Chu, D. T. W.; Shen, L. L.; Pernet, A. G. *J. Med. Chem.* **1987**, *30*, 2283.
(66) Li, Q. M.; Mitscher, L. A.; Shen, L. L. *Med. Res. Rev.* **2000**, *20*, 231.
(67) Lomaestro, B. M.; Bailie, G. R. *Drug Saf.* **1995**, *12*, 314.
(68) Polk, R. E. *Am. J. Med.* **1989**, *87*, 76S.
(69) Fresta, M.; Guccione, S.; Beccari, A. R.; Furneri, P. M.; Puglisi, G. *Bioorg. Med. Chem.* **2002**, *10*, 3871.
(70) Fresta, M.; Spadaro, A.; Cerniglia, G.; Ropero, I. M.; Puglisi, G.; Furneri, P. M. *Antimicrob. Agents Chemother.* **1995**, *39*, 1372.
(71) Hayashi, N.; Nakata, Y.; Yazaki, A. *Antimicrob. Agents Chemother.* **2004**, *48*, 799.
(72) Albini, A.; Monti, S. *Chem. Soc. Rev.* **2003**, *32*, 238.
(73) Vargas, F.; Rivas, C.; Machado, R. *J. Photochem. Photobiol., B* **1991**, *11*, 81.
(74) Morimura, T.; Ohno, T.; Matsukura, H.; Nobuhara, Y. *Chem. Pharm. Bull. (Tokyo)* **1995**, *43*, 1000.
(75) Fasani, E.; Profumo, A.; Albini, A. *Photochem. Photobiol.* **1998**, *68*, 666.
(76) Shibata, T.; Nagasawa, M.; Iwai, N.; Miyazaki, M.; Kawamura, Y.; Kodama, T. *Jpn. J. Antibiot.* **1995**, *48*, 861.
(77) Martinez, L. J.; Sik, R. H.; Chignell, C. F. *Photochem. Photobiol.* **1998**, *67*, 399.
(78) Monti, S.; Sortino, S.; Fasani, E.; Albini, A. *Chem.—Eur. J.* **2001**, *7*, 2185.
(79) Howard, W.; Biedenbach, D. J.; Jones, R. N. *Clin. Microbiol. Infect.* **2002**, *8*, 340.
(80) Miolo, G.; Viola, G.; Vedaldi, D.; Dall'Acqua, F.; Fravolini, A.; Tabarrini, O.; Cecchetti, V. *Toxicol. in Vitro* **2002**, *16*, 683.
(81) Jones, R. N.; Biedenbach, D. J. *Diagn. Microbiol. Infect. Dis.* **2003**, *45*, 273.
(82) Matsumoto, J.; Miyamoto, T.; Minamida, A.; Nishimura, Y.; Egawa, H.; Nishimura, H. *J. Med. Chem.* **1984**, *27*, 292.
(83) Koikeda, S.; Okabe, H.; Shibata, H.; Hase, T.; Nakanishi, N.; Okezaki, E.; Kato, H.; Masamune, Y. *Jpn. J. Antibiot.* **1989**, *42*, 193.
(84) Peric, M.; Jacobs, M. R.; Appelbaum, P. C. *Antimicrob. Agents Chemother.* **2004**, *48*, 3188.
(85) Fu, L. M.; Fu-Liu, C. S. *Omics* **2002**, *6*, 199.
(86) Tomb, J. F.; White, O.; Kerlavage, A. R.; Clayton, R. A.; Sutton, G. G.; Fleischmann, R. D.; Ketchum, K. A.; Klenk, H. P.; Gill, S.; Dougherty, B. A.; Nelson, K.; Quackenbush, J.; Zhou, L.; Kirkness, E. F.; Peterson, S.; Loftus, B.; Richardson, D.; Dodson, R.; Khalak, H. G.; Glodek, A.; McKenney, K.; Fitzegerald, L. M.; Lee, N.; Adams, M. D.; Venter, J. C.; et al. *Nature* **1997**, *388*, 539.
(87) Albrecht, R. *Prog. Drug Res.* **1977**, *21*, 9.
(88) Chu, D. T.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E., Jr.; Pernet, A. G. *J. Med. Chem.* **1985**, *28*, 1558.
(89) Fernandes, P. B.; Chu, D. T.; Bower, R. R.; Jarvis, K. P.; Ramer, N. R.; Shipkowitz, N. *Antimicrob. Agents Chemother.* **1986**, *29*, 201.
(90) Rosen, T.; Chu, D. T.; Lico, I. M.; Fernandes, P. B.; Shen, L.; Borodkin, S.; Pernet, A. G. *J. Med. Chem.* **1988**, *31*, 1586.
(91) Alghasham, A. A.; Nahata, M. C. *Ann. Pharmacother.* **1999**, *33*, 48.
(92) Lumish, R. M.; Norden, C. W. *Antimicrob. Agents Chemother.* **1975**, *7*, 159.
(93) Segawa, J.; Kitano, M.; Kazuno, K.; Matsuoka, M.; Shirahase, I.; Ozaki, M.; Matsuda, M.; Tomii, Y.; Kise, M. *J. Med. Chem.* **1992**, *35*, 4727.
(94) Chu, D. T.; Hallas, R.; Clement, J. J.; Alder, J.; McDonald, E.; Plattner, J. J. *Drugs Exp. Clin. Res.* **1992**, *18*, 275.
(95) Kondo, H.; Sakamoto, F.; Inoue, Y.; Tsukamoto, G. *J. Med. Chem.* **1989**, *32*, 679.
(96) Kondo, H.; Sakamoto, F.; Kawakami, K.; Tsukamoto, G. *J. Med. Chem.* **1988**, *31*, 221.
(97) Kondo, H.; Sakamoto, F.; Uno, T.; Kawahata, Y.; Tsukamoto, G. *J. Med. Chem.* **1989**, *32*, 671.
(98) Li, Z. R.; Guo, H. Y.; Zhang, Z. P. *Yao Xue Xue Bao* **1991**, *26*, 111.
(99) Vincent, J.; Venitz, J.; Teng, R.; Baris, B. A.; Willavize, S. A.; Polzer, R. J.; Friedman, H. L. *J. Antimicrob. Chemother.* **1997**, *39* (Suppl. B), 75.
(100) Li, Q.; Chu, D. T.; Claiborne, A.; Cooper, C. S.; Lee, C. M.; Raye, K.; Berst, K. B.; Donner, P.; Wang, W.; Hasvold, L.; Fung, A.; Ma, Z.; Tufano, M.; Flamm, R.; Shen, L. L.; Baranowski, J.; Nilius, A.; Alder, J.; Meulbroek, J.; Marsh, K.; Crowell, D.; Hui, Y.; Seif, L.; Melcher, L. M.; Plattner, J. J.; et al. *J. Med. Chem.* **1996**, *39*, 3070.
(101) Miyamoto, T.; Matsumoto, J.; Chiba, K.; Egawa, H.; Shibamori, K.; Minamida, A.; Nishimura, Y.; Okada, H.; Kataoka, M.; Fujita, M.; et al. *J. Med. Chem.* **1990**, *33*, 1645.
(102) Imada, T.; Miyazaki, S.; Nishida, M.; Yamaguchi, K.; Goto, S. *Antimicrob. Agents Chemother.* **1992**, *36*, 573.
(103) Kaminsky, D.; Meltzer, R. I. *J. Med. Chem.* **1968**, *11*, 160.
(104) Nagate, T.; Komatsu, T.; Izawa, A.; Ohmura, S.; Namiki, S.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1980**, *17*, 763.
(105) Braveny, I.; Machka, K. *Arzneimittelforschung* **1980**, *30*, 1476.
(106) Corelli, F.; Massa, S.; Stefancich, G.; Artico, M.; Panico, S.; Simonetti, N. *Farmaco, Ed. Sci.* **1984**, *39*, 95.
(107) Matsumoto, J.; Minami, S. *J. Med. Chem.* **1975**, *18*, 74.
(108) Hayashi, K.; Takahata, M.; Kawamura, Y.; Todo, Y. *Arzneimittelforschung* **2002**, *52*, 903.
(109) Ledoussal, B.; Bouzard, D.; Coroneos, E. *J. Med. Chem.* **1992**, *35*, 198.
(110) Gerster, J. R.; Rohlfing, S. R.; Pecore, S. E.; Winandy, R. M. *Proc. North Am. Med. Chem. Symp., Toronto* **1982**, 153.
(111) Ishikawa, H.; Tabusa, F.; Miyamoto, H.; Kano, M.; Ueda, H.; Tamaoka, H.; Nakagawa, K. *Chem. Pharm. Bull. (Tokyo)* **1989**, *37*, 2103.
(112) Takahashi, N.; Shiragiku, T.; Itoh, T.; Kaneko, E.; Shibahara, T.; Kanbe, T.; Yamashita, S. *Arzneimittelforschung* **1994**, *44*, 1265.
(113) Fukuyama, M.; Oonaka, K.; Hara, M.; Imagawa, Y. *Kansenshogaku Zasshi* **1996**, *70*, 51.
(114) Coughlin, S. A.; Danz, D. W.; Robinson, R. G.; Klingbeil, K. M.; Wentland, M. P.; Corbett, T. H.; Waud, W. R.; Zwelling, L. A.; Altschuler, E.; Bales, E.; et al. *Biochem. Pharmacol.* **1995**, *50*, 111.
(115) Biedenbach, D. J.; Jones, R. N. *Antimicrob. Agents Chemother.* **1995**, *39*, 1636.
(116) Georgopapadakou, N. H.; Dix, B. A.; Angehrn, P.; Wick, A.; Olson, G. L. *Antimicrob. Agents Chemother.* **1987**, *31*, 614.
(117) Ariens, E. J. *Trends Pharmacol. Sci.* **1993**, *14*, 68.
(118) Shen, L. L.; Mitscher, L. A.; Sharma, P. N.; O'Donnell, T. J.; Chu, D. W.; Cooper, C. S.; Rosen, T.; Pernet, A. G. *Biochemistry* **1989**, *28*, 3886.
(119) Morrissey, I.; Hoshino, K.; Sato, K.; Yoshida, A.; Hayakawa, I.; Bures, M. G.; Shen, L. L. *Antimicrob. Agents Chemother.* **1996**, *40*, 1775.
(120) Hoshino, K.; Sato, K.; Akahane, K.; Yoshida, A.; Hayakawa, I.; Sato, M.; Une, T.; Osada, Y. *Antimicrob. Agents Chemother.* **1991**, *35*, 309.
(121) Akahane, K.; Sekiguchi, M.; Une, T.; Osada, Y. *Antimicrob. Agents Chemother.* **1989**, *33*, 1704.
(122) Vorbach, H.; Weigel, G.; Robibaro, B.; Schaumann, R.; Hlousek, M.; Beil, W. J.; Griesmacher, A.; Georgopoulos, A.; Graninger, W. *Int. J. Clin. Pharmacol. Ther.* **1997**, *35*, 235.
(123) Fleisch, F.; Hartmann, K.; Kuhn, M. *Infection* **2000**, *28*, 256.
(124) Shakibaei, M.; de Souza, P.; van Sickle, D.; Stahlmann, R. *Arch. Toxicol.* **2001**, *75*, 369.
(125) Williams, R. J., III; Attia, E.; Wickiewicz, T. L.; Hannafin, J. A. *Am. J. Sports Med.* **2000**, *28*, 364.
(126) Kim, J. C.; Yun, H. I.; Shin, H. C.; Han, S. S.; Chung, M. K. *Arch. Toxicol.* **2000**, *74*, 120.
(127) Donders, G. G. *Drugs* **2000**, *59*, 477.
(128) Patmore, L.; Fraser, S.; Mair, D.; Templeton, A. *Eur. J. Pharmacol.* **2000**, *406*, 449.
(129) Kang, J.; Wang, L.; Chen, X. L.; Triggle, D. J.; Rampe, D. *Mol. Pharmacol.* **2001**, *59*, 122.
(130) Chiba, K.; Sugiyama, A.; Satoh, Y.; Shiina, H.; Hashimoto, K. *Toxicol. Appl. Pharmacol.* **2000**, *169*, 8.
(131) Allen, A.; Bygate, E.; Faessel, H.; Isaac, L.; Lewis, A. *Int. J. Antimicrob. Agents* **2000**, *15*, 283.
(132) Bowles, S. K.; Popovski, Z.; Rybak, M. J.; Beckman, H. B.; Edwards, D. J. *Antimicrob. Agents Chemother.* **1988**, *32*, 510.
(133) Randinitis, E. J.; Alvey, C. W.; Koup, J. R.; Rausch, G.; Abel, R.; Bron, N. J.; Hounslow, N. J.; Vassos, A. B.; Sedman, A. J. *Antimicrob. Agents Chemother.* **2001**, *45*, 2543.
(134) Cole, S. T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S. V.; Eiglmeier, K.; Gas, S.; Barry, C. E., III; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Barrell, B. G.; et al. *Nature* **1998**, *393*, 537.
(135) Bates, A. D.; Maxwell, A. *DNA Topology*; Oxford University Press: New York, 1993.
(136) Nelson, D. L.; Cox, M. M. *Lehninger Principles of Biochemistry*, 4th ed.; W. H. Freeman and Co.: New York, 2005.
(137) Berg, J. M.; Tymoczko, J. L.; Stryer, L. *Biochemistry*, 5th ed.; W. H. Freeman and Co.: New York, 2002.
(138) Aubry, A.; Pan, X.-S.; Fisher, L. M.; Jarlier, V.; Cambau, E. *Antimicrob. Agents Chemother.* **2004**, *48*, 1281.
(139) Chen, C. R.; Malik, M.; Snyder, M.; Drlica, K. *J. Mol. Biol.* **1996**, *258*, 627.
(140) Krasin, F.; Hutchinson, F. *J. Mol. Biol.* **1977**, *116*, 81.
(141) Mouton, R. P.; Koelman, A. *Chemotherapy* **1966**, *11*, 10.

(142) Gradelski, E.; Kolek, B.; Bonner, D. P.; Valera, L.; Minassian, B.; Fung-Tomc, J. *Int. J. Antimicrob. Agents* **2001**, *17*, 103.
(143) Fraser, C. M.; Norris, S. J.; Weinstock, G. M.; White, O.; Sutton, G. G.; Dodson, R.; Gwinn, M.; Hickey, E. K.; Clayton, R.; Ketchum, K. A.; Sodergren, E.; Hardham, J. M.; McLeod, M. P.; Salzberg, S.; Peterson, J.; Khalak, H.; Richardson, D.; Howell, J. K.; Chidambaram, M.; Utterback, T.; McDonald, L.; Artiach, P.; Bowman, C.; Cotton, M. D.; Venter, J. C.; et al. *Science* **1998**, *281*, 375.
(144) Gushchin, A. E.; Ladygina, V. G.; Govorun, V. M.; Taraskina, A. M.; Savicheva, A. M. *Mol. Gen. Mikrobiol. Virusol.* **2000**, 33.
(145) Reece, R. J.; Maxwell, A. *J. Biol. Chem*. **1991**, *266*, 3540.
(146) Ali, J. A.; Jackson, A. P.; Howells, A. J.; Maxwell, A. *Biochemistry* **1993**, *32*, 2717.
(147) Brown, P. O.; Peebles, C. L.; Cozzarelli, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 6110.
(148) Tingey, A. P.; Maxwell, A. *Nucleic Acids Res.* **1996**, *24*, 4868.
(149) Chatterji, M.; Unniraman, S.; Maxwell, A.; Nagaraja, V. *J. Biol. Chem.* **2000**, *275*, 22888.
(150) Bernard, P.; Kezdy, K. E.; Van Melderen, L.; Steyaert, J.; Wyns, L.; Pato, M. L.; Higgins, P. N.; Couturier, M. *J. Mol. Biol.* **1993**, *234*, 534.
(151) Mizuuchi, K.; O'Dea, M. H.; Gellert, M. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5960.
(152) Nakada, N.; Gmunder, H.; Hirata, T.; Arisawa, M. *Antimicrob. Agents Chemother.* **1994**, *38*, 1966.
(153) Tsai, F. T.; Singh, O. M.; Skarzynski, T.; Wonacott, A. J.; Weston, S.; Tucker, A.; Pauptit, R. A.; Breeze, A. L.; Poyser, J. P.; O'Brien, R.; Ladbury, J. E.; Wigley, D. B. *Proteins* **1997**, *28*, 41.
(154) Angehern, P.; Buchmann, S.; Funk, C.; Goetschi, E.; Gmeunder, H.; Hebeisen, P.; Kostrewa, D.; Link, H.; Luebbers, T.; Masciadri, R.; Nielsen, J.; Reindl, P.; Ricklin, F.; Schmitt-Hoffmann, A.; Theil, F.-P. *J. Med. Chem.* **2004**, *47*, 1487.
(155) Heddle, J. G.; Blance, S. J.; Zamble, D. B.; Hollfelder, F.; Miller, D. A.; Wentzell, L. M.; Walsh, C. T.; Maxwell, A. *J. Mol. Biol.* **2001**, *307*, 1223.
(156) Pierrat, O. A.; Maxwell, A. *J. Biol. Chem*. **2003**, *278*, 35016.
(157) Tanitame, A.; Oyamada, Y.; Ofuji, K.; Fujimoto, M.; Iwai, N.; Hiyama, Y.; Suzuki, K.; Ito, H.; Terauchi, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J. *J. Med. Chem.* **2004**, *47*, 3693.
(158) Tanitame, A.; Oyamada, Y.; Ofuji, K.; Kyoya, Y.; Suzuki, K.; Ito, H.; Kawasaki, M.; Nagai, K.; Wachi, M.; Yamagishi, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2857.
(159) Tanitame, A.; Oyamada, Y.; Ofuji, K.; Suzuki, K.; Ito, H.; Kawasaki, M.; Wachi, M.; Yamagishi, J. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 2863.
(160) Elsea, S. H.; Osheroff, N.; Nitiss, J. L. *J. Biol. Chem*. **1992**, *267*, 13150.
(161) Wigley, D. B.; Davies, G. J.; Dodson, E. J.; Maxwell, A.; Dodson, G. *Nature* **1991**, *351*, 624.
(162) Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *Cell* **1997**, *90*, 65.
(163) Morais Cabral, J. H.; Jackson, A. P.; Smith, C. V.; Shikotra, N.; Maxwell, A.; Liddington, R. C. *Nature* **1997**, *388*, 903.
(164) Albertini, S.; Chetelat, A. A.; Miller, B.; Muster, W.; Pujadas, E.; Strobel, R.; Gocke, E. *Mutagenesis* **1995**, *10*, 343.
(165) Champoux, J. J. *Annu. Rev. Biochem*. **2001**, *70*, 369.
(166) Anderson, V. E.; Zaniewski, R. P.; Kaczmarek, F. S.; Gootz, T. D.; Osheroff, N. *Biochemistry* **2000**, *39*, 2726.
(167) Zunino, F.; Capranico, G. *Anticancer Drug Des*. **1990**, *5*, 307.
(168) Okuda, J.; Okamoto, S.; Takahata, M.; Nishino, T. *Antimicrob. Agents Chemother.* **1991**, *35*, 2288.
(169) Argaman, M.; Bendetz-Nezer, S.; Matlis, S.; Segal, S.; Priel, E. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 789.
(170) Gangloff, S.; de Massy, B.; Arthur, L.; Rothstein, R.; Fabre, F. *EMBO J*. **1999**, *18*, 1701.
(171) Shen, L. L.; Pernet, A. G. *Proc. Natl. Acad. Sci. U.S.A*. **1985**, *82*, 307.
(172) Shen, L. L. *Methods Mol. Biol*. **2001**, *95*, 171.
(173) Gould, K. A.; Pan, X. S.; Kerns, R. J.; Fisher, L. M. *Antimicrob. Agents Chemother.* **2004**, *48*, 2108.
(174) Shen, L. L.; Baranowski, J.; Pernet, A. G. *Biochemistry* **1989**, *28*, 3879.
(175) Tornaletti, S.; Pedrini, A. M. *Biochem. Biophys. Acta* **1988**, *949*, 279.
(176) Critchlow, S. E.; Maxwell, A. *Biochemistry* **1996**, *35*, 7387.
(177) Sissi, C.; Andreolli, M.; Cecchetti, V.; Fravolini, A.; Gatto, B.; Palumbo, M. *Bioorg. Med. Chem*. **1998**, *6*, 1555.
(178) Palumbo, M.; Gatto, B.; Zagotto, G.; Palu, G. *Trends Microbiol*. **1993**, *1*, 232.
(179) Noble, C. G.; Barnard, F. M.; Maxwell, A. *Antimicrob. Agents Chemother.* **2003**, *47*, 854.
(180) Wentland, M. P.; Perni, R. B.; Dorff, P. H.; Rake, J. B. *J. Med. Chem*. **1988**, *31*, 1694.
(181) Yoshida, H.; Bogaki, M.; Nakamura, M.; Yamanaka, L. M.; Nakamura, S. *Antimicrob. Agents Chemother.* **1991**, *35*, 1647.
(182) Heddle, J.; Maxwell, A. *Antimicrob. Agents Chemother.* **2002**, *46*, 1805.
(183) Llorente, B.; Leclerc, F.; Cedergren, R. *Bioorg. Med. Chem.* **1996**, *4*, 61.
(184) Gatto, B.; Capranico, G.; Palumbo, M. *Curr. Pharm. Des.* **1999**, *5*, 195.
(185) Heddle, J. G.; Barnard, F. M.; Wentzell, L. M.; Maxwell, A. *Nucleosides, Nucleotides, Nucleic Acids* **2000**, *19*, 1249.
(186) Kwok, Y.; Zeng, Q.; Hurley, L. H. *J. Biol. Chem.* **1999**, *274*, 17226.
(187) Fan, J. Y.; Sun, D.; Yu, H.; Kerwin, S. M.; Hurley, L. H. *J. Med. Chem*. **1995**, *38*, 408.
(188) Zeng, Q.; Kwok, Y.; Kerwin, S. M.; Mangold, G.; Hurley, L. H. *J. Med. Chem.* **1998**, *41*, 4273.
(189) Tuma, J.; Connors, W. H.; Stitelman, D. H.; Richert, C. *J. Am. Chem. Soc.* **2002**, *124*, 4236.
(190) Tran, J. H.; Jacoby, G. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5638.
(191) Hooper, D. C.; Wolfson, J. S.; McHugh, G. L.; Winters, M. B.; Swartz, M. N. *Antimicrob. Agents Chemother*. **1982**, *22*, 662.
(192) Saiki, A. Y.; Shen, L. L.; Chen, C. M.; Baranowski, J.; Lerner, C. G. *Antimicrob. Agents Chemother.* **1999**, *43*, 1574.
(193) Barrett, J. F.; Bernstein, J. I.; Krause, H. M.; Hilliard, J. J.; Ohemeng, K. A. *Anal. Biochem*. **1993**, *214*, 313.
(194) Hooper, D. C. *Drug Resist Updates* **1999**, *2*, 38.
(195) Moelbak, K.; Gerner-Smidt, P.; Wegener, H. *Emerging Infect. Dis.* **2002**, *8*, 514.
(196) Anderson, A.; McClellan, J.; Rossiter, S.; Angulo, F. J. The National Academies Press: National Academies of Sciences, Washington, DC, 2003; Appendix A, p 231.
(197) Drlica, K.; Malik, M. *Curr. Top. Med. Chem*. **2003**, *3*, 249.
(198) Hooper, D. C. *Emerging Infect. Dis*. **2001**, *7*, 337.
(199) Zhanel, G. G.; Walkty, A.; Nichol, K.; Smith, H.; Noreddin, A.; Hoban, D. J. *Diagn. Microbiol. Infect. Dis.* **2003**, *45*, 63.
(200) Hawkey, P. M. *J. Antimicrob. Chemother.* **2003**, *51 Suppl 1*, 29.
(201) Bhatt, K.; Banerjee, S. K.; Chakraborti, P. K. *Eur. J. Biochem*. **2000**, *267*, 4028.
(202) Lomovskaya, O.; Warren, M. S.; Lee, A.; Galazzo, J.; Fronko, R.; Lee, M.; Blais, J.; Cho, D.; Chamberland, S.; Renau, T.; Leger, R.; Hecker, S.; Watkins, W.; Hoshino, K.; Ishida, H.; Lee, V. J. *Antimicrob. Agents Chemother.* **2001**, *45*, 105.
(203) Abramycheva, N.; Govorun, V. M. *Antibiot. Khimioter*. **2000**, *45*, 14.
(204) Chatterji, M.; Nagaraja, V. *EMBO Rep*. **2002**, *3*, 261.
(205) Wang, M.; Sahm, D. F.; Jacoby, G. A.; Hooper, D. C. *Antimicrob. Agents Chemother.* **2004**, *48*, 1295.
(206) Pan, X. S.; Yague, G.; Fisher, L. M. *Antimicrob. Agents Chemother.* **2001**, *45*, 3140.
(207) Yamagishi, J.; Yoshida, H.; Yamayoshi, M.; Nakamura, S. *Mol. Gen. Genet.* **1986**, *204*, 367.
(208) Nakamura, S.; Nakamura, M.; Kojima, T.; Yoshida, H. *Antimicrob. Agents Chemother.* **1989**, *33*, 254.
(209) Cullen, M. E.; Wyke, A. W.; Kuroda, R.; Fisher, L. M. *Antimicrob. Agents Chemother.* **1989**, *33*, 886.
(210) Bast, D. J.; Low, D. E.; Duncan, C. L.; Kilburn, L.; Mandell, L. A.; Davidson, R. J.; de Azavedo, J. C. *Antimicrob. Agents Chemother.* **2000**, *44*, 3049.
(211) Oram, M.; Howells, A. J.; Maxwell, A.; Pato, M. L. *Mol. Microbiol.* **2003**, *50*, 333.
(212) Sreedharan, S.; Oram, M.; Jensen, B.; Peterson, L. R.; Fisher, L. M. *J. Bacteriol.* **1990**, *172*, 7260.
(213) Tanaka, M.; Onodera, Y.; Uchida, Y.; Sato, K. *Antimicrob. Agents Chemother.* **1998**, *42*, 3044.
(214) Dong, Y.; Xu, C.; Zhao, X.; Domagala, J.; Drlica, K. *Antimicrob. Agents Chemother.* **1998**, *42*, 2978.
(215) Sindelar, G.; Zhao, X.; Liew, A.; Dong, Y.; Lu, T.; Zhou, J.; Domagala, J.; Drlica, K. *Antimicrob. Agents Chemother.* **2000**, *44*, 3337.
(216) Pillai, S.; Pillai, C.; Shankel, D. M.; Mitscher, L. A. *Mutat. Res.* **2001**, *496*, 61.
(217) Vincent, J.; Teng, R.; Dalvie, D. K.; Friedman, H. L. *Am. J. Surg.* **1998**, *176*, 8S.
(218) Zhanel, G. G.; Noreddin, A. M. *Curr. Opin. Pharmacol.* **2001**, *1*, 459.
(219) Andes, D.; Craig, W. A. *Antimicrob. Agents Chemother.* **2003**, *47*, 3935.
(220) Kunin, C. M.; Ellis, W. Y. *Antimicrob. Agents Chemother.* **2000**, *44*, 848.
(221) Kashiwase, H.; Momota, K.; Ohmine, T.; Komai, T.; Kimura, T.; Katsube, T.; Nishigaki, T.; Kimura, S.; Shimada, K.; Furukawa, H. *Chemotherapy* **1999**, *45*, 48.

CR030101Q