Structural Features of New Quinolones and Relationship to Antibacterial Activity Against Gram-positive Bacteria

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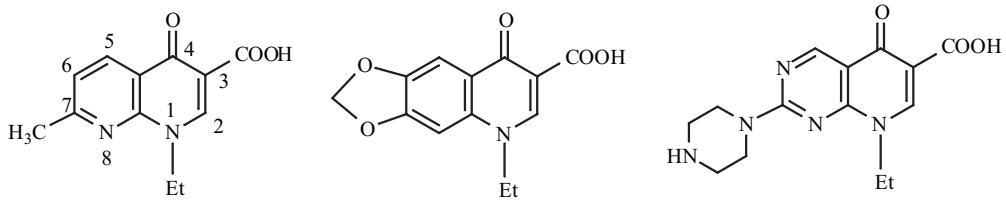
Abstract: A major recent focus of quinolone antibacterials has been the development of agents with enhanced activity against Gram-positive bacteria. The extensive research efforts have enabled a better definition of the structural features of quinolones that offer the best combination of clinical efficacy and reduced resistance selection in Gram-positive bacteria. This review considers the structural features of new quinolones and relationship to antibacterial activity against Gram-positive bacteria, while trying to position them in the context of recent and possible future advances based on an understanding of their chemical structure and how these impact on target specificity, avoidance of efflux and prevention of emergence of quinolone-resistant mutants in Gram-positive bacteria.

Keywords: Quinolone, fluoroquinolone, gram-positive bacteria, structure-activity relationships, resistance.

INTRODUCTION

Gram-positive bacteria are responsible for a wide range of diseases, and rising antibiotic resistance in this group is causing increasing concern [1, 2]. Gram-positive bacteria lack many of the complex permeation and export pathways found in Gram-negative bacteria, making them usually more amenable to antibacterial therapy. Yet, despite this, Gram-positive cocci have become a major problem, with resistance growing to β-lactams and to macrolides in strains of *Streptococcus pneumoniae* and staphylococci, especially in methicillin-resistant *S. aureus* (MRSA) strains. Resistance to macrolides is now widespread among strains of *Streptococcus pyogenes*, though they remain susceptible to penicillins. Enterococci, intrinsically resistant to many agents, have developed resistance to vancomycin [1, 2].

The quinolone class of antibacterial agents has evolved rapidly to emerge as one of the most effective classes of drugs in the treatment of infectious diseases [3-5]. Over the past 10 years quinolone research has been aimed at generally improving activity against Gram-positive cocci (particularly against staphylococci and pneumococci), whilst retaining the activity against Gram-negative organisms. Thus, with the recent introduction of newer quinolones, the traditional Gram-negative coverage of quinolones has been expanded to include specific Gram-positive organisms [6-10]. However, the isolation of quinolone-resistant bacteria has become a normal outcome. These problems of multi-drug resistance have been the driving force for the development of newer quinolones. The extensive research efforts have enabled a better definition of the structural moieties or elements around the basic pharmacophore of quinolones that offer the best combination of clinical efficacy and reduced resistance selection in Gram-positive bacteria.



Nalidixic acid (**1**) Oxolinic acid (**2**) Pipemidic acid (**3**)

Fig. (1). Primary quinolones with almost no activity against Gram-positive organisms.

This review considers the structural features of new quinolones and relationship to antibacterial activity against Gram-positive bacteria, while trying to position them in the context of recent and possible future advances based on an understanding of their chemical structure and how these impact on target specificity, avoidance of efflux and prevention of emergence of quinolone-resistant mutants in Gram-positive bacteria.

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DEVELOPMENT OF QUINOLONE ACTIVITY AGAINST GRAM-POSITIVES

Over three decades ago, a new milestone in the field of antibacterial drug discovery was reached by the introduction of a novel class of molecules, known as quinolones. The first of these compounds was the naphthyridine agent, nalidixic acid (**1**), an antibacterial by-product of chloroquine synthesis (Fig. **1**). Nalidixic acid (**1**) demonstrated limited antibacterial activity against Gram-negative cocci with a promise of clinical utility in urinary tract infections [11]. Structural modification of this first-generation quinolone ensued, in an effort to expand the spectrum of activity. Nalidixic acid (**1**) has several structural features retained by many of the newer compounds, and is based on an 4-oxo-1,8-naphthyridin (commonly, 4-quinolone) nucleus. Subsequent derivations, such as oxolinic acid (**2**) and pipemidic acid (**3**, the first 7-piperazinyl quinolone) were discovered in the 1970s, and represented only marginal improvements over nalidixic acid and these compounds were restricted in their use to urinary tract infections caused by the majority of Gram-negative bacteria. Gram-positive organisms are usually resistant to the early quinolones [12-13].

Flumequine (**4**) was the first compound to be developed with a fluoro- group at position 6 (Fig. **2**), and gave the first indications that modifications of the basic chemical structure could improve Gram-positive activity [6]. The newer fluoroquinolones arose with the development of norfloxacin (**5**), which combined structural features of flumequine (**4**) (C-6 fluorine atom) and pipemidic acid (**3**) (C-7 piperazine ring) [12-16]. During the 1980s, a great number of fluoroquinolones were developed, several of which reached the market and are still used today, including pefloxacin (**6**), enoxacin (**7**), fleroxacin (**8**), ciprofloxacin (**9**), lomefloxacin (**10**), ofloxacin (**11**) and levofloxacin (**12**) (Fig. **2**). The advantages of these compounds are that their spectrum includes Gram-positive species as well as Gram-negatives, and that they are well absorbed from the gastrointestinal

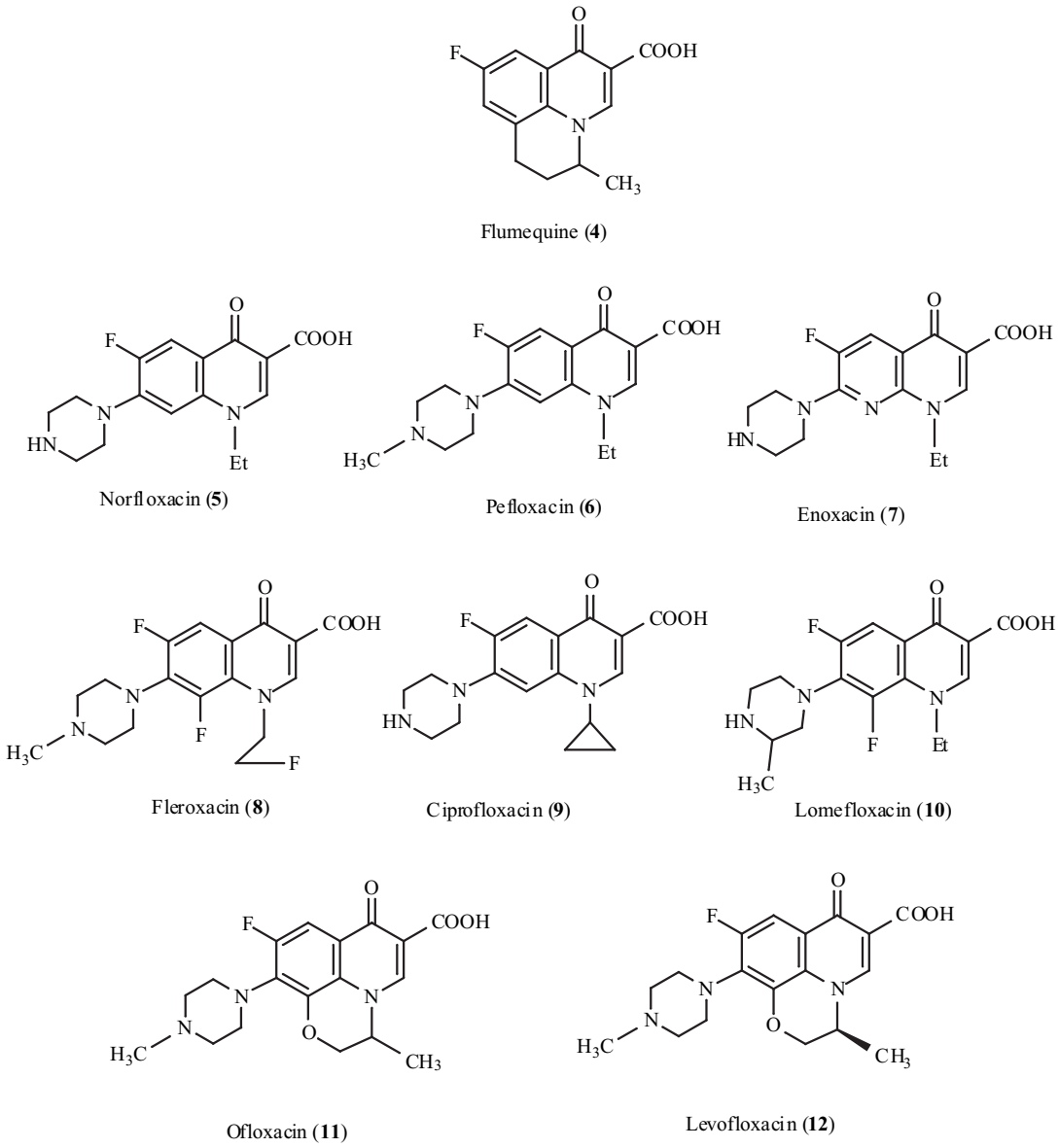

Fig. (2). Older fluoroquinolones possessing limited potency against Gram-positives.

tract, providing adequate blood levels to allow their use for systemic infections [7, 17, 18]. However, these early 6-fluoro- compounds were most potent against Gram-negative organisms; thus their activity against *Streptococcus pneumoniae* was too marginal to warrant clear indications for use in the treatment of respiratory tract infections, and the emergence of resistance soon reduced their potential against *Staphylococcus aureus*.

The fluoroquinolones were further enhanced against Gram-positives by structural modifications at the C-5, C-7 and C-8 positions (Fig. **3**). One of the first modifications was the introduction of NH_2 group at position C-5, which resulted in a general increase in anti-Gram-positive activity [7, 15-18]. This is seen with sparfloxacin (**13**), which also has fluorine at position C-6, a piperazine at position C-7 and is alkylated [19, 20]. Grepafloxacin (**14**) is also substituted at position C-5 but by a methyl group and has improved anti-Gram-positive potency compared with ciprofloxacin (**9**) [21]. Sparfloxacin (**13**) and grepafloxacin (**14**) had far better activity against a number of Gram-positives, particularly pneumococci, and improved activity against anaerobes, but their activity against staphylococci, although better than

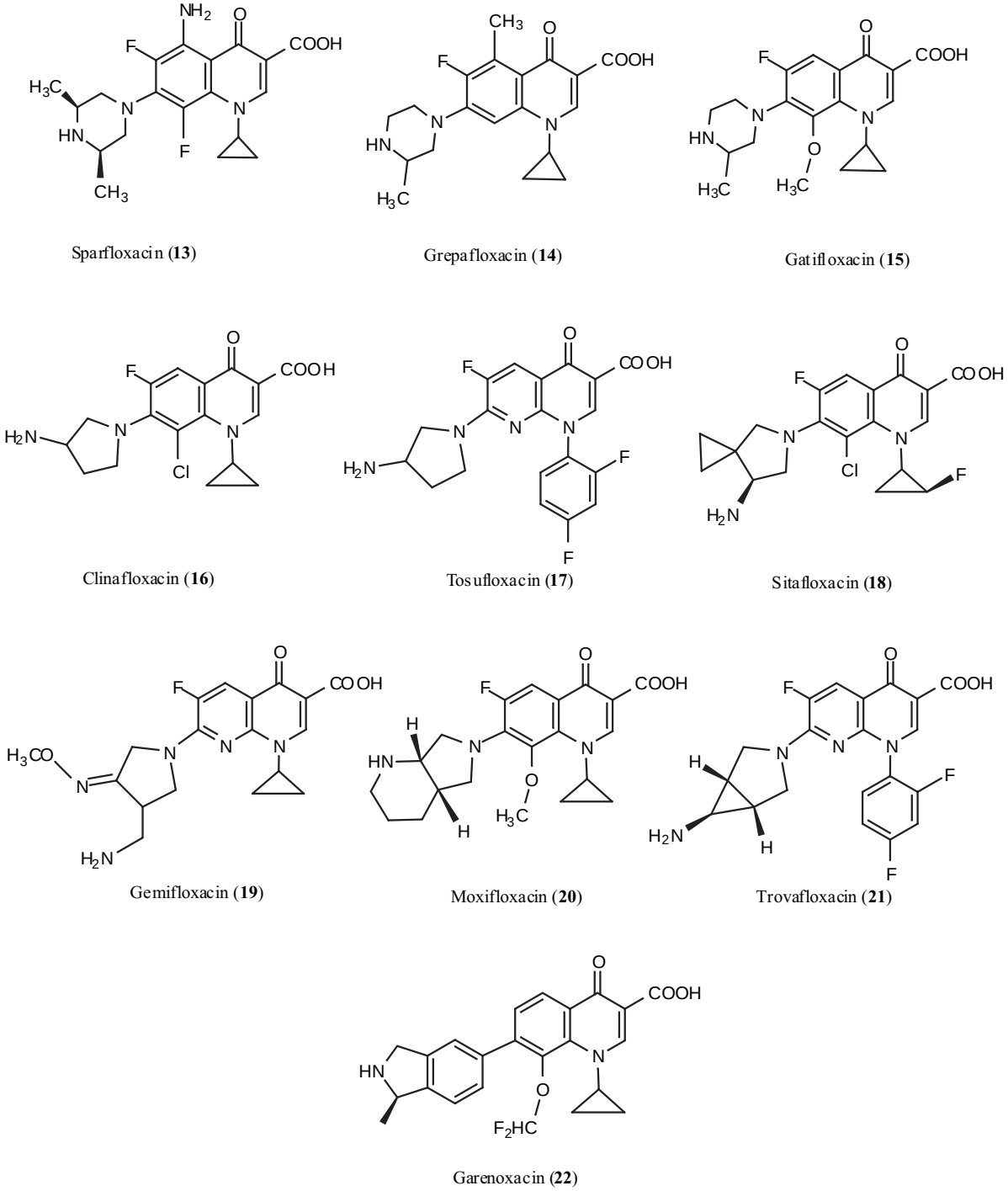

Fig. (3). New quinolones with improved Gram-positive coverage.

ciprofloxacin (**9**), is still less than ideal. Equally, the improvement in their activity against fluoroquinolone-resistant strains of staphylococci is only of academic interest and is not sufficiently great to have major clinical significance. However, both of these agents were soon withdrawn or restricted for toxicological reasons [6-8]. Since then, further improvement in activity against Gram-positive bacteria, together with significant anti-anaerobe activity, was seen with gatifloxacin (**15**), caused by the presence of a methyl piperazine at position 7, and of a methoxy at position 8 [22]. Introduction of pyrrolidine rings at C-7 were associated with enhanced potency against Gram-positive bacteria. Clinafloxacin (**16**), tosufloxacin (**17**), sitafloxacin (**18**) and gemifloxacin (**19**) are example of the advantages associated with a pyrrolidine ring. The addition of bicyclic amine groups onto position 7 has resulted in agents [moxifloxacin (**20**) and trovafloxacin (**21**)] with significant anti-Gram-positive activity, marked lipophilicity and long half-lives [9, 23-25].

Optimizing all other substituents has permitted the removal of the fluorine atom at position 6 (which has been claimed to be involved in genotoxicity), giving rise to the newer generation of quinolones, termed non-fluorinated quinolones (or des-fluoroquinolones), with garenoxacin (**22,** BMS-284756, T-3811ME) as its first representative. Garenoxacin (**22**) has a difluoromethoxy substituent at position 8, instead of a methoxy group, which has been shown to improve bacteriostatic and bactericidal activity. Garenoxacin (**22**) has exceptional activity against Gram-positive cocci including *S. aureus*; it is the most active quinolone against methicillin-susceptible and -resistant staphylococci, being more active than ciprofloxacin (**9**), ofloxacin (**11**), levofloxacin(**12**), and moxifloxacin (**20**) [26, 27].

STRUCTURE-ACTIVITY RELATIONSHIPS

Extensive efforts in the synthesis of new compounds in this class have been made in attempts to optimize the biological activity of the quinolones against Gram-positive bacteria. As has been pointed out previously [6], a similarity is seen in many quinolones with the β-lactam field, in that a reciprocal relationship is seen with the increasing the Gram-positive activity associated with the decreasing Gram-negative activity. However, some of the newer compounds seem to have greatly improved the activity against many Gram-positive species without compromising their activity against Gram-negatives [3-10]. Through this research, the breadth of allowable structural modification has been defined, while a number of optimal structural features have been kept relatively constant.

The 1,4- dihydro-4-oxopyridine-3-carboxylic acid associated with a 5,6-fused aromatic ring is the common chemical feature of quinolones (Fig. **4**). The nitrogen at N-1 is almost indispensable for activity and the carboxylic acid at position 3 and the carbonyl group at position 4 are considered critical for binding to cleaved or perturbed DNA, and no useful substitutions have yet been reported. Therefore, the 3-carboxylate and 4-carbonyl groups are considered essential for antimicrobial activity [4, 24]. As position 2 is very close to the binding site so it is believed that any added bulk inhibits access and results in a lower level of activity [14, 17].

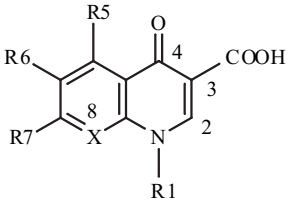

Fig. (4). Common pharmacophore of quinolones.

Much of the improved potency of modern quinolones against Gram-positive microorganisms has been achieved by tinkering with the N-1, C-5, C-6, C-7, and C-8 substituents on the quinolone ring system (Fig. **4**).

Position N-1

Hydrophobic substituents are essential. They have some impact on pharmacokinetics and control overall potency [14, 28-30]. A cyclopropyl substituent is now considered the most potent modification at this position; most of the successful compounds developed or under development contain this substituent including ciprofloxacin (**9**), sparfloxacin (**13**), grepafloxacin (**14**), gatifloxacin (**15**), clinafloxacin (**16**), gemifloxacin (**19**), moxifloxacin (**20**), and garenoxacin (**22**). Sitafloxacin (**18**) contains a fluorinated cyclopropyl group [*cis*-oriented (1*R*,2*S*)-2-fluorocyclopropyl] at N-1 [31, 32]. The addition of a 2,4-difluorophenyl group at position 1 [e.g. tosufloxacin (**17**) and trovafloxacin (**21**)] also improved activity against Gram-positive bacteria and anaerobes [23]. Another structure at this position is found in ofloxacin (**11**) and levofloxacin (**12**), which has a fused ring between positions 1 and 8. Indeed, ofloxacin has a tricyclic ring structure with a methyl group attached to the asymmetric C-3 position on the oxazine ring, thus connecting positions 1 and 8 with a fused ring. Although this has been a useful alternative to the cyclopropyl substituent, the *S*- isomer (levofloxacin) exhibits twice the order of magnitude of activity as the *R*- isomer [33].

Position 5

It has been shown that introduction of bulky substituents, halogen and methoxy at this position reduce activity markedly; this is probably a consequence of interference with the active binding site at positions 3 and 4 [14, 24]. Modestly sized substituents, such as an amino [as in sparfloxacin (**13**)], methyl [as in grepafloxacin (**14**)], or hydroxyl group can markedly increase *in vitro* activity against Gram-positive bacteria [14, 34]. Thus, substitutions at this position are thought to contribute to potency against Gram-positive organisms [19-21]. However, the influence of C-5 substituent markedly depends on the substitution pattern at N-1, C-7 and C-8, because nearly all the current quinolones [including ciprofloxacin (**9**), ofloxacin (**11**), levofloxacin (**12**), gatifloxacin (**15**), clinafloxacin (**16**), sitafloxacin (**18**), gemifloxacin (**19**), moxifloxacin (**20**), trovafloxacin (**21**) and garenoxacin (**22**)] have only a hydrogen at this position. Many of these compounds also have a cyclopropyl at position 1, but their activity against Gram-positives varies markedly, with some, particularly

clinafloxacin (**16**), sitafloxacin (**18**), gemifloxacin (**19**), moxifloxacin (**20**) and garenoxacin (**22**) having far greater activity than others, indicating that the Gram-positive activity is also influenced by substitutions at other positions.

Position 6

The addition of a fluorine at C-6 position markedly improved antimicrobial activity [28, 29]. Flumequine (**4**) was the first compound to be developed with a fluoro- group at position 6, and gave the first indications that modifications of the basic chemical structure could improve Gram-positive activity [6]. The influence of fluorine at this position is essential for high activity as evidenced by its enhanced gyrase inhibition and cell penetration which has become the basis for generic name fluoroquinolones [28, 29]. However, there has been a recent interest in quinolones without a fluorine at this position. More recently, a series of 6-H quinolones (non-fluorinated quinolones) was disclosed [35, 36]. The objective of the non-fluorinated quinolones optimization effort was to design compounds with potent activity against Gram-positive pathogens and adequate activity against quinolone-resistant strains, while maintaining broad-spectrum antibacterial activity. For the fluoroquinolones, it was probably difficult to achieve high potency against Gram-positive pathogens because of the apparent similarity between the type of substitutions at the 6 and 8 positions required for improved activity against Gram-positive pathogens and those leading to increased levels of genotoxicity and cytotoxity [4, 37, 38]. During the initial investigation of the non-fluorinated quinolones, it was noticed that removal of the fluorine at the position C-6 systematically lowered the level of genotoxicity, based on a comparison of the non-fluorinated quinolones and the corresponding 6-fluoroquinolones [38]. It then became clear that the non-fluorinated quinolones series was a useful platform for reoptimization of the quinolone backbone toward improved potency against Gram-positive pathogens. The non-fluorinated quinolones, for example garenoxacin (**22**), show greater potency than the newer fluoroquinolone moxifloxacin (**20**) against both sensitive and resistant Gram-positive organisms thus casting doubt on the validity of the necessity of the C-6 fluorine [26, 27].

Another group of agents with novel substituents at C-6 position are the 6-nitro and 6-amino quinolones, which have expanded activity against Gram-positive cocci [39, 40]. For the 6-amino compounds as well, the overall potency is highly dependent on substituents at positions C-7 and C-8. Similar to the fluoroquinolones, a methyl (and probably a methoxy), at position 8 enhances Gram-positive activity, at least *in vitro* [40].

Position 7

The substituents at position C-7 are associated with a number of key attributes, such as antibacterial spectrum, bioavailability and adverse effects. The most common substituents are cyclic amino- groups, for example piperazine or pyrrolidine rings [13-17, 28-30]. Unsubstituted piperazine rings [as in norfloxacin (**5**), enoxacin (**7**) or ciprofloxacin (**9**)] confer potency against Gram-negative bacteria, while the addition of methyl groups can improve both oral absorption and anti-Gram-positive activity. However, the improved activity against Gram-positive bacteria can sometimes be at the expense of activity against *Pseudomonas aeruginosa* [4-6]. Pefloxacin (**6**), fleroxacin (**8**), ofloxacin (**11**) and levofloxacin (**12**) have a 4-methyl piperazine, lomefloxacin (**10**), grepafloxacin (**14**) and gatifloxacin (**15**) have a 3-methyl piperazine, and sparfloxacin (**13**) has a 3,5-dimethyl piperazine. These substituted piperazine-containing compounds have greater activity against Gram-positives, and are believed to have enhanced penetration into the bacterial cell [14, 41].

Amino pyrrolidine rings are also common substitution at position 7, and are associated with enhanced activity against Gram-positives. Introduction of methyl groups on the pyrrolidine ring also enhances activity against Gram-positive bacteria and helps to overcome some physical and pharmacokinetics disadvantages [4, 14, 17]. Clinafloxacin (**16**) and tosufloxacin (**17**) both have a 3-amino pyrrolidine substituent and sitafloxacin (**18**) a spiro-aminopyrrolidine group [24, 31]. Gemifloxacin (**19**) carries a novel 3-aminomethyl-4-methoxyimino-1-pyrrolidinyl substituent at the C-7 position of the 6-fluoro-1,8-naphthyridone core, thought to be associated with reduced cognitive effects and perhaps accounting for the dramatic improvement in antipneumococcal activity [25, 42]. DW286 (**23**), a methyl analog of gemifloxacin (**19**) which possessing 3-aminomethyl-3-methyl-4-methoxyimino-1-pyrrolidinyl substituent at the C-7 position (Fig. **5**) has potent antibacterial activity against Gram-positive bacteria, its activity is stronger than that of gemifloxacin (**19**) [43-45].

It has been shown that the size and lipophilicity of the oximinoalkyl group on the pyrrolidine at position 7 has a marked effect on the antibacterial activity and pharmacokinetics of fluoroquinolones. As the length of the alkyl chain is increased, so does the Gram-positive activity increase, but the activity against Gram-negatives decreases. Bulky substitutions, such as benzyl, whilst retaining activity, had poor pharmacokinetic and physicochemical properties [6].

The addition of bicyclic amino- groups onto position 7 has resulted in agents [moxifloxacin (**20**), trovafloxacin (**21**) and CFC-222 (**24**)] with significant anti-Gram-positive activity [9, 23, 46]. Indeed, in this unusual type of substituent at C-7, a second ring being fused to a pyrrolidine ring. Moxifloxacin (**20**) has a diazabicyclic ring, while trovafloxacin (**21**) and CFC-222 (**24**) have an amino-azabicyclic ring [9, 23, 46]. Generally, among new fluoroquinolones, clinafloxacin (**16**), tosufloxacin (**17**), sitafloxacin (**18**), gemifloxacin (**19**), moxifloxacin (**20**), and trovafloxacin (**21**), have a pyrrolidine skeleton attached to the C-7 position and display excellent activity against Gram-positives. In the field of non-fluorinated quinolones, PGE 9262932 (**25**) and PGE 4175997 (**26**) containing certain 3-(aminoalkyl)-7-pyrrolidinyl moiety (Fig. **5**), demonstrated broad spectrums of antibacterial activity that are similar to that of trovafloxacin (**21**). They are more active than trovafloxacin (**21**) against Gram-positive pathogens, somewhat less active against the Enterobacteriaceae [47]. In addition, unlike these non-fluorinated quinolones and fluoroquinolones, garenoxacin (**22**) has a certain aryl moiety

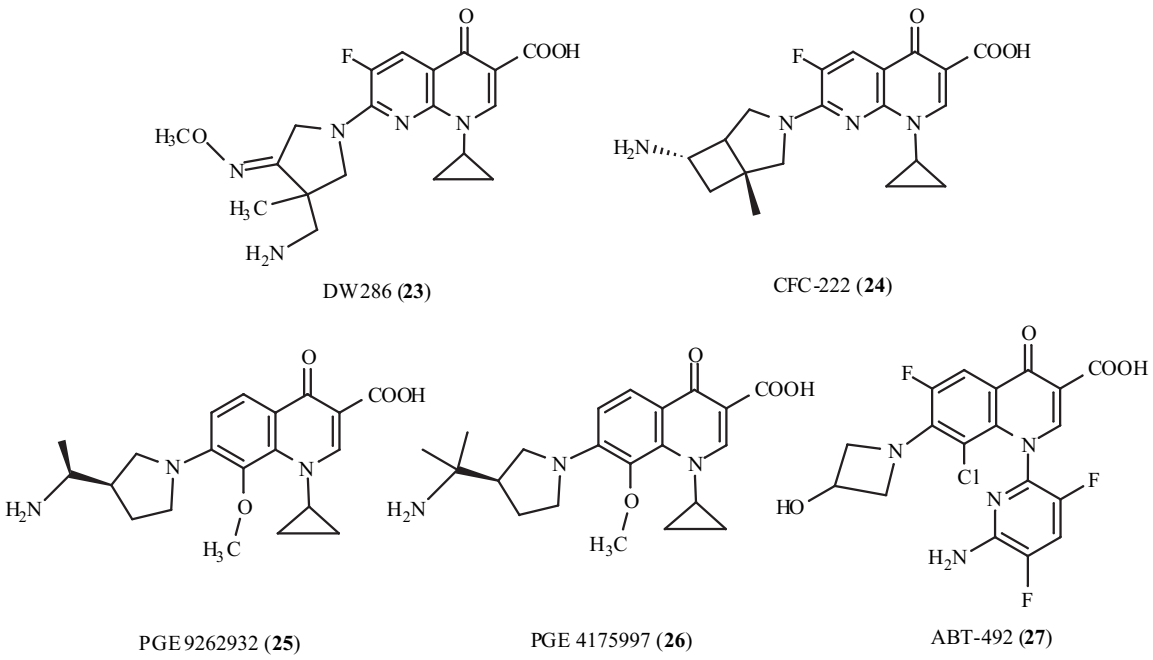

Fig. (5).

(isoindolin-5-yl) instead of cyclic amine at C-7. Garenoxacin (**22**) has outstanding Gram-positive coverage, especially against *S. pneumoniae*, as well as a balanced spectrum of Gram-negative activity [26, 27].

ABT-492 (**27**) currently being developed by Abbott Laboratories and has a small cyclic amine ring at C-7 position. Indeed, this new quinolone differs from other members of the class by two structural features: the 6-amino-3,5-difluoropyridine at the N-1 position and the 3-hydroxyazetidine-1-yl substituent at the C-7 position (Fig. **5**). Preliminary data indicate that ABT-492 (**27**) has potent activity against Gram-positive organisms with enhanced anti-staphylococcal activity compared with earlier fluoroquinolones [ciprofloxacin (**9**), levofloxacin (**12**) and trovafloxacin (**21**)], in addition to activity against β-haemolytic streptococci, pneumococci including penicillin- and fluoroquinolone-resistant strains and vancomycin-susceptible and -resistant *Enterococcus faecalis* [48, 49].

The 7-cyclic amine moiety of quinolones possesses enough structural flexibility to allow product optimization. In addition, a position on the quinolone molecule, where substitutions of bulky groups are permitted, is C-7 position [50, 51]. Furthermore, it has been proposed that for Gram-positive organisms, increasing molecular mass and bulkiness of substituent at C-7 position is not a barrier to penetration [52]. Accordingly, we described a number of *N*-substituted piperazinyl quinolones (**28a, b**) containing certain bulky substituent in the piperazine unit of 7-piperazinyl quinolones (Fig. **6**), to identify a particular chemical modification that allows manipulation of potency particularly in Gram-positive bacteria. Some of these derivatives exhibit high activity against staphylococci more potent than their parent *N*-piperazinyl quinolones [51, 53-55]. Although the nature of the C-7 substituent is known to influence quinolone activity, but improvement of anti-Gram-positive activity in this type of *N*-substituted piperazinyl quinolones may be due to more favorable accumulation in Gram-positive bacteria.

There is an approach consisting of combining two pharmacophores in one molecule. These two pharmacophores, by addressing the active site of two different targets, offer the possibility to overcome the current resistance and in addition to reduce the appearance of new resistant strains. Because of high tolerance for structural variation at the 7-positions of the quinolone ring, this

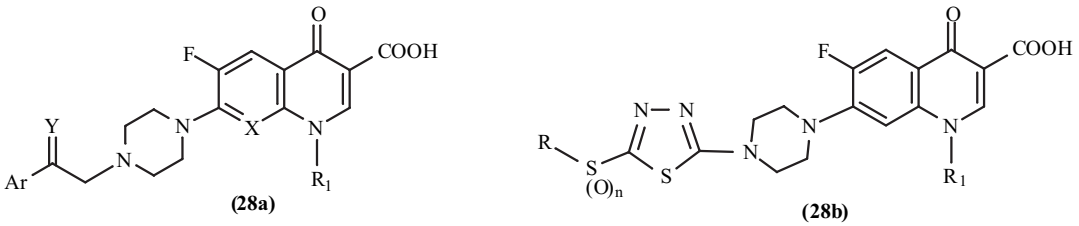

Fig. (6).

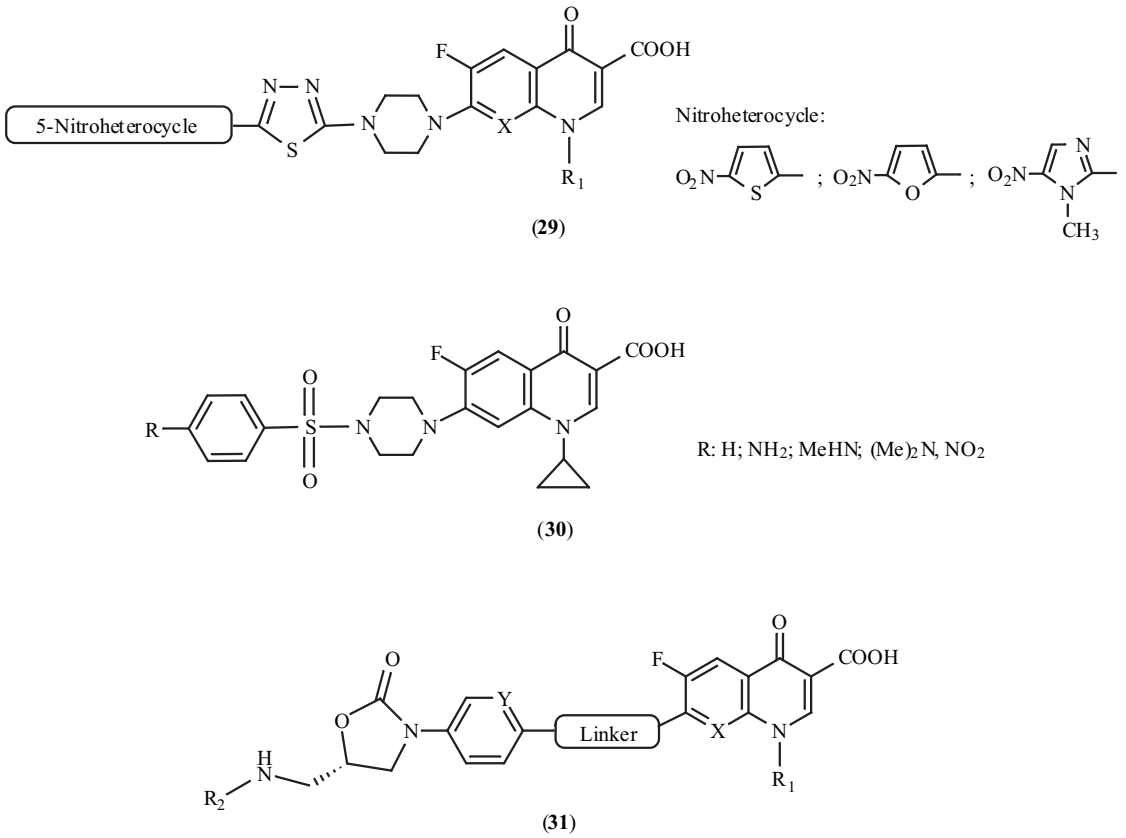

Fig. (7).

strategy was already applied to quinolone-containing hybrids via C-7 connection. Based on these considerations several types of hybrids, including quinolone-nitroheterocycle (**29**) [56-58], quinolone-sulfonamide (**30**) [59-61] and quinolone-oxazolidinone (**31**) [62-64] hybrides have been synthesized and evaluated (Fig. **7**). Representative analogues of these hybrids displayed an improved potency against Gram-positive pathogens.

Previously, Kerns *et al*. [65, 66] described symmetric and asymmetric C-7-piperazinyl-linked dimers of quinolones possess potent antibacterial activity against drug-resistant strains of *S. aureus* (Fig. **8**).

Position 8

A number of quinolones have no substituent at the position 8, but have a good activity against Gram-positive organisms; these include ciprofloxacin (**9**) and grepafloxacin (**14**). The naphthyridones have a nitrogen in the place of the carbon in the ring [tosufloxacin (**17**), gemifloxacin (**19**), and trovafloxacin (**21**)].

Manipulation of the group at position 8 has also been shown to play a role in altering oral pharmacokinetics, broadening the spectrum of activity and reducing the selection of mutants [40, 67-69]. Halogen substituents, as well as a methyl or methoxy also increase the *in vitro* activity against Gram-positive cocci, even in those bacteria resistant to older fluoroquinolones. In addition, these substitutions at this position confer good anaerobic activity. Early work showed that the 8-fluoro group [as in lomefloxacin (**10**) and sparfloxacin (**13**)] and 8-chloro group [as in clinafloxacin (**16**) and sitafloxacin (**18**)] were the most favorable substituents in terms of antibacterial activity; however, quinolones containing C-8 halogens tend to exhibit phototoxicity and other unacceptable side-effects. In contrast, newer quinolones such as gatifloxacin (**15**) and moxfloxacin (**20**), with a methoxy group at the C-8 position exhibit both enhanced activity against Gram-positives and

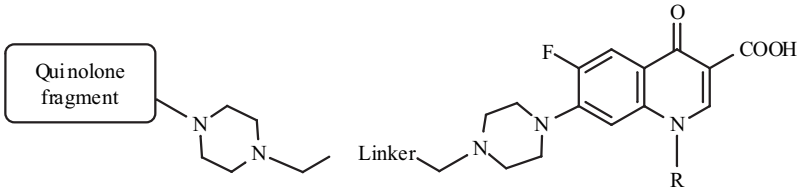

Fig. (8).

anaerobes but does not seen to carry any risk of phototoxicity and cytotoxicity [9, 10, 15-19, 67-69].

The increasing potency of the newer quinolones against Gram-positive organisms offers hope that we can remain on step ahead of resistance development. At the present, SAR studies demonstrated that numerous structural modifications of quinolones particularly at the C-6, C-7 and C-8 positions increase the number of intracellular targets on type II topoisomerases and impair the action of exporter proteins (efflux pumps) and thus lower the propensity for development of quinolone resistance in Gram-positive pathogens.

Briefly, the implications of target preference and resistance mechanisms in Gram-positives will be discussed below, as well as structural features of quinolones related to them and possible future strategies that may be used to limit resistance emergence.

Target Preference in Gram-Positives and Relationship to Quinolone Structure

Quinolones have two targets of action, DNA gyrase and topoisomerase IV, in all Gram-positive bacteria that have been studied. Both enzymes are necessary for DNA replication; DNA gyrase acting by altering DNA supercoiling; and topoisomerase IV acting to separate interlocked DNA strands to allow segregation of daughter chromosomes into daughter cells [70, 71]. DNA gyrase is a tetrameric enzyme composed of two A subunits and two B subunits, encoded by *gyrA* and *gyrB*, respectively. Topoisomerase IV is an A_2B_2 enzyme as well, encoded by *parC* and *parE* (referred to as *grlA* and *grlB* in *Staphylococcus aureus*). These subunits (*ParC* and *ParE*) are highly homologous to *GyrA* and *GyrB*, respectively. Quinolones form a ternary drug-topoisomerase- DNA complex and interfere with DNA breakage-reunion mediated by the two *GyrA* (*ParC*) subunits [70, 71].

A key to understanding quinolone action in Gram-positive bacteria is the observation that the drugs can act preferentially through topoisomerase IV, through gyrase, or through both targets in a manner dependent on quinolone structure [72]. The difference in quinolone target arises because gyrase from Gram-positive bacteria is less susceptible to inhibition by quinolones than gyrase from Gram-negative bacteria [71]. For most of the quinolones tested against Gram-positive bacteria, including ciprofloxacin (**9**), levofloxacin (**12**), and trovafloxacin (**21**), topoisomerase IV appears to be the primary target [73]. However, some studies indicate that the DNA gyrase may act as the primary target in Gram-positive microorganisms for some quinolones, such as sparfloxacin (**13**) and nadifloxacin [72, 74, 75]. Data from trials in resistant mutant species suggest that newer quinolones likely have a dual-binding mechanism of action, inhibiting both DNA gyrase and topoisomerase IV, in Gram-positive species [76]. Although some have debated the dual activity of newer quinolones based on enzymic and genetic studies [77], the general consensus is that some newer agents, such as gatifloxacin (**15**), clinafloxacin (**16**), gemfloxacini (**19**), moxifloxacin (**20**) and non-fluorinated quinolones do possess dual-activity [37, 78-80]. Furthermore, this target preference appears to depend upon the species of Gram-positive organism. For example, gatifloxacin (**15**) that target gyrase in *Streptococcus pneumoniae* [81] appear to target topoisomerase IV in *S. aureus* [82]. Thus, relative target preference cannot necessarily be generalized even within Gram-positive species.

As mentioned above, the newer generation of fluoroquinolones and non-fluorinated quinolones exhibits enhanced activity against Gram-positive organisms compared to the older members of this drug class. The improved activity of the newer quinolones is probably a combination of an enhanced activity against the target's DNA gyrase and topoisomerase IV and this lower rate of selection of resistant mutants [37, 78-80]. Such a compound would be highly desirable because if it can attack both topoisomerase IV and gyrase simultaneously, two mutations, one in each target, would be required for a cell to become resistant. Thus, the design of derivatives that target both enzymes (dual-action) my be a preferred drug design, as opposed to having a drug with high potency against one enzyme and weak potency against the other [28, 79, 83].

Although the structural features responsible for the interaction of quinolones with the binding sites on DNA gyrase or topoisomerase IV are not yet understood fully, position 7 of quinolone structure is considered to be one that directly interacts with DNA gyrase, or topoisomerase IV, and determines target preference of quinolones [59, 84]. Shen *et al*. have demonstrated the importance of the C-7 substituent in drug-enzyme interactions [50]. According to this model, four fluoroquinolone molecules are envisaged to bind to the single stranded DNA regions opened up in the gyrase-DNA complex by covalent attachment of the two *GyrA* subunits to each complementary DNA strand. Two fluoroquinolones are envisaged to lie above the other pair of drug molecules, making hydrophobic interactions with each other through substituents on N-1, C-2, and C-8. Binding to DNA strands is suggested to involve a hydrogen-bonding domain on the drug comprising the C-3 carboxyl group, the ketone at C-4, and C-6 fluorine substituent. Furthermore, Shen *et al*. postulated that bulky C-7 substituents were tolerated and the substituent on C-7 is involved in drug-enzyme interactions. Although the rules governing selectivity are poorly understood, it has been shown that the intracellular targeting of ciprofloxacin (**9**) in *S. pneumoniae* can be switched from topoisomerase IV to gyrase by the addition of a benzenesulfonylamido moiety to the C-7 piperazinyl ring of quinolone (compound **30**) [59]. Observations with the dimers (Fig. **8**) again highlight the key role of the C-7 group in determining target preference [65, 66, 84]. These dimmers (**32**) involve linkage of monomers through their C-7 piperazinyl groups and act through gyrase as the primary target in *S. pneumoniae*, whereas monomeric ciprofloxacin targets topoisomerase IV [65, 66, 84]. Gould *et al*. suggested that these dimers bind to the enzyme-DNA complex in a monomeric fashion. In the context of monomeric binding, dimers could be viewed as being ciprofloxacin but carrying a very bulky C-7 side chain, which may favor the targeting of gyrase [84].

More recently, garenoxacin (**22**) was disclosed as a new 6-H quinolone (non-fluorinated quinolone), which bears a unique aryl type substituent (methylisoindolin-5-yl) at the 7-

position. While this type of substitution has been used in quinolones before, it was believed to be a type of group that lowered the selectivity for the bacterial enzyme. However the unique type of substitution of this series allowed the identification of selective inhibitors. Studies with purified topoisomerase IV and gyrase from *S. aureus* showed that garenoxacin (**22**) had similar activity against topoisomerase IV and gyrase, and although its activity against topoisomerase IV was 2-fold greater than that of ciprofloxacin (**9**), its activity against gyrase was 10-fold greater [26].

Another useful development in the dual-targeting mechanism of action has been achieved with changes at C-8 position. Interestingly, specific changes at C-8 appear to dramatically alter the initial target of fluoroquinolones in Gram-positive cocci. A hydrogen substituent [as in ciprofloxacin (**9**)], or a fused ring [e.g., ofloxacin (**11**) and levofloxacin (**12**)] typically leads to high activity against topoisomerase IV, with little benefic activity against DNA gyrase [83]. In contrast, a halogen substituent shifts the initial target to that of DNA gyrase and markedly reduces anti-topoisomerase IV action. This was shown for the 8-fluoro- quinolone, sparfloxacin (**13**) [72]. Furthermore, Clinafloxacin (**16**, an 8-chloro-quinolone) has a greater ability to exploit both DNA gyrase and topoisomerase IV, respect to ciprofloxacin (**9**) [37, 79]. More beneficial development in the dual-targeting mechanism of action has been the introduction of a methoxy group at C-8 position [such as in gatifloxacin (**15**) and moxifloxacin (**20**), where this group actually replaced chlorine that had similar properties, but caused phototoxicity] [68, 79]. A C-8-methoxy moiety improves fluoroquinolone action against gyrase at both the bacteriostatic and bactericidal levels and also improved the attack of topoisomerase IV, which should make 8-methoxyquinolones better able to attack species in which topoisomerase IV is the primary quinolone target [67, 68, 82].

Resistance Among Gram-positives and Impact of Quinolone Structure

Resistance to quinolones among Gram-positive cocci has emerged as these antimicrobial agents have become extensively used in clinical medicine. Two main mechanisms of quinolone resistance have been established: alterations in the targets of quinolones, and decreased accumulation inside the bacteria due to impermeability of the membrane and/or an over expression of efflux pump systems. Both of these mechanisms are chromosomally mediated [85, 86]. Although plasmid-mediated resistance to quinolones has been seen in some Gram-negative bacteria, it has not yet been seen in any Gram-positive bacteria [85].

Alterations in the drug target enzymes may be sub-classified as mutations in DNA gyrase and mutations in topoisomerase IV. DNA gyrase mutations, both in the *GyrA* and *GyrB* subunits, also occur in Gram-positive bacteria; however, as DNA gyrase typically serves as a secondary target to topoisomerase IV in these bacterial species, the relative importance of these mutations is questionable. Furthermore, in quinolone-resistant Gram-positive bacteria, alterations in either subunit of DNA gyrase have typically been found only in the presence of concomitant topoisomerase IV mutations [85, 86]. Topoisomerase IV mutations have been most extensively studied in quinolone-resistant Gram-positive bacteria, particularly in *S. aureus* and *S. pneumoniae* species. As with DNA gyrase, alterations may occur in either subunit, mutations in *ParC* (*GrlA* in *S. aureus*) occur more commonly than *ParE* (*GrlB* in *S. aureus*) mutations, and *ParC* mutations are believed to play a more critical role in resistance development [85]. A mutation in the more sensitive enzyme results in an increase in the MIC of a quinolone, whereas a mutation in the less sensitive enzyme generally causes resistance only in the presence of resistance mutations in the primary target. A quinolone with similar affinities for both targets is little affected by a mutation in one of the enzymes, and concurrent mutations in both enzymes are necessary for resistance to develop [26, 28, 79, 83].

Several studies direct attention to identify the structural characteristics of quinolones and provide a rationale for dissociated propensity for development of resistance amongst Gram-positive bacteria.

Focusing on the C-6 position, the primary objective of the non-fluorinated quinolones design effort was achieved with a series of quinolones that are relatively unaffected by the serine mutation in DNA gyrase. However, the data reviewed by Roychoudhury *et al*. showed that those non-fluorinated quinolones also have highly desirable properties against the second target of quinolones, topoisomerase IV, especially in *S. aureus*. They found a series of non-fluorinated quinolones with very potent antibacterial activity against several important Gram-positive pathogens, combined with potency against clinically prevalent quinolone-resistant isolates [37, 38]. Hartman-Neumann *et al*. found that the non-fluorinated quinolone, garenoxacin (**22**) to select mutants with the known changes in *GyrA* and *ParC* [87].

Regarding the C-7 substituent, Alovero *et al*. examined the antipneumococcal mechanisms of a series of novel fluoroquinolones (**30**) that are identical to ciprofloxacin (**9**) except for the addition of a benzenesulfonyl group to the C-7 piperazinyl ring. A substance bearing a 4-(4-aminophenylsulfonyl)- 1-piperazinyl group at C-7 showed enhanced activity against a pneumococcal strain and, in contrast to ciprofloxacin (**9**), a mutation in *par*C could not confer resistance against the new substance, while a mutation in *gyr*A made the mutant strain four- to eight-fold more resistant against it. Moreover, the new substance selected a *gyr*A mutant and showed enhanced activity in inhibiting supercoiling [59].

Fukuda *et al*. demonstrated the importance of an 8-methoxy substituent by comparing gatifloxacin (**15**) and AM-1147 (an 8-methoxy quinolone) and their respective 8-H counterparts. They showed the 8-methoxy derivatives to select mutants at a lower frequency than their 8-H counterparts and to prefer DNA gyrase [88]. Zhao *et al*. showed that 8-methoxy ciprofloxacin derivatives are more lethal than 8-bromine, 8-ethoxy, and 8-H derivatives for *Staphylococcus aureus*, especially when topoisomerase IV is resistant. The methoxy group also increases lethality against wild-type cells when protein synthesis was inhibited [69]. The unique effect of a methoxy substituent at C-8 has been demonstrated for moxifloxacin (**20**). With this substituent,

even a double *ParC* and *GyrA* mutation did not cause the MIC against *S. pneumoniae* to go above clinically attainable moxifloxacin (**20**) concentrations [89].

Moreovere, garenoxacin (**22**) that lacks the classical C-6 fluorine and has a difluoromethoxy substituent at position 8, instead of a methoxy group, has been shown to improve bacteriostatic and bactericidal activity and decrease the selection of resistant mutants [26].

The second resistance mechanism in Gram-positive bacteria is mediated through active drug efflux by the over expression of certain efflux pumps. The efflux pump system is a mechanism that allows immediate survival of bacteria in the presence of an antimicrobial agent by actively expelling that agent across the cell membrane, thereby reducing the intracellular concentrations to sub lethal levels [85, 90].

Low-level resistance to quinolones can occur by increased expression of these efflux pumps, usually due to mutations that increase transcription of the structural gene for the pump either from effects on the gene promoter or from alterations in other regulatory proteins that affect pump expression. In the case of *S. aureus*, low-level resistance to ciprofloxacin (**9**) can result from increased expression of a pump called *NorA* [85]. Mutations causing this increase and low-level resistance have been seen in the *norA* gene promoter causing increased stability of *norA* mRNA and in other regulatory proteins [85]. The presence of *NorA*-like efflux systems has also been described or suggested in other Gram-positive microorganisms such as *S. pneumoniae* and *Bacillus subtilis* (called *PmrA* in *S. pneumoniae* and *Bmr* or *Blt* in *B. subtilis* which their overexpression provides a similar resistance spectrum to that of *NorA*) [85, 86, 90].

The pump's action is dependent on the quinolone's ability to bind to the bacterial efflux protein and be exported [91]. Norfloxacin (**5**), ciprofloxacin (**9**) and ofloxacin (**11**) seem to be affected more than sparfloxacin (**13**), gatifloxacin (**15**), sitafloxacin (**18**), gemifloxacin (**19**), moxifloxacin (**20**), trovafloxacin (**21**) and garenoxacin (**22**), suggesting that these last quinolones may be poorer substrates for these pumps [22, 85, 90, 92- 94]. Thus, efflux-mediated resistance mechanisms seem to affect quinolone agents to different extents, depending on the physicochemical properties and structural characteristics of the individual quinolones. Previously, only hydrophilicity of the fluoroquinolones was thought to be an important factor for *NorA*-mediated transport. It was found that the *NorA* inhibitors reserpine and omeprazole dramatically improve the activities of the more hydrophilic quinolones [norfloxacin (**5**) and ciprofloxacin (**9**)] [93]. In addition, Hydrophilic quinolones, such as norfloxacin (**5**) and ciprofloxacin (**9**), appear more prone to efflux than more hydrophobic molecules like grepafloxacin (**14**) and gatifloxacin (**15**) in Gram-positive organisms such as *S. pneumoniae* [95, 96]. However, Takenouchi *et al*. demonstrated that the bulkiness of the C-7 substituent and the hydrophobicity and bulkiness of the C-8 substituent, not the molecular hydrophobicity, was correlated with the activity of quinolones [97]. Of the quinolones tested by Beyer *et al*. and Madras-Kelly *et al*., levofloxacin (**12**) has the least bulky C-7 substituent, sparfloxacin (**13**) a slightly larger derivative and moxifloxacin (**20**), the bulkiest C-7 substituent. On the other hand, sparfloxacin (**13**) is a rather hydrophobic drug, while levofloxacin (**12**) and moxifloxacin (**20**) are much more hydrophilic. Sparfloxacin (**13**) and moxifloxacin (**20**) were relatively unaffected by *PmrA*- or *NorA*-mediated efflux, whereas levofloxacin (**12**) was highly affected [92, 98].

In order to investigating novel approaches to find quinolones that are refractory to efflux-mediated resistance in Gram-positive bacteria, Kerns *et al*. discovered a novel piperazinyl-linked quinolone dimers (**32**), which exhibited potent antibacterial activity against drug resistant strains of *S. aureus*, including strains possessing resistance due to the *NorA* multidrug efflux pump and a mutation in the quinolone resistance-determining region of topoisomerase IV. They suggested that a bulky C-7 side chain might account for the lack of effective dimer efflux through the *norA* pump [65, 66].

These data demonstrate that the bulk at C-7 appears to be the key structural characteristic for avoidance of efflux. Gemifloxacin (**19**), moxifloxacin (**20**), trovafloxacin (**21**), and garenoxacin (**22**) are the currently available agents with the greatest bulk at C-7 position, and they appear least affected by efflux-mediated resistance in Gram-positive organisms [22, 90].

CONCLUSIONS

The current knowledge of structure-activity relationships has been gained through the past development of a large number of compounds within the quinolone class. A major recent focus of this class has been the development of quinolones with enhanced activity against Gram-positive bacteria. With the recent introduction of agents such as gatifloxacin (**15**), gemifloxacin (**19**), moxifloxacin (**20**), trovafloxacin (**21**), and garenoxacin (**22**), the traditional Gram-negative coverage of quinolones has been expanded to include specific Gram-positive organisms. At the present, the structural modification of quinolones at the C-5, C-6, C-7 and C-8 positions to alter target specificity and avoidance of efflux has yielded results. Thus, the improved activity of the newer quinolones is probably a combination of an enhanced activity against the target's DNA gyrase and topoisomerase IV (dual mechanism of action) in Gram-positive bacteria, in addition to reducing efflux from the bacterial cell. While we now have a considerable variety of clinically useful agents, it seems clear that design of new compounds with potent activity against Gram-positive pathogens and adequate activity against quinolone-resistance strains, while maintaining broad-spectrum antibacterial activity are still possible. The next generation of quinolones will be potent against multi-drug resistant Gram-positive bacteria and provide a lower rate of emergence in resistance.

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