Quinolones: structure–activity relationships and future predictions

G. S. TILLOTSON
Pharmaceutical Division, Bayer plc, Strawberry Hill, Newbury, Berkshire RG13 1JA

Development of the first clinically useful quinolone – nalidixic acid – occurred in 1962, but the significant breakthrough with this class of agents occurred almost 20 years after the original discovery when the addition of a fluorine molecule at position C6 of the pharmacore created the ‘fluoroquinolones’. It has been estimated that over 10 000 analogues of nalidixic acid or the fluoroquinolones have now been synthesised. The benefits of some of these new compounds include: oral and parenteral dosing, a much broader spectrum of antibacterial activity, good tissue distribution, improved pharmacokinetic profiles, stability and a comparatively low incidence of adverse effects. This review considers the structure of the core fluoroquinolone molecule, some of the changes that feature on current class members under development, and the effects that these chemical modifications may have on the interaction of these compounds with man.

Historical development of the quinolones

A group of scientists in the laboratories of the Sterling Company, while pursuing new chemical entities based on the structure of quinine in an effort to expand the armamentarium against malaria, discovered that derivatives of the 1,8-naphthyridine molecule possessed antibacterial activity. By 1962, George Lesher and colleagues had developed nalidixic acid [1]. This was the first clinically useful compound in the series, and by 1964 it was available in the UK for the treatment of urinary tract infections. This narrow clinical indication was a consequence of two factors: the poor serum and tissue concentrations achieved after oral administration, and the limited spectrum of activity, restricted primarily to the Enterobacteriaceae [1, 2].

This first 4-quinolone – also known as naphthyridine carboxylic acid – was succeeded by molecules with minor modifications, including compounds such as oxolinic acid, cinoxacin, pipemidic acid and others. However, none of these compounds constituted a major advance in terms of improved antibacterial spectrum or pharmacokinetics, and hence were still restricted largely to the treatment of urinary tract infections. It was not until almost 20 years after the original development of nalidixic acid that there was a significant breakthrough with this class of agents. Koga et al. [3] observed that improvements in absorption and activity were achieved by making modifications at positions C6 and C7 of the pharmacore (Fig. 1). The addition of a fluorine molecule at C6 created the ‘fluoroquinolones’. This category of agents is one of the most intensively investigated group of compounds in the field of medicine; indeed, since Lesher’s original report, it has been estimated that over 10 000 analogues of nalidixic acid or the fluoroquinolones have been synthesised [4]. The benefits of these newer compounds include: oral and parenteral dosing, a much broader spectrum of antibacterial activity, good tissue distribution, improved pharmacokinetic profiles which favour once or twice a day dosing, good stability and a comparatively low incidence of adverse effects. The major breakthroughs have resulted from a markedly improved understanding of the molecule and how its

Fig. 1. The quinolone pharmacore.
structure interacts with both its target site in bacteria and metabolic systems in man.

Despite the plethora of research and publications regarding this class of antimicrobials, only a few have so far been licensed for clinical use. Table 1 shows the agents available in Europe and the USA. The value of structure–activity relationship (SAR) studies should be considered in the context of the approved agents and how certain modifications on new, yet to be licensed widely, molecules may constitute a step forward. The remainder of this review will discuss the core fluoroquinolone molecule and some of the changes that feature on class members under development.

**Structure–activity relationships**

Fig. 1 shows the core molecule and the positions at which key changes are engineered. Some of these molecular substitutions should not be altered as they would interface with or reduce markedly the basic mode of action of the drug. These are positions 2, 3 and 4; at position 2, a hydrogen moiety is optimal — any larger molecular additions may create a steric hindrance at the adjacent positions 3 and 4 which must be a carboxyl group and oxygen molecule, respectively. Binding to the DNA bases occurs at these positions, which are then made available for new hydrogen-binding partners by the action of the enzyme, DNA gyrase. The moiety at position 6 should be small, and a fluorine atom is optimal as it confers between five- and 100-fold greater potency than any other potential halogen moiety.

The four other positions can receive a wide range of potential substituents. SAR studies have enabled the recognition of features that lead to specific changes, as summarised below:

- **Position 1**—has some effect on the pharmacokinetics of an agent and exerts control on its overall potency;
- **Position 5**—specific moieties substituted at this position have resulted in increased activity against gram-positive bacteria;
- **Position 7**—both spectrum of activity and pharmacokinetics are controlled at this point; five- and six-membered rings containing a ‘N’ atom yield the most activity;
- **Position 8**—pharmacokinetic and specific activity against anaerobic bacteria can be adjusted from this point.

It is intriguing for molecular scientists to explore the effects of a wide range of potential moieties at each of the four more variable molecule locations in an attempt to create ‘wondaflaxacin’; however, it is essential that an overall molecular balance be maintained and that each substituent should contribute to antibacterial activity in some form. A detailed examination of the quinolone pharmacore may help to explain some of the features found on the quinolones available currently, as well as those under development.

**Position 1**

It was realised that a cyclopropyl moiety (e.g., as seen in ciprofloxacin and sparfloxacin) at this point conferred significant activity against gram-negative bacteria. The groups 2,4-difluorophenyl (temafloxacin) and t-butyl (BMY 40062) are slightly less potent; however, the 2,4-difluorophenyl group heightens activity against anaerobes [5].

**Position 5**

Considerable changes have been concentrated at this position in an effort to improve the activity of the fluoroquinolones against gram-positive bacteria. The most advanced compounds that carry significant changes at position 5 are sparfloxacin and PD 124816, both of which carry an -NH₂ moiety, whereas OPC 17116 (grepafloxacin) possesses a -CH₃ molecule [5].

**Position 7**

This is one of the most influential points on the molecule and, almost without exception, the possession of a five- or six-membered nitrogen heterocycle at this position has improved a molecule’s activity and pharmacokinetic profile. The most popular heterocycles employed at position 7 are aminopyrrolidines and piperazines. Anti-bacterial agents that contain an aminopyrrolidine moiety are tosufloxacin, clinafloxacín, Du6859a, Bay 3118 and several compounds from the Parke-Davis company [6]. In contrast, the piperazine substituent is found on ciprofloxacin, lomefloxačín, temafloxacin, sparfloxacin and BMY 40062. The former moiety tends to confer better activity against gram-positive bacteria, whereas piperazine offers improved activity against gram-negative bacteria [5]. Substitution with an alkyl moiety will improve gram-

---

**Table 1. Fluoroquinolones licensed currently**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Country in which licensed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enoxacin</td>
<td>UK, USA</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>UK, USA</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>F</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>UK, USA, &gt;120 countries</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>UK, USA, &gt;100 countries</td>
</tr>
<tr>
<td>Lomefloxacín</td>
<td>USA</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>J, F, UK, Be, Fin, CH</td>
</tr>
<tr>
<td>Tosufloxacin</td>
<td>J</td>
</tr>
<tr>
<td>Levofloxacín</td>
<td>J</td>
</tr>
<tr>
<td>Temafloxacin</td>
<td>[UK, USA, now withdrawn]</td>
</tr>
</tbody>
</table>

Be, Belgium; CH, Switzerland; F, France; Fin, Finland; J, Japan; UK, United Kingdom; USA, United States of America.
positive potency and lengthen the serum half-life; this
type of change has been employed in lomefloxacin,
sparfloxacin and several other quinolones now under
development.

Position 8

Various substituents at this position have led to marked
improvements in activity, particularly against anaerobic
bacteria; the most useful groups employed on this
position are CF, CCl and COMe.

Examples of specific substituents to the quinolone
pharmacore are shown in Fig. 2. In addition to the role
that certain moieties may play in enhancing activity
against specific groups of bacteria and altering the
pharmacokinetic properties of a drug, these chemical
modifications also play a significant role in the
specific interaction of these compounds with man.

Adverse effects

Fluoroquinolones are known to produce various side-
effects in man and certain test animal species. Perhaps
the most frequent side-effects are those affecting the
gastrointestinal system, such as nausea, vomiting,
gastric irritation and, occasionally, diarrhoea. Despite
the transient unpleasant consequences of these effects,
no specific structure has yet been identified as their
main cause and thus no quinolone has yet been
designed which is free of gastrointestinal effects.

Adverse dermatological symptoms, such as skin rash
or pruritis, occur in 0.5–3% of patients [7], and may be
consequences of histamine release, as has been
suggested for BMY 40062 [8]. An animal model
developed to ascertain the effect of alkylation of the
piperazinyl at position 8 showed this alkylation to be
beneficial, [5] and it appears that such an animal model
may be of value in examining future quinolones.

Table 2. Relative degree of GABA inhibition and incidence of spontaneously reported CNS events in the UK*

<table>
<thead>
<tr>
<th>Agent</th>
<th>Position 7</th>
<th>Degree of GABA inhibition</th>
<th>Incidence of CNS side-effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>High</td>
<td>62/10^6 scrips</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td></td>
<td>High</td>
<td>49/10^6 scrips</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>CH,N N</td>
<td>Low</td>
<td>89/10^6 scrips</td>
</tr>
</tbody>
</table>

*See references 5 and 7.
Effects within the central nervous system (CNS) are generally some of the more frequent adverse consequences of quinolone therapy [7]. It is convenient to categorise the quinolones into those that act directly on the CNS receptors, and those that exert an effect when given with other agents. The direct effects include headache, sleep disorders, dizziness, agitation and, very occasionally, convulsions. These effects correlate with the quinolone binding to the receptors for \( \gamma \)-aminobutyric acid (GABA) in the brain, thereby preventing normal binding of GABA and heightening CNS stimulation. Several models have been developed to measure the interactions of quinolones with GABA receptors. From these models it transpires that the substituent at position 7 has most effect on the direct CNS interaction. Curiously, the predicted level of GABA interaction does not always correlate with the frequency at which adverse events are reported [9]. Table 2 shows the relative degree of GABA inhibition and the frequency of spontaneous adverse CNS events reported in the UK.

One of the earliest adverse events recognised with quinolones was arthropathic damage in weight-bearing joints of animals, particularly canine species [10]. These changes were seen most often after prolonged exposure to high doses. Certain quinolones seem to be more likely to cause these changes, but insufficient evidence from substituent modifications has been gathered. Thus it is assumed that these arthropathies are class effects but, fortunately, there have been very few instances of irreversible joint damage in children treated with quinolones [11].

The adverse event that has caused most recent attention is phototoxicity [12]. All fluoroquinolones can cause a non-immunogenic phototoxic reaction, but there are clearly some compounds that cause the problem more frequently and more severely. In an effort to predict how new agents will react, an animal model, the mouse phototolerance system, has been developed [13]. This model can incorporate all the influencing factors, such as photoactivity, half-life, skin toxicity and skin penetration. It has been shown that the possession of a halogen, such as fluorine or chlorine, at position 8 leads to the highest incidence of phototoxicity. Table 3 shows some of the current and imminent quinolones in terms of the least concentration required to cause a phototoxic response in the mouse model.

<table>
<thead>
<tr>
<th>Quinolone</th>
<th>( X_\alpha ) substituent</th>
<th>Dosage associated with phototoxicity (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>CH</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>COR</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>COR</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Lomefloxacin*</td>
<td>CF</td>
<td>10</td>
</tr>
<tr>
<td>Clinafloxacin(^1)</td>
<td>CCl</td>
<td>18</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>CF</td>
<td>18</td>
</tr>
<tr>
<td>Des-amino</td>
<td>CF</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Sparfloxacin Bay 3118(^1)</td>
<td>CF</td>
<td>3</td>
</tr>
</tbody>
</table>

*Subject of FDA doctor ‘warning letter’.
\(^1\)Development discontinued.

Issued warnings to doctors regarding the phototoxic potential of sparfloxacin [15, 16]. In these two countries, 52 and 208 cases of phototoxicity, respectively, were reported spontaneously to the health authorities within the first 8 months of clinical use. These reports corroborate the mouse phototolerance model findings.

In addition to specific activity, pharmacokinetic and side-effect features, certain interactions have been shown to be associated closely with some moieties. Interaction with theophylline or non-steroidal anti-inflammatory drugs (NSAIDs) are among the best-recognised clinically significant situations. For the NSAID interaction, position 7 seems to have the greatest significance, with unsubstituted piperazines (ciprofloxacin, enoxacin, norfloxacin) showing the greatest effect. Alkylated piperazines and pyrrolidines have less effect. Despite these strong in-vitro and model interactions, there are few NSAID-induced adverse CNS events [17].

So far as the theophylline interaction is concerned, this compound is metabolised by the cytochrome P450 enzymes. These enzymes are inhibited by some quinolones, thus leading to an accumulation of theophylline and possible toxic reactions such as convulsions. Again, the position 7 substituent has a major bearing on this interaction, although not exclusively, as the moieties at positions 1 and 8 also have an effect. It is also important to note that quinolones which are metabolised hepatically may lead to metabolites that can also interact with theophylline, e.g., the M-1 metabolite of ciprofloxacin [5].

In summary, although there is now a profound understanding of the basic quinolone molecule and precisely what effects will be exerted when specific substituents are added at certain positions, the ‘ultimate’ molecule with a very broad spectrum of activity, an excellent pharmacokinetic profile (i.e., long half-life and vast volume of distribution) and an acceptable safety profile still seems to be elusive amongst the fluoroquinolone molecules of today.
Possible future developments

Of the fluoroquinolones currently licensed, ciprofloxacin has the broadest spectrum of activity, while ofloxacin is well-distributed into tissues. Both of these compounds are tolerated well by man. A variety of unforeseen effects, including the temafloxacin syndrome, have resulted in the disappearance of many potential 'block-busters'. Therefore, it may be time to look for alternative molecules that also attack bacteria via DNA gyrase. Such a group of molecules were 'showcased' at the 1994 Interscience Conference on Antimicrobial Agents and Chemotherapy by the Abbott company. This new group – 2-pyridones (Fig. 3) – act on DNA gyrase and, probably, topoisomerase IV (which is a homologue of DNA gyrase). This enzyme appears to play a role in the separation of the daughter DNA molecules after replication. The precise interplay between quinolones and this enzyme is unknown. Of these new 2-pyridones, ABT 719 has considerable antibacterial activity, including gram-positive bacteria and anaerobes. It possesses greater activity than the best current gram-negative quinolone, ciprofloxacin, against both enterobacteria and pseudomonads. Animal models have shown ABT719 (also known as A-86719.1) to be effective in vivo in several systemic infections [19]. It remains to be seen whether the 2-pyridones are set to take over from the quinolones, which in the space of 10 years have become some of the most relied-upon antibiotics throughout the world.

References


