Synthetic antibacterial agents

Quinolones

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Synthetic antibacterial agents

• Several synthetic antibacterial agents were synthesized based on model compounds, these include:
  - Sulfonamides
  - Quinolones
  - Nitroheterocyclic compounds (nitrofurans, metronidazole)

• Some agents can be used for systematic infections

• Others are unsuitable for treating systematic infections due to inadequate concentrations achieved in plasma and tissues following oral and parenteral administrations.

• Some agents are excreted mainly unchanged in the urine, thus can be used to treat urinary tract infections e.g. nitrofurantoin and nalidixic acid.
Quinolones

• The quinolone antibacterial agents were not isolated from growing organisms but were synthesized by chemists.

• The history of the newer quinolone agents began with the discovery of nalidixic acid in 1962 as an accidental byproduct during the synthesis of the antimalarial compound chloroquine, which led to the development of the newer quinolones.

• The quinolones are a group of synthetic antibacterial agents derived from nalidixic acid.

• Nalidixic acid is a 1,8-naphthyridine derivative, synthesized in 1962 and used mainly for UTI.
Quinolones (Cont.)

• Nalidixic acid is the lead compound for this group.

• According to the heterocyclic core can be divided into:

<table>
<thead>
<tr>
<th>Naphthyridones</th>
<th>Quinolines</th>
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<td>nalidixic acid and enoxacin</td>
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![Chemical structures of nalidixic acid and enoxacin](attachment:image1.png)

![Chemical structures of norfloxacin, ciprofloxacin, ofloxacin, lemofolexacin](attachment:image2.png)
The quinolones and fluoroquinolones inhibit the replication and transcription of bacterial DNA by stabilizing DNA-topoisomerase complex.

The synthetic fluoroquinolones mostly end in the suffix -floxacin.

Conversion of N → C:
- ↓ adverse reactions
- ↑ and increased activity against Gram-positive cocci,

**Figure 1.** Common structure of 4-quinolones.

The presence of a **nitrogen** at position 8 identifies the naphthyridones, The presence of a **carbon** at position 8 identifies the quinolones.
The quinolones and napththyridones were further improved by the addition of groups to the N-1, C-5, C-6 and C-7 positions of their respective basic molecules.

None of the introduced groups provides significant improvements over nalidixic acid until the development of Fluoroquinolones (Flumequine).

Flumequine

Figure 2. Clinical development of quinolones
• Derivatives of nalidixic acid were developed which showed improved broad-spectrum activity such as 
  **enoxacin** which is based on:
  - C6-F $\xrightarrow{\uparrow}$ activity & $\uparrow$ cellular uptake by bacteria
  - C7-piperazine $\xrightarrow{\uparrow}$ basicity $\xrightarrow{\text{zwitterion with C3-COOH}}$ (affects pharmacokinetics)

& **ciprofloxacin** which is based on:
  - N1-cyclopropyl $\xrightarrow{\uparrow}$ spectrum of activity
  - N8 is changed to C8 $\xrightarrow{\downarrow}$ adverse reactions & $\uparrow$ activity vs S. aureus

**FIGURE 19.71** Quinolones and fluoroquinolones.
Mechanism of action

- DNA topoisomerase (or gyrase) alters the conformation of DNA by catalyzing transient double-strand cuts, passing the uncut portion of the molecule through the gap, and resealing the molecule back together.
- Topoisomerase IV seems more important to some Gram +ve, and DNA gyrase seems more important to some Gram –ve bacteria.
- Topoisomerase and gyrase are targets for quinolones.
- Human has topoisomerase II, which has low affinity to quinolones at normal doses.
Quinolones (Cont.)

Stacking with DNA bases

Hydrogen bonding to DNA bases

Enzyme interaction domain

Self-association
Fluroquinolone-Gyrase-DNA complex
The essential pharmacophore for activity is the carboxy-4-pyridone nucleus.

- Apparently, the carboxylic acid and the ketone are involved in binding to the DNA/DNA-gyrase enzyme system.
- Reduction of the 2,3-double bond or the 4-keto group inactivates the molecule.
- Substitution at C-2 interferes with enzyme–substrate complexation.

C6-F
- ↑ lipophilicity
- ↑ penetration of cell wall
- ↑ antimicrobial activity (binding to DNA gyrase)

C7- Heterocyclic substitution piperazinyl (ciprofloxacin)
- ↑ binding to GABA of CNS → CNS side effects
- ↑ spectrum vs Gram –ve bacteria.

N-alkylated piperazinyl → ↓CNS side effects
- ↑ half-life & oral absop

Aminopyrrolidine → ↑ spectrum vs Gram +ve bac

Bulky group reduces bacterial efflux

Ring condensation at 1-8, 5-6, 6-7 and 7-8 also lead to better activity.

Addition of third ring (ofloxacin) → ↑ binding to DNA-gyrase complex.

C8-Fluorine
- ↑ absorption, half-life
- ↑ drug-induced photosensitivity.

C8-OCH3 → ↓ phototoxicity

N1-cyclopropyl (or small alkyl or aryl) substitution broaden spectrum of activity
SAR for quinolones

- **Position 1.**
- This position is part of the enzyme-DNA binding complex, and has a hydrophobic interaction with the major grove of DNA. The optimum substituents at position 1 appear to be ethyl, butyl. The most potent is cyclopropyl (ciprofloxacin) followed by addition of a 2,4-difluorophenyl.

- Ofloxacin is tricyclic ring structure. It contains oxazine ring between positions 1 and 8. Oxazine has asymmetric C3 position (S isomer is more active than R isomer, which affects binding to DNA hydrophobic pocket).

Ciprofloxacin

(±)-Ofloxacin

Levofloxacin

**SAR for quinolones**

- *Position 2.*
  - It is close to DNA binding site of gyrase (or topoisomerase IV). Therefore, bulky substitutions inhibit binding and antimicrobial activity.

- *Positions 3 and 4.*
  - These two positions on the quinolone nucleus are considered critical for binding to cleaved or perturbed DNA, and no useful substitutions at these positions have yet been reported.
  - 4-thioxo or sulphonyl group leads to a loss of activity.

SAR for quinolones

• **Position 5.**
• Substituents at this position is beneficial for activity
• Bulk groups affects overall stearic configuration (planar structure) of the molecule which affects the activity
• NH2, OH, CH3 groups ➔ increase activity vs Gram +ve bacteria
• OCH3 ➔ reduces the activity
• NH2 ➔ reduces the phototoxicity of Fluroquinolones
SAR for quinolones

• **Position 6.**

• 6-F produces the fluoroquinolone class with
  1. Enhanced antibacterial activity against Gram +ve and Gram –ve bacteria (including *P. aeruginosa*)
  2. Increased incidence of phototoxicity

• 6-OCH$_3$
  1. Increase activity
  2. Reduce phototoxicity

• 6-NH$_2$
  • with 8-CH$_3$ quinolones ⇒ expand activity against Gram +ve cocci
  • With C7-tetrahydroisoquinoline ⇒ increase the potency up to 100 fold compared to ciprofloxacin

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SAR for quinolones

- **Position 7.**
- This position is directly interacts with DNA gyrase (or topoisomerase IV).
- Optimum substituents are 5- to 6-membered nitrogen heterocycles.
- Piperazine substituents \( \rightarrow \) increase activity against Gram –ve bacteria (especially *P. aeruginosa*) & affinity to gamma-aminobutyric acid (GABA) receptor, which contributes to central nervous system (CNS) side effects. N-alkylated piperazine produces lower CNS side effects.
- Aminopyrrolidine or alkyl moieties substituents \( \rightarrow \) increase activity against Gram +ve bacteria
- Bulkier groups at 7 position reduces the bacterial ability to efflux antibiotic, thus prevent resistance development.

\[ \text{Piperazine} \quad \text{Aminopyrrolidine} \]

**SAR for quinolones**

- **Position 8.**

  - This position affects stearic configuration (similar to position 5), and thus accessibility to enzyme or DNA binding sites
  - Free halogen (F or Cl) \(\rightarrow\) improves activity against Gram \(-\)ve & anaerobes
  - Halogen, CH\(_3\) or OCH\(_3\) \(\rightarrow\) increase activity against Gram +ve cocci
  - Replacement of C8 with N8 \(\rightarrow\) increase antimicrobial potency
  - Large substitution (e.g. ethyl derivatives) \(\rightarrow\) reduces activity against gram +ve and \(-\)ve bacteria.
  - Replacement of C8 to N8 as well as C8-CH\(_3\) substitutions \(\rightarrow\) reduces the development of resistance especially if combined with bulky group at C7
  - A halogen (F or Cl) at the C-8 position improves oral absorption.
  - Removal of F from C8 drastically reduces activity unless it is compromised by C8-OCH\(_3\) or C8-OCHF\(_2\).

Figure 2. Clinical development of quinolones

Figure 3. Structural development of 7-piperazinylquinolones from primary quinolones

Structure-activity relationship
Figure 1. Quinolone structure–side effect relationships. GABA, γ-aminobutyric acid. Modified from [12].

Chemical incompatibility common to all of the quinolones involves the ability of these drugs to chelate polyvalent metal ions (Ca$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Al$^{3+}$), resulting in decreased solubility and reduced drug absorption.

Chelation occurs between the metal and the 3-carboxylic acid and 4-keto groups.

Agents containing polyvalent metals should be administered separately from the quinolones.
Spectrum of activity for quinolons

• Nalidixic acid and the earliest members of the quinolone class (e.g., oxolinic acid, cinoxacin) are largely confined to
  - **Effective against** Gram-ve bacteria, including common urinary pathogens such as *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Proteus* spp. *Shigella*, *Salmonella*, and *Providencia* are also susceptible.
  - **Ineffective against** Strains of *P. aeruginosa*, *Neisseria gonorrhoeae*, and *Haemophilus influenza*, Gram+Ve cocci and anaerobes.

• Newer members of the class possessing 6-fluoro and 7-piperazinyl substituents exhibit an extended spectrum of activity that includes effectiveness against
  - Gram-ve pathogens (e.g., *P. aeruginosa*, *H. influenzae*, *N. gonorrhoeae*),
  - Gram+ve cocci (e.g., *S. aureus*), and some streptococci.

• The quinolones generally exhibit poor activity against most anaerobic bacteria, including most *Bacteroides* and *Clostridium* species.

• In many cases, bacterial strains that have developed resistance to the antibacterial antibiotics, such as penicillin-resistant gonococci, methicillin-resistant *S. aureus*, and aminoglycoside resistant *P. aeruginosa* are susceptible to the quinolones.
Resistance to quinolones

Resistance is developed through:

1. Mutation in gyrase (or topoisomerase)
2. Mutation in porins that mediates entrance of quinolones
4. QSAR showed inverse relationship between log P and uptake of quinolones by Gram –ve bacteria, and positive relationship between log P and uptake by Gram+ve bacteria
1. **CNS effects** (irritability, tremor, anxiety, convulsions) due to antagonism of gamma-aminobutyric acid (GABA) receptors in brain by quinolones especially with 7-piperazine

   - Piperazino
   - 3-amino-1-pyrrolidino

   CNS effect is present in fluoroquinolones having basic property at 7-position such as:
   - Piperazino
   - 3-amino-1-pyrrolidino

   Note: substitution of CH3 at piperazine reduces GABA binding

2. **Phototoxicity** is associated with quinolones having C8-halogen IF NOT accompanied with either of: 5-OCH3, 8-OCH3 or 5-NH2

3. **Crystalurea** due to formation of insoluble zwitterions at physiological pH for quinolones having C3-COOH, C7-piperazino and C6-F (e.g. norfloxacin)
Side effects

5. **Cytotoxicity** due to binding to human topoisomerase II. This toxicity is most particularly associated with those quinolones that have substitutions at N1, C7 and 8-position.

Usually those substitutions which increases activity against Gram +ve bacteria (e.g. N1-cyclopropyl, C7-pyrrolidine, C8-F) decreased the selectivity for bacterial topoisomerase (i.e. increased binding to human topoisomerase)
Solubility

• Fluoroquinolones are generally insoluble in water.
• They are exist as zwitterions due to the presence of carboxylic acid (pKa=5.5-6.3) and distal amino group (pKa 7.6-9.3).
• They are more soluble at acidic and basic pH than at physiological pH.
Figure 6.6 • Ionization equilibria in the quinolone antibacterial drugs.
Naphthyridines

- **Nalidixic Acid**
- 1-Ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid occurs as a pale buff crystalline powder that is sparingly soluble in water and ether but soluble in most polar organic solvents.
- Mainly used for UTI against Gram –ve bacteria.
- Rapidly absorbed, metabolized and excreted (t$_{0.5}$ = 6 to 7 hrs)
Metabolism of Nalidixic acid

Nalidixic acid

More active

7-hydroxymethyl metabolite

Inactive

Glucuronide

7-methoxyglucuronide metabolite

Inactive

7-carboxylic acid metabolite
Naphthyridines (Cont.)

- **Enoxacin**
- 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid
- Well absorbed following oral administration (90%).
- Well distributed through the body
- Concentrations of the drug in the kidneys, prostate, cervix, fallopian tubes, and myometrium typically exceed those in the plasma, therefore used for infections of reproductive systems
- About 50% is excreted unchanged in urine
- 15-20% is metabolized by CYP450
Fluroquinolones

- They are 6-fluoro-7-piperazinoquinolones derivatives.
- They exhibit extended spectrum of activity that covers most of gram +ve and gram –ve bacteria especially *P. aeruginosa*.
- C6-F ➔ increase activity against Gram-ve
- Members:
  - Norfloxacin
  - Ciprofloxacin.
  - Ofloxacin.
  - Pefloxacin.
  - Lomefloxacin.
  - Enofloxacin.
  - Levofloxacin
Fluroquinolones (Cont.)

- **Norfloxacín**

- 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid is a pale yellow crystalline powder that is sparingly soluble in water.

- Broad spectrum activity. The fluorine atom provides increased potency against Gram-positive organisms, whereas the piperazine moiety improves anti-pseudomonal activity.

- Well absorbed after oral administration (30%) excreted in urine including 7% inactive metabolites.
Fluroquinolones (Cont.)

- **Ciprofloxacin**
- 1-Cyclopropyl0-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylicacid.
- Used orally (70% is absorbed) and parenterally
- 15% of it is metabolized to less active metabolites
- 40 to 50% exerted unchanged in urine.
- Significant amount is excreted unchanged in feces
- Highly distributed to all body fluids including CS fluid.
- Highly potent against gram –ve especially *P. aeruginosa* (why?).
- Used in gastroenteritis, skin, soft tissues (bone and joints) infections and UTI.
- Causes crystalurea if urine is alkalinized (pH >7) by some agents
**Fluroquinolones (Cont.)**

- **Ofloxacin and Levofloxacin**
  - 9-Fluoro-2,3-dihydro-3-methyl-10(4-methyl-1-piperazin-yl)-7-oxo-7H-pyrido[1,2,3-de]-1,4,-benzoxazine-6-carboxylic acid
  - 1- and 8-positions are joined in the form of a 1,4-oxazine ring
  - Has better penetration to CNS than ciprofloxacin
  - The structure has asymmetric carbon atom, normally ofloxacin is given as racemate, although the $3S(-)$ isomer is 125x more active than the $3R(+) \text{ isomer (WHY ?)}$.
  - Recently the $3S(-)$ isomer was purified to be sold as Levofloxacin
Fluroquinolones (Cont.)

- **Lomefloxacin**
  - 1-Ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid
  - Is a difluorinated quinolone with a longer elimination half-life (7–8 hours) than other members of its class due to:
    - High tissue distribution
    - High renal reabsorption
  - High incidence of phototoxicity due to the presence of two fluorine atoms.
  - Phototoxicity: is the formation of highly reactive oxygen radicals due to the exposure to light.
Phototoxicity of fluoroquinolones

Free radicals which cause phototoxicity
Fluroquinolones (Cont.)

• **Sparfloxacin**

• *(cis)-5-amino-1-cyclopropyl-7-(3,5-dimethyl)-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid, is a newer fluoroquinolone*

• Highly active against Gram+ve as well as Gram-ve bacteria. It is also active against anerobes.

• It has high tissue distribution and long elimination half-life of 18 hours, which permits single daily dosing.

• The incidence of phototoxicity of sparfloxacin is the lowest of the fluoroquinolones, because of the presence of the 5-amino group, which counteracts the effect of the 8-fluoro substituent.

(5-amino or 5-OCH₃ → reduce phototoxicity of fluoroquinolones)
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Nitro-heterocyclic compounds

- Nitroheterocyclic compounds was introduced as chemotherapy such as nitrofurans and nitroimidazoles.
- Examples of nitrofurans are nitrofurazone, furazolidone, and nitrofurantoin which were used to treat bacterial infections for almost 50 years.
- Example of nitroimidazole is metronidazole which is used as antiprotozoal e.g. amebicide (trichomonicide) as well as used for the treatment of systemic infections caused by anaerobic bacteria.

\[ \text{Nitrofurans} \quad \text{Nitroimidazole} \]
The mechanism of antimicrobial action of the nitrofurans is not fully understood, however, it may involve DNA damage.
Synthesis of nitrofurans

• The nitrofurans are derivatives of 5-nitro-2-furaldehyde, formed on reaction with the appropriate hydrazine or amine derivative. Antimicrobial activity is present only when the nitro group is in the 5-position.

5-nitro-2-furaldehyde

H₂N—NHR
Hydrazine derivative

HO⁻·N=O⁻·N⁺—N—R

Nitrofurazone R=

Furazolidone R=

Nitrofurantoin R=
Examples of nitrofurans

- **Nitrofurazone**
- 5-Nitro-2-furaldehyde semicarbazone (Furacin) occurs as a lemon-yellow crystalline solid that is sparingly soluble in water and practically insoluble in organic solvents.
- It is chemically stable, but moderately light sensitive.
- Used for burns as ointment
- It has a broad spectrum of activity against Gram-positive and Gram-negative bacteria
Furazolidone

3-[(5-Nitrofurylidene)amino]-2-oxazolidinone

It is active against Gram+ve, Gram-ve bacteria as well as protozoa such as Giardia lamblia

It is recommended for the oral treatment of bacterial or protozoal diarrhea caused by susceptible organisms

Only small fraction is absorbed after oral use. About 5% of the oral dose is detected in urine.
Examples of nitrofurans (Cont.)

- **Nitrofurantoin**
- Nitrofurantoin, 1-(5-nitro-2-furfurylidene)-1-aminohydantoin
- is a nitrofuran derivative that is suitable for oral use. Used mainly for urinary tract infections caused by susceptible strains of *E. coli*, enterococci, *S. aureus*, *Klebsiella*, *Enterobacter*, and *Proteus* spp.
• Metronidazole is a nitroimidazole structure used initially as anti-protozoal and later as antibacterial agents for anaerobes

• Nitro group is reduced to NH₂ after entry to bacterial cell which makes concentration gradient for more metronidazole molecules to flow inside the bacteria.

• The aromatic amine can attack DNA and cause bacterial death.

Metronidazole, R = OH (Flagyl)
Tinidazole R = SO₂C₂H₅ (Fasigyn)
Metabolism of metronidazole (human)

In the liver, metabolism of metronidazole leads to two major metabolites:
1. Hydroxylation of the 2-methyl group to 2-hydroxymethyl metronidazole
2. Oxidation to metronidazole acetic acid (MAA).
Metabolism of metronidazole (anaerobes)

- Anerobes have more reduction capability than aerobes (such as human)
- Thus anaerobic bacteria formed reduced metabolite of metronidazole more than aerobic bacteria
- Thus anaerobic bacteria is more affected by metronidazole than aerobic bacteria.

Interact with DNA and causes death
Methenamine (synthetic antimicrobial agent)

- Methenamine is a drug that can be used for the disinfection of acidic urine.
- Structurally it is a low molecular weight polymer of ammonia and formaldehyde which reverts to its components under mildly acid conditions.
- Formaldehyde is the active antimicrobial component.
- Methenamine can be used for recurrent urinary tract infections.

\[
\text{Methenamine (Prosed, Urimax, Urised, Uroqid-Acid)} \xrightarrow{\text{H}_3\text{O}^+} 4 \text{NH}_3 + 6 \text{CH}_2\text{O}
\]

Antimicrobial effect
1. Aminoglycosides
2. Macrolides
3. Tetracyclines
4. Chloramphenicol
5. Quinolones
6. Anti-mycoplasma
7. Anti-viral
8. Anti-fungal