Synthetic antibacterial agents

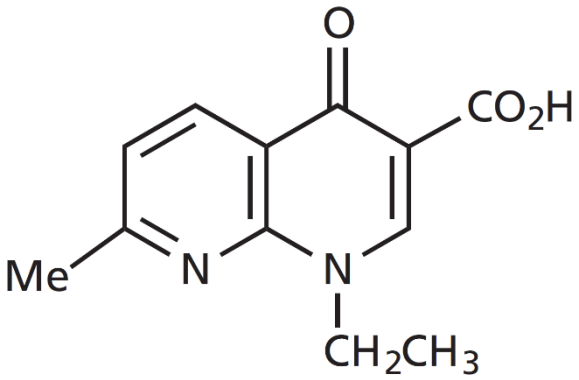
Quinolones

Synthetic antibacterial agents

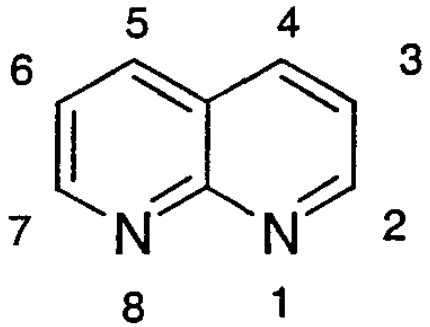
- Several synthetic antibacterial agents were synthesized based on model compounds, these include:
  - Sulfonamides
  - Quinolones
  - Nitroheterocyclic compounds (nitrofurans, metronidazole)
- Some agents can be used for systematic infections
- Others are unsuitable for treating systematic infections 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 quinolones are group of synthetic antibacterial agents derived from nalidixic acd.
- Nalidixic acid is 1,8-naphthyridine derivative, synthesized in 1962 used mainly for UTI.

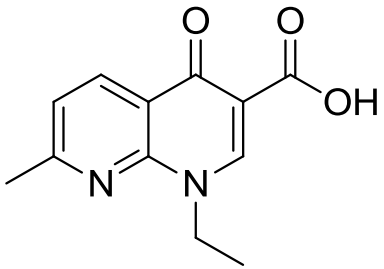


Nalidixic acid

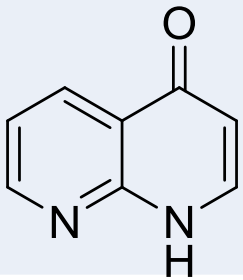
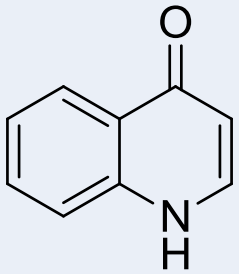


Quinolones (Cont.)

- Nalidixic acid is the lead compound for this group.

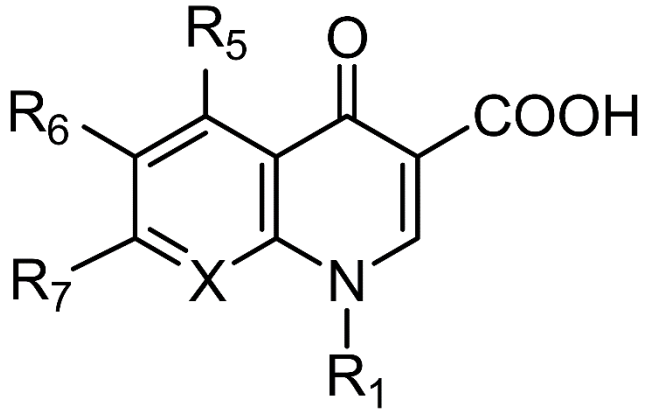


- According to the heterocyclic core can be divided into:

Naphthyridines	Quinolines
nalidixic acid and enoxacin	norfloxacin, ciprofloxacin, ofloxacin, lemofloxacin
	

Quinolones (Cont.)

- The quinolones and fluoroquinolones inhibit the replication and transcription of bacterial DNA by stabilizing
- The synthetic fluoroquinolones mostly end in the suffix **-floxacin**.



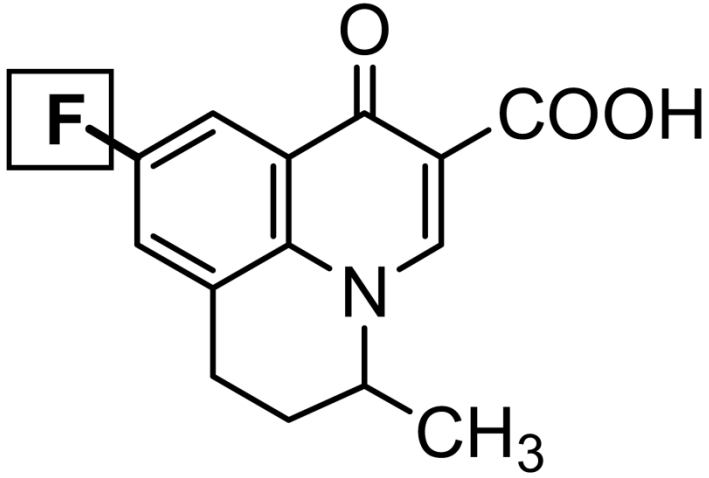
quinolones: X= CH or C-R_8
naphthyridones: X= N

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

Quinolones (Cont.)

- The quinolones and napthhyridones 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 Fuoroquinolones (Flumequine).



Flumequine

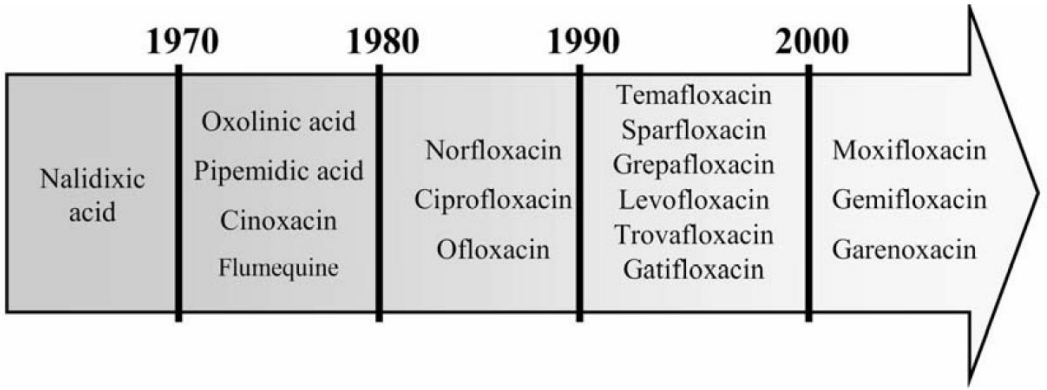
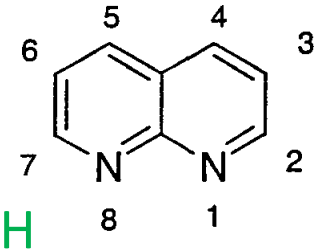


Figure 2. Clinical development of quinolones

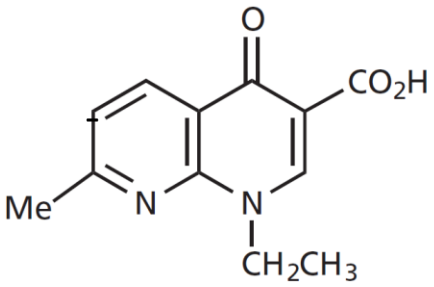
Quinolones (Cont.)

- Derivatives of nalidixic acid were developed which showed improved broad-spectrum activity such as **enoxacin** which is based on:
  - C6-F ➔ ↑activity &↑cellular uptake by bacteria
  - C7-piperazine ➔ ↑basicity ➔ zwitterion with C3-COOH (affects pharmacokinetics)

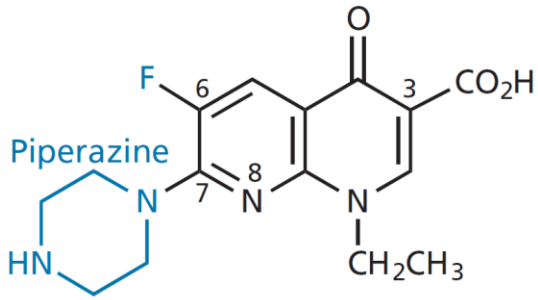


& **ciprofloxacin** which is based on:

- N1-cyclopropyl ➔ ↑ spectrum of activity
- N8 is changed to C8 ➔ ↓ adverse reactions & ↑ activity vs S. aureus



Nalidixic acid

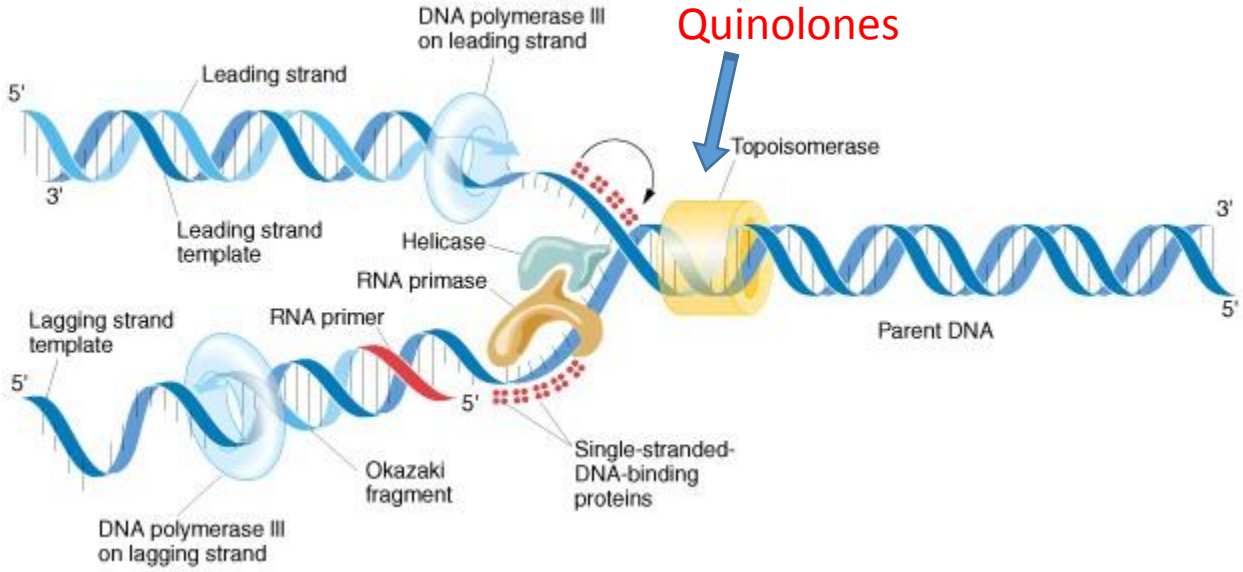


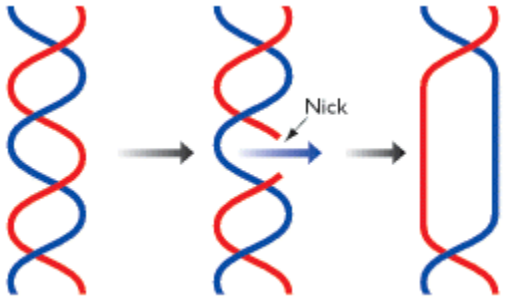
Enoxacin

FIGURE 19.71 Quinolones and fluoroquinolones.

Mechanism of action

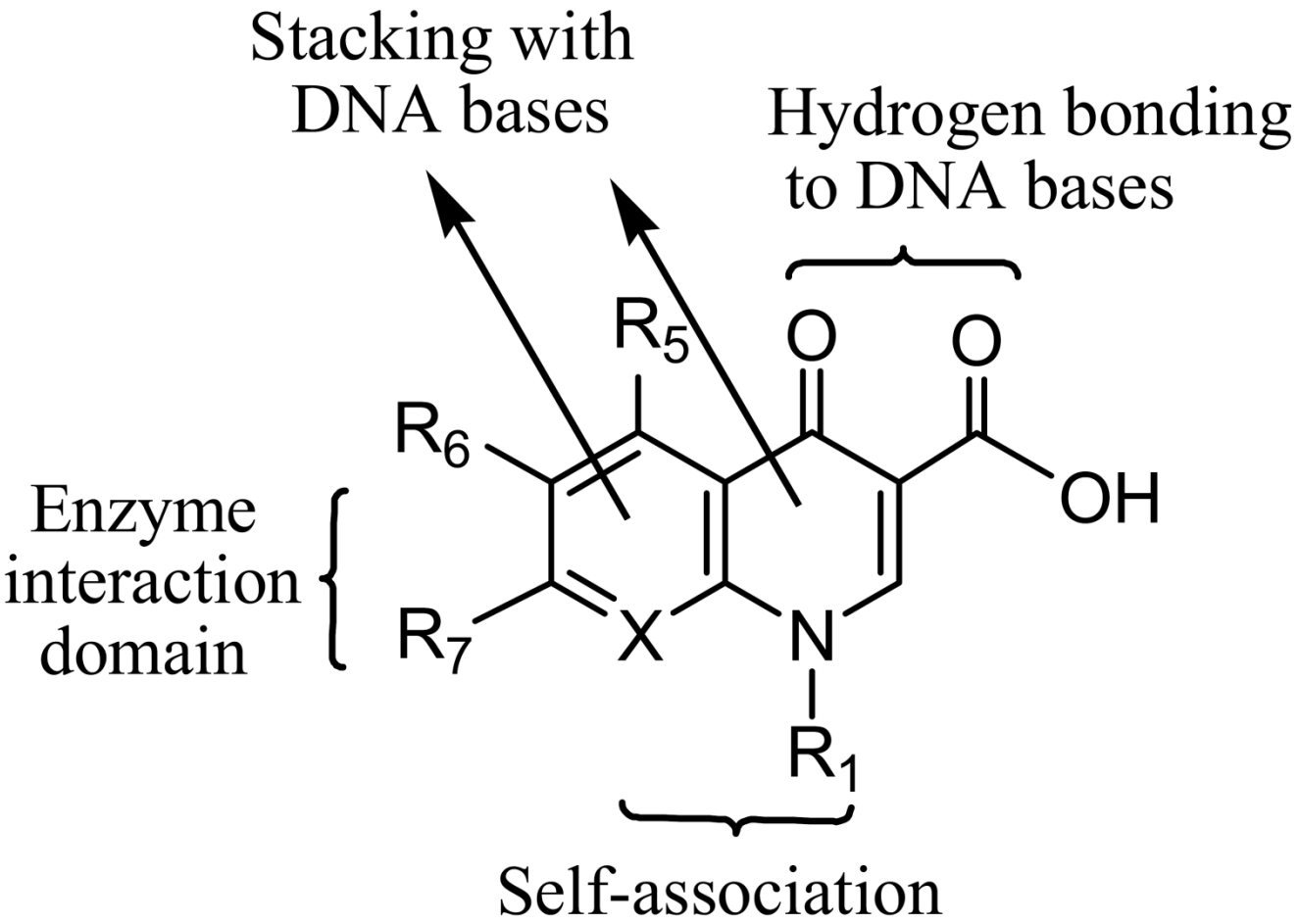
- DNA topoisomerase (or gyrase) alters the conformation of DNA by catalyzing transient double-strand cuts, passing the uncut portion of the molecule through the gap, and resealing the molecule back together.
- Topoisomerase IV seems more important to some Gram +ve, and DNA gyrase seems more important 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



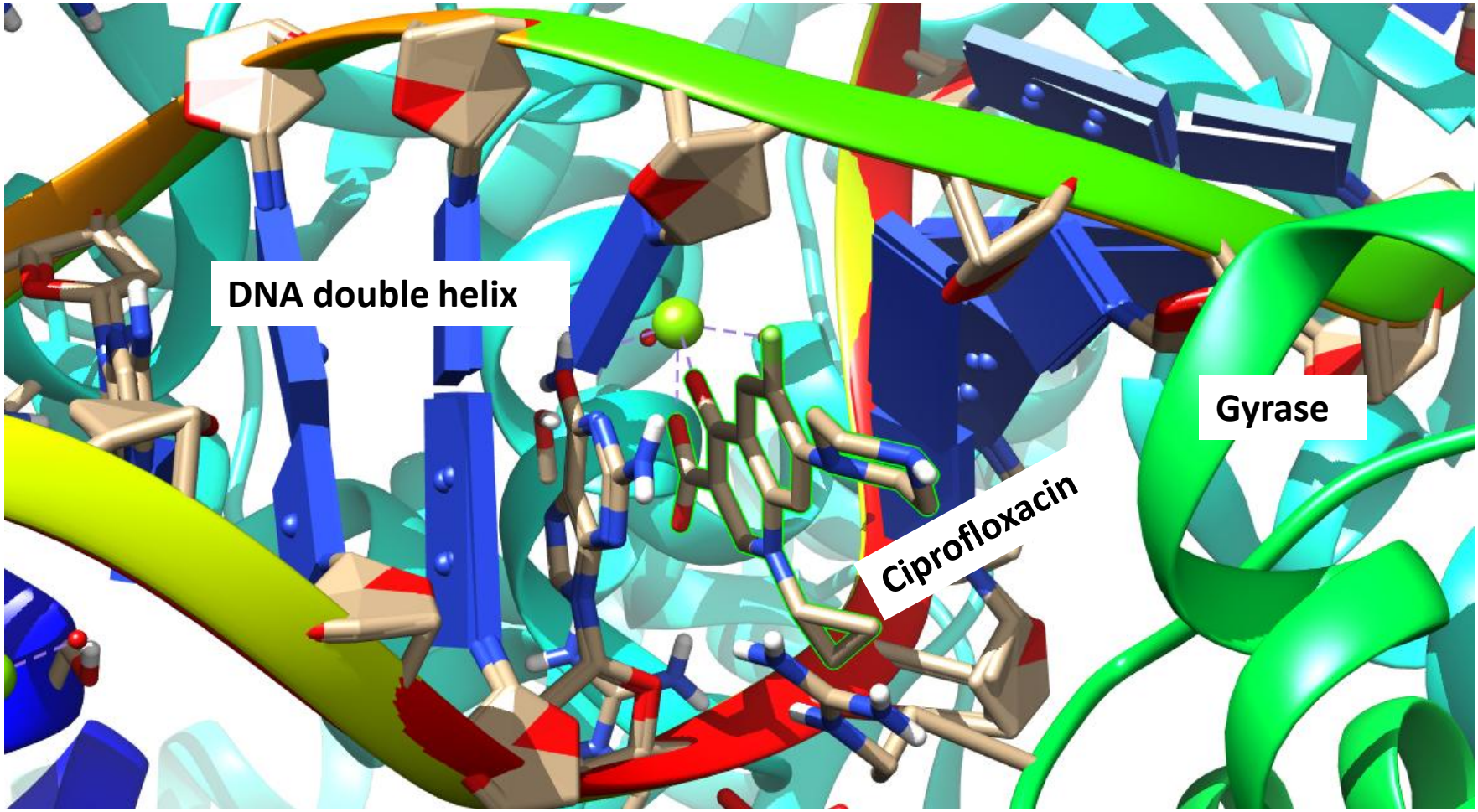


Topoisomerase makes cuts, unwind, then stick DNA again

Quinolones (Cont.)



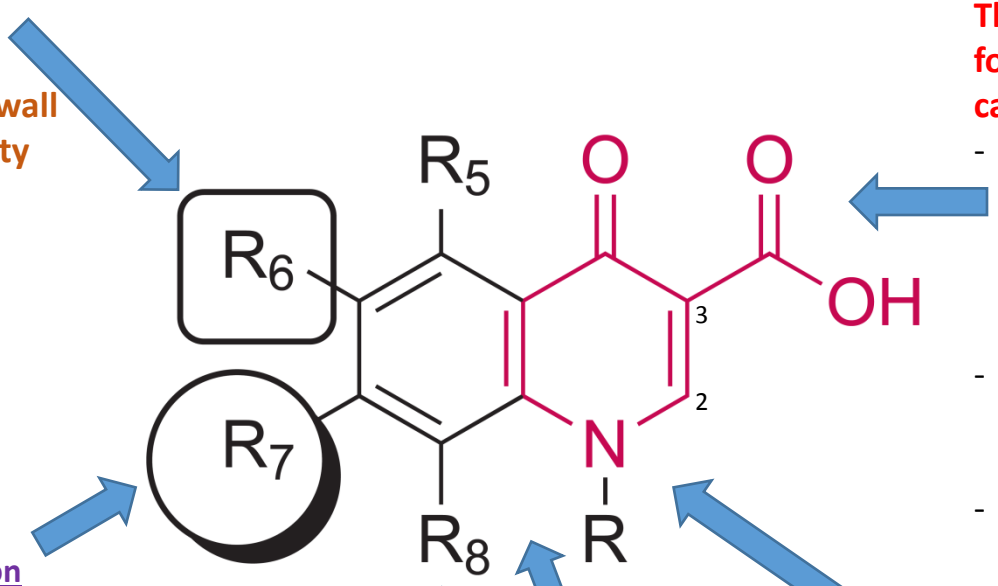
Fluroquinolone-Gyrase-DNA complex



SAR for quinolones

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

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



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

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

Aminopyrrrolidine ➔↑ spectrum vs Gram +ve bac

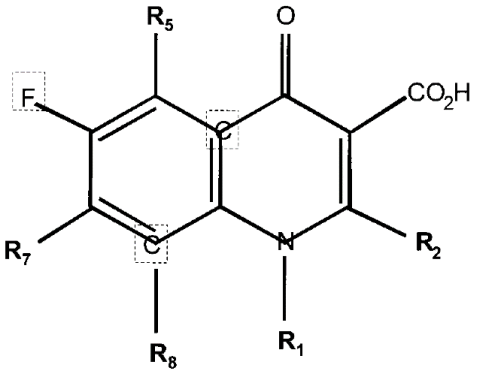
Bulky group reduces bacterial efflux

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

C8-F
➔↑ absorption, half-life
➔↑drug-induced photosensitivity.
C8-OCH3 ➔↓ phototoxicity

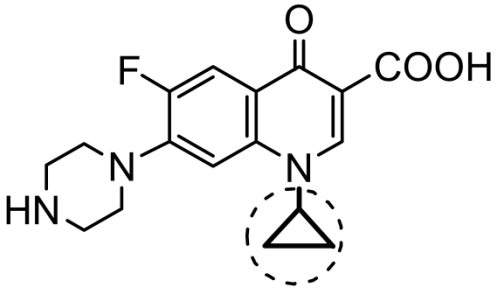
N1-cyclopropyl (or small alkyl or aryl) substitution broaden spectrum of activity

SAR for quinolones

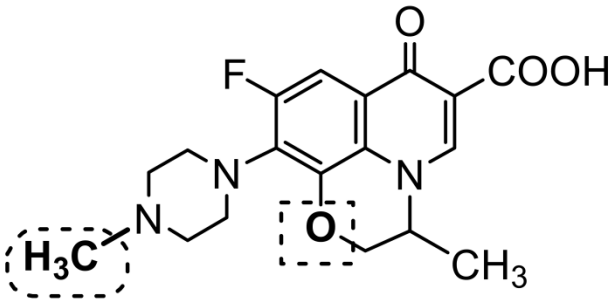


- ***Position 1.***
- This position is part of the enzyme-DNA binding complex, and has a hydrophobic interaction with the major grove of DNA. The optimum substituents at position 1 appear to be ethyl, butyl. The most potent is cyclopropyl (ciprofloxacin) followed by addition of a 2,4-difluorophenyl

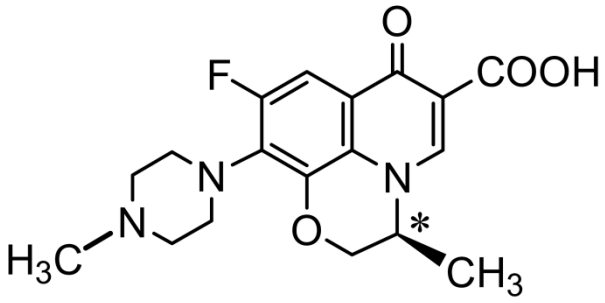
- Ofloxacin is tricyclic ring structure. It contains oxazine ring between positions 1 and 8. oxazine has asymmetric C3 position (S isomer is more active than R isomer, which affects binding to DNA hydrophobic pocket)



Ciprofloxacin



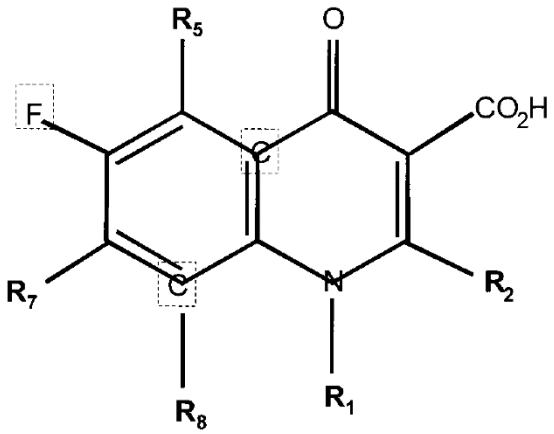
(±)-Ofloxacin



Levofloxacin

Peterson, Lance R. "Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity." *Clinical Infectious Diseases* 33.Supplement 3 (2001): S180-S186.

SAR for quinolones



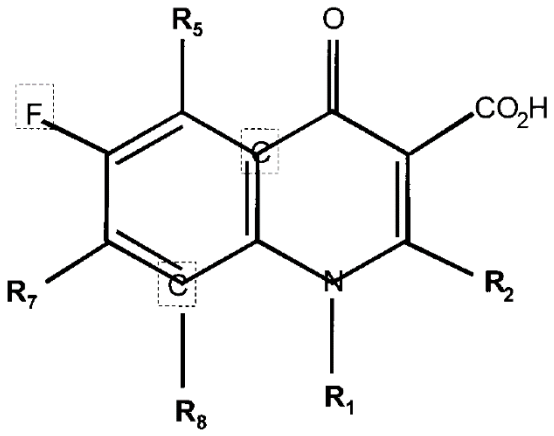
- ***Position 2.***

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

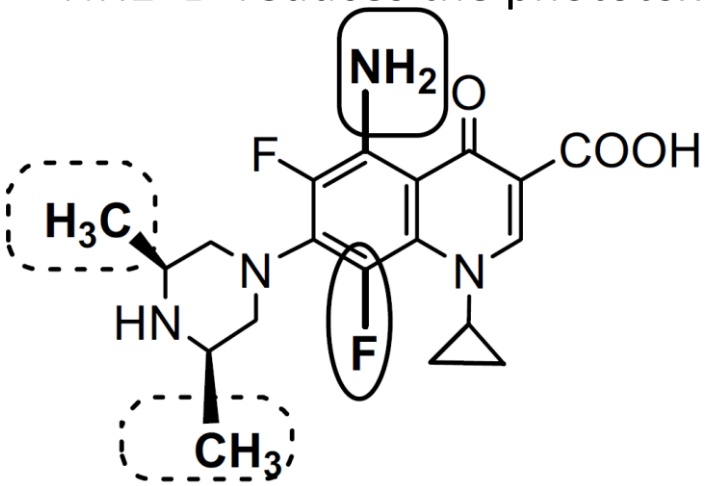
- ***Positions 3 and 4.***
- These two positions on the quinolone nucleus are considered critical for binding to cleaved or perturbed DNA, and no useful substitutions at these positions have yet been reported.
- 4-thioxo or sulphonyl group leads to a loss of activity

Peterson, Lance R. "Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity." *Clinical Infectious Diseases* 33.Supplement 3 (2001): S180-S186.

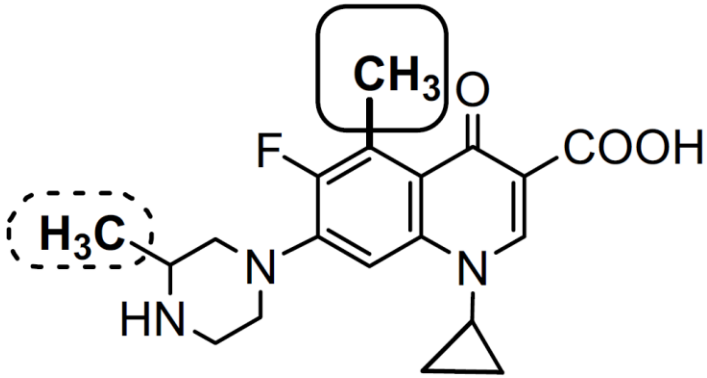
SAR for quinolones



- ***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



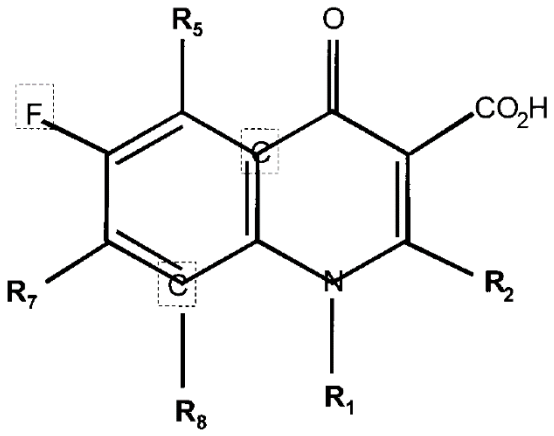
Sparfloxacin



Grepafloxacin

Peterson, Lance R. "Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity." *Clinical Infectious Diseases* 33.Supplement 3 (2001): S180-S186.

SAR for quinolones



- ***Position 6.***

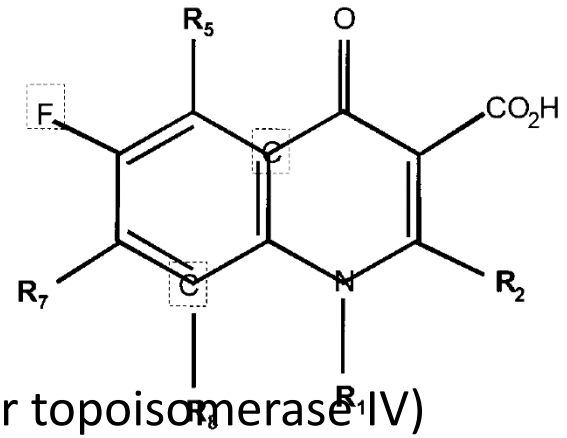
- Addition of Fluorine produces the fluoroquinolone class with enhanced antibacterial activity against Gram +ve and Gram –ve bacteria (including *P. aeruginosa*)
- 6-NH2
  - with 8-CH3 quinolones ➔ expand activity against Gram +ve cocci
  - With C7-tetrahydroisoquinoline ➔ increase the potency up to 100 fold compared to ciiprofloxacin

Emami, Saeed, Abbas Shafiee, and Alireza Foroumadi. "Quinolones: recent structural and clinical developments." *Iranian Journal of Pharmaceutical Research* (2010): 123-136.

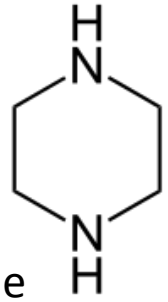
Tillotson, G. S. "Quinolones: structure-activity relationships and future predictions." *Journal of medical microbiology* 44.5 (1996): 320-324.

Peterson, Lance R. "Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity." *Clinical Infectious Diseases* 33.Supplement 3 (2001): S180-S186.

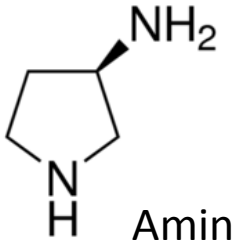
SAR for quinolones



- ***Position 7.***
- This position is directly interacts with DNA gyrase (or topoisomerase IV)
- Optimum substituents are 5- to 6-membered nitrogen heterocycles.
- Piperazine substituents ➔ 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 ➔ increase activity against Gram +ve bacteria
- Bulkier groups at 7 position reduces the bacterial ability to efflux antibiotic, thus prevent resistance development.



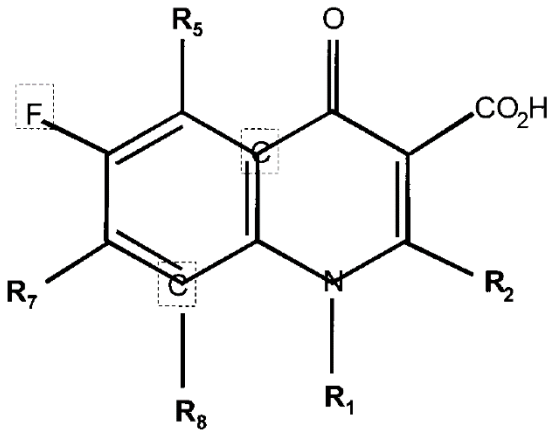
Piperazine



Aminopyrrolidine

Peterson, Lance R. "Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity." *Clinical Infectious Diseases* 33.Supplement 3 (2001): S180-S186.

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) ➔ improves activity against Gram –ve & anaerobes
- Halogen, CH3 or OCH3 ➔ increase activity against Gram +ve cocci
- Replacement of C8 with N8 ➔ increase antimicrobial potency
- Large substitution (e.g. ethyl derivatives) ➔ reduces activity against gram +ve and –ve bacteria.
- Replacement of C8 to N8 as well as C8-CH3 substitutions ➔ 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.

Peterson, Lance R. "Quinolone molecular structure-activity relationships: what we have learned about improving antimicrobial activity." *Clinical Infectious Diseases* 33.Supplement 3 (2001): S180-S186.

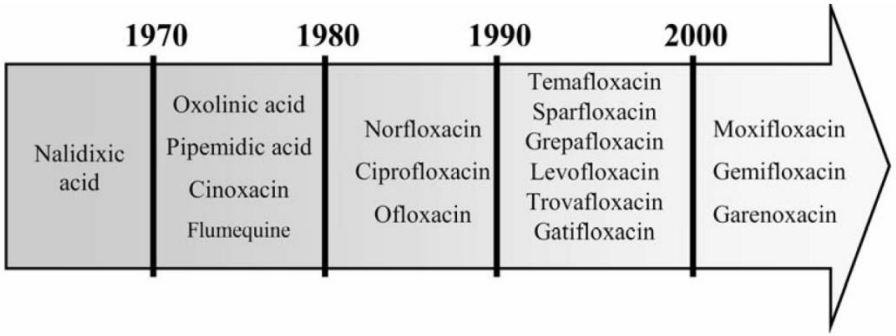


Figure 2. Clinical development of quinolones

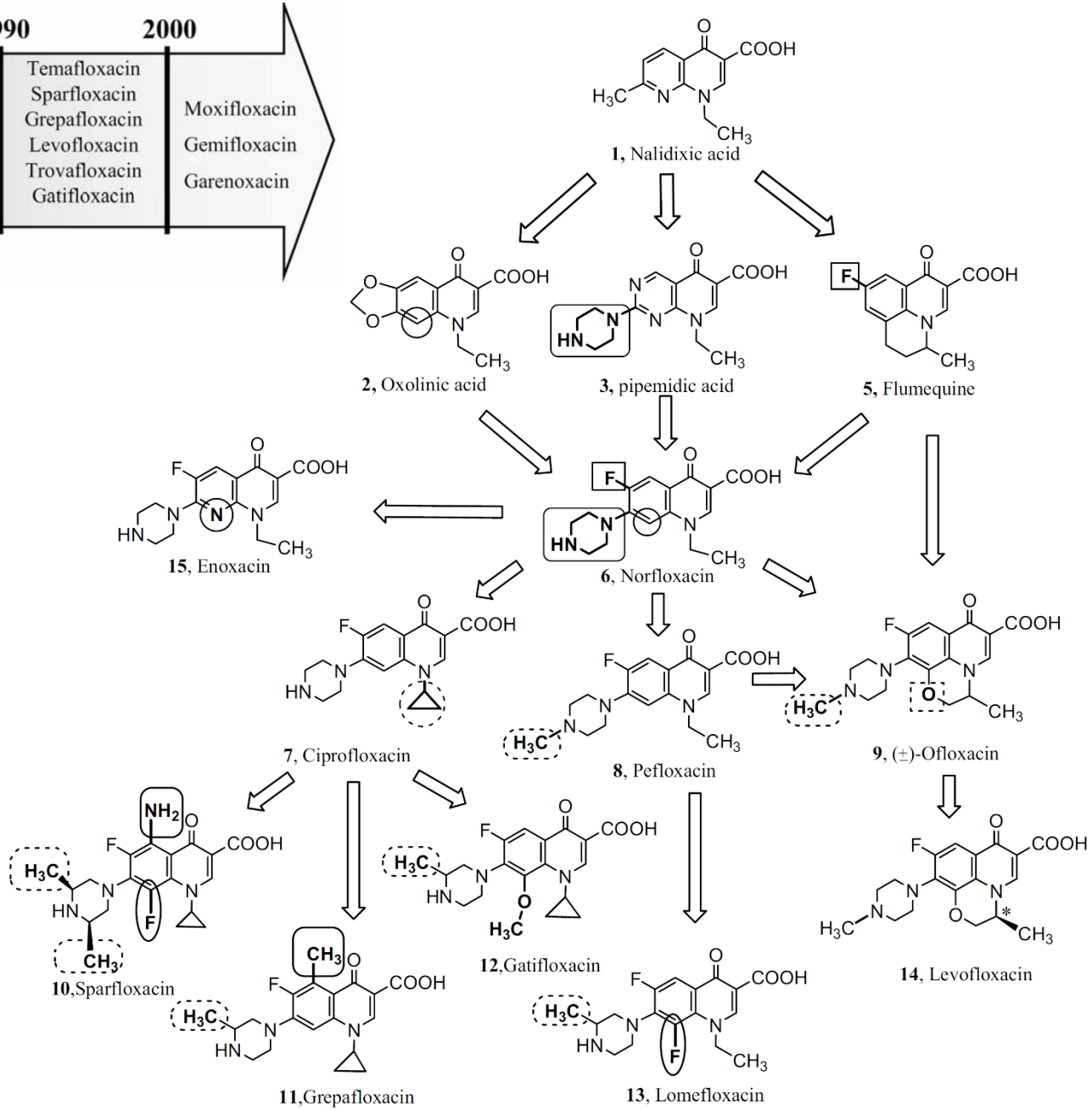
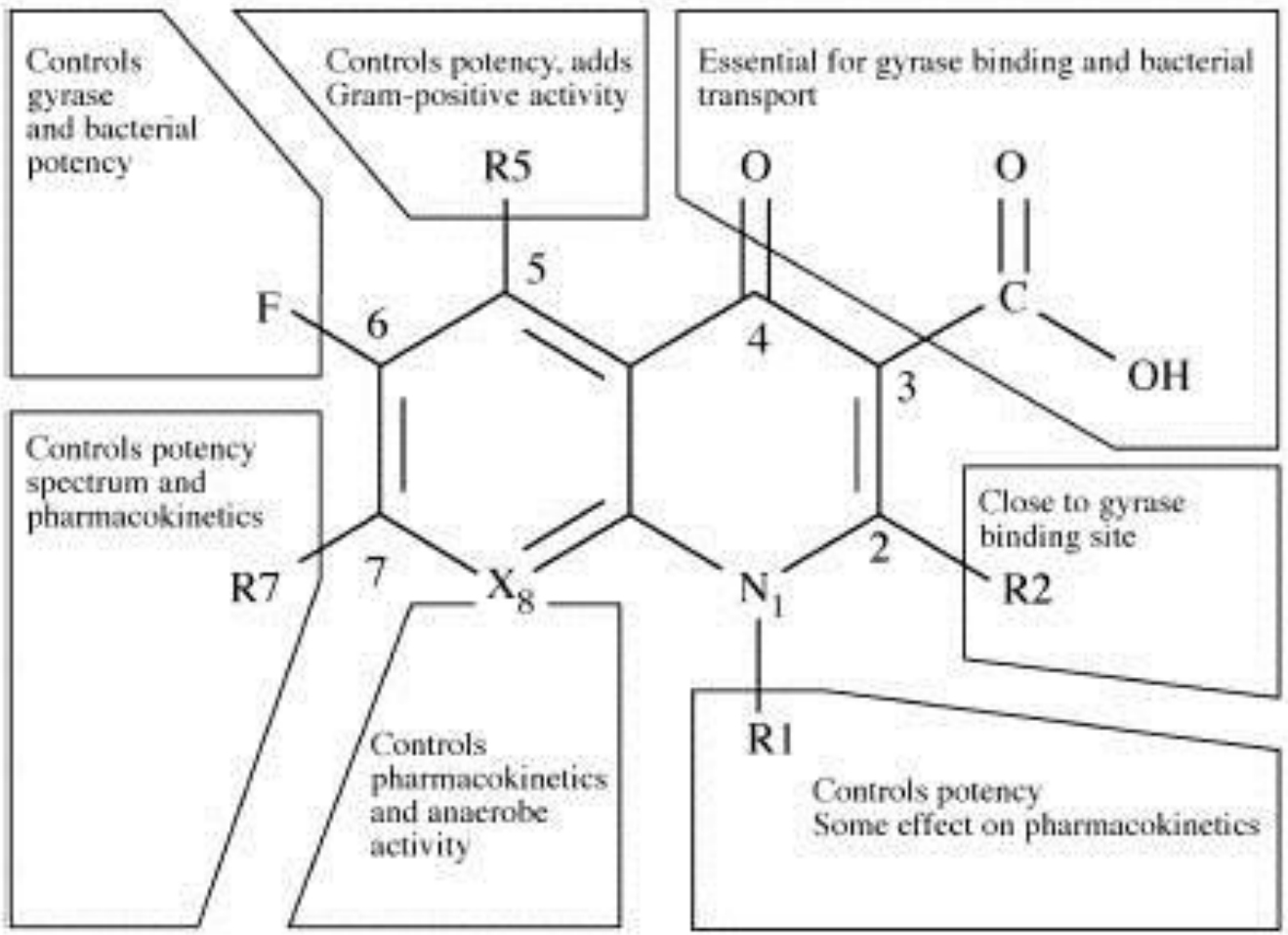


Figure 3. Structural development of 7-piperazinylquinolones from primary quinolones

Emami, Saeed, Abbas Shafiee, and Alireza Foroumadi. "Quinolones: recent structural and clinical developments." *Iranian Journal of Pharmaceutical Research* (2010): 123-136.

Structure-activity relationship



Structure-adverse effect relationship

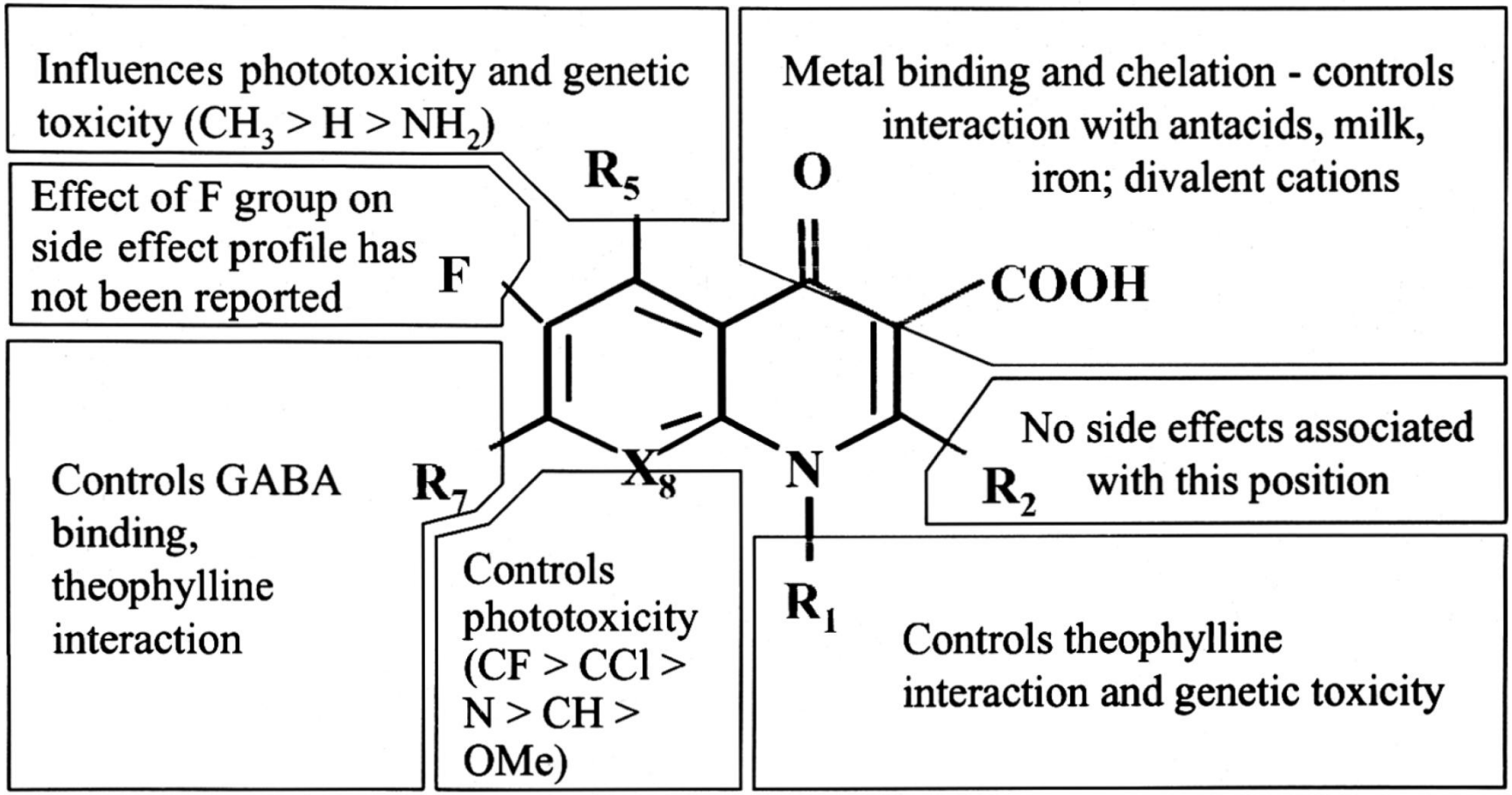
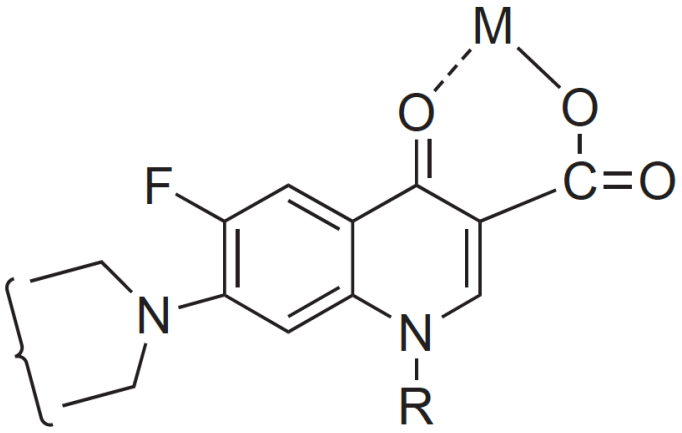


Figure 1. Quinolone structure–side effect relationships. GABA, γ-aminobutyric acid. Modified from [12].

Mandell, Lionel A., Peter Ball, and Glenn Tillotson. "Antimicrobial safety and tolerability: differences and dilemmas." *Clinical infectious diseases* 32.Supplement 1 (2001): S72-S79.

Chelation of quinolones wit polyvalent ions

- Chemical incompatibility common to all of the quinolones involves the ability of these drugs to chelate polyvalent metal ions (Ca2+, Mg2+, Zn2+, Fe2+, Al3+), 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 activtiy 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.

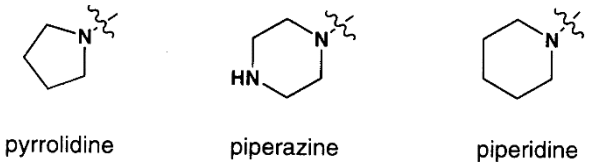
Resistance to quinolones

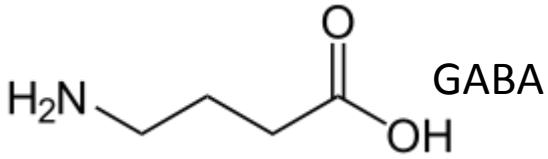
Resistance is developed through:

1. Mutation in gyrase (or topoisomerase)
2. Mutation in porins that mediates entrance of quinolones
3. Energy-dependent efflux of quinolones by some bacterial species.
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

Side effects

1. CNS effects (irritability, tremor, anxiety, convulsions) due to antagonism of gamma-aminobutyric acid (GABA) receptors in brain by quinolones especially with 7-piperazine





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

Note: substitution of CH3 at piperazine reduces GABA binding

3. CNS effect is almost absent in quinoliones due to inability to penetrate the blood-brain barrier
4. Phototoxicity is associated with quinolones having C8-halogen if not accompanies with OCH_3 at C5 and C8 or NH_2 at C5
5. Crystalurea due to formation of insoluble zwitterions at physiological pH for quinolones having C3-COOH, C7-piperazino and C6-F (e.g. norfloxacin)

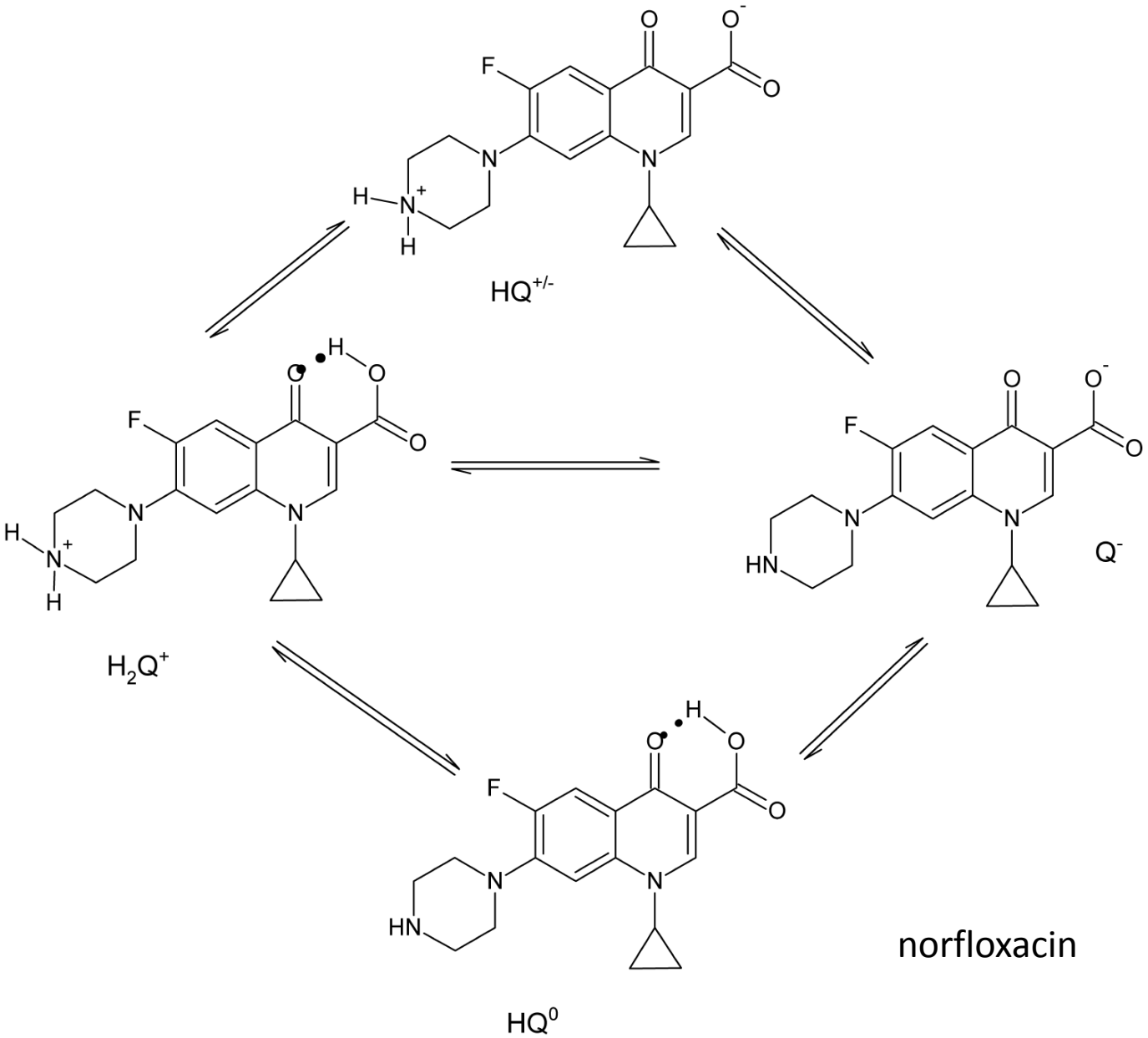
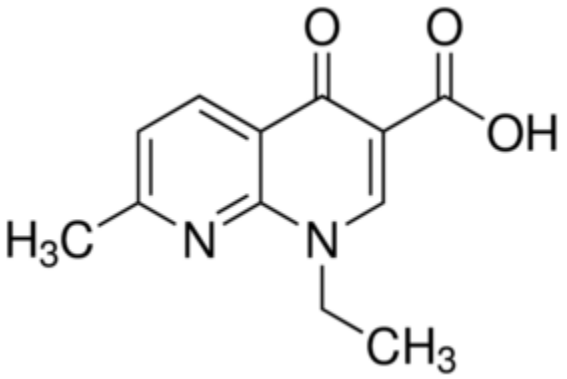


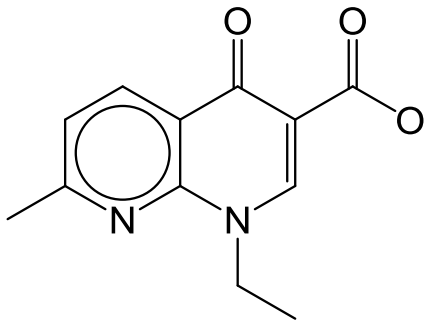
Figure 6.6 ● Ionization equilibria in the quinolone antibacterial drugs.

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)

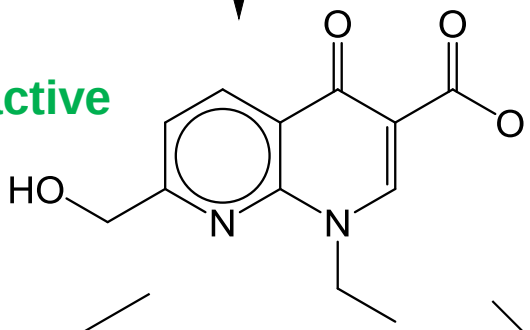


Metabolism of Nalidixic acid



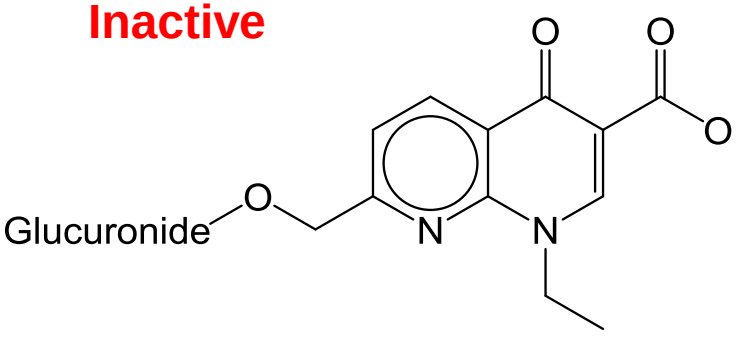
Nalidixic acid

More active



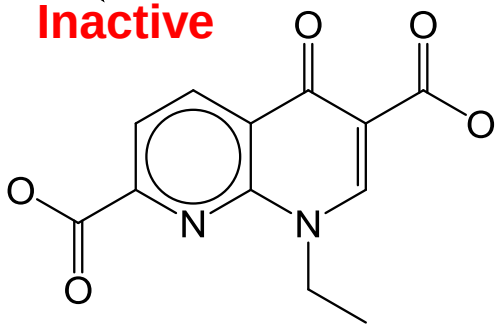
7-hydroxymethyl metabolite

Inactive



7-methyoxyglucuronide metabolite

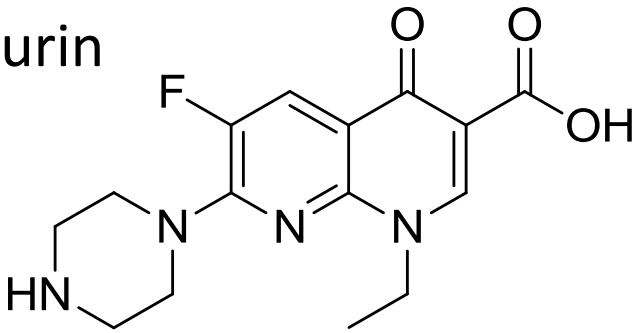
Inactive



7-carboxylic acid metabolite

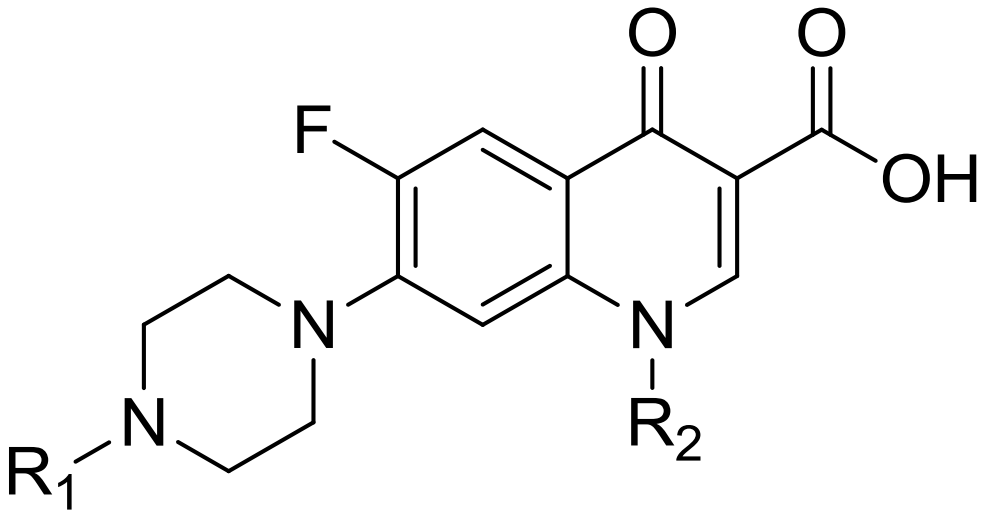
Naphthyridines (Cont.)

- ***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 urin
- 15-20% is metabolized by CYP450



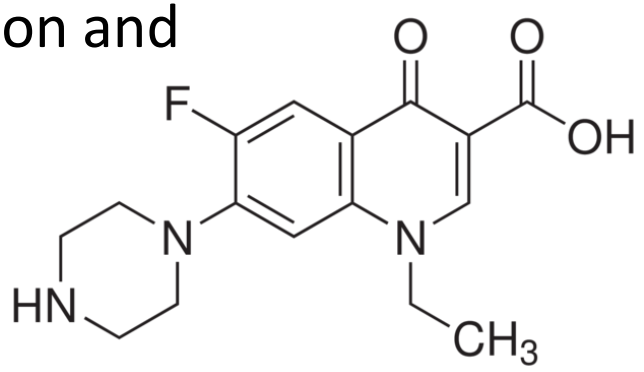
Fluroquinolones

- 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



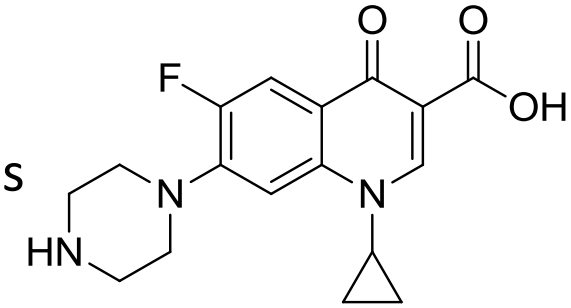
Fluroquinolones (Cont.)

- ***Norfloxacin***
- 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 and (30%) excreted in urine including 7% inactive metabolites



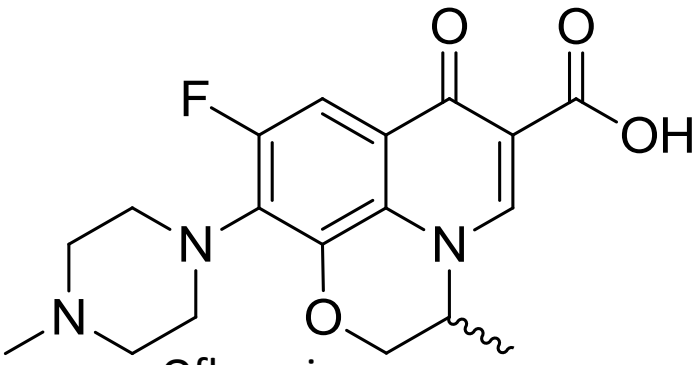
Fluroquinolones (Cont.)

- ***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

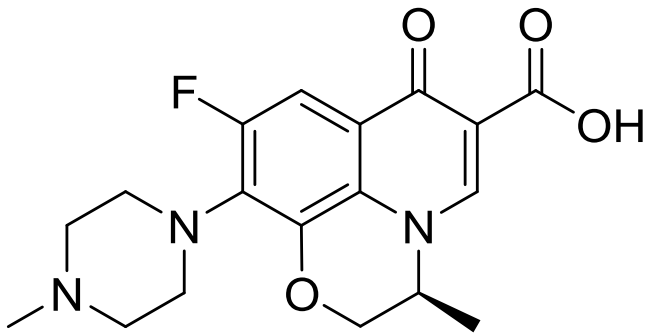


Fluroquinolones (Cont.)

- ***Ofloxacin and Levofloxacin***
- 9-Fluoro-2,3-dihydro-3-methyl-10(4-methyl-1-piperazin-yl)-7-oxo-7*H*-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 3*S*(-) isomer is 125x more active than the 3R(+) isomer (WHY ?).
- Recently the 3S(-) isomer was purified to be sold as Levofloxacin



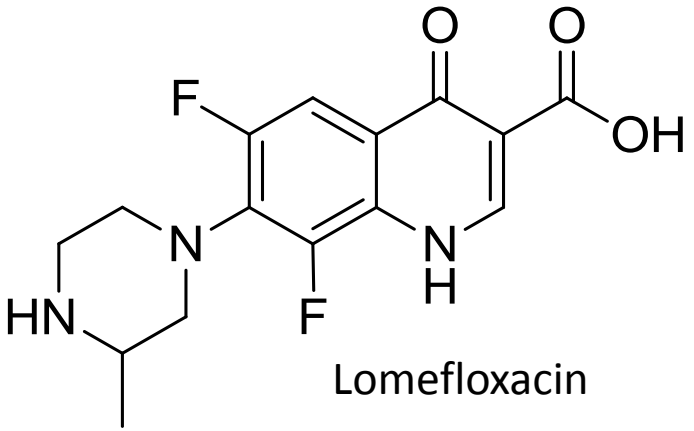
Ofloxacin



Levofloxacin

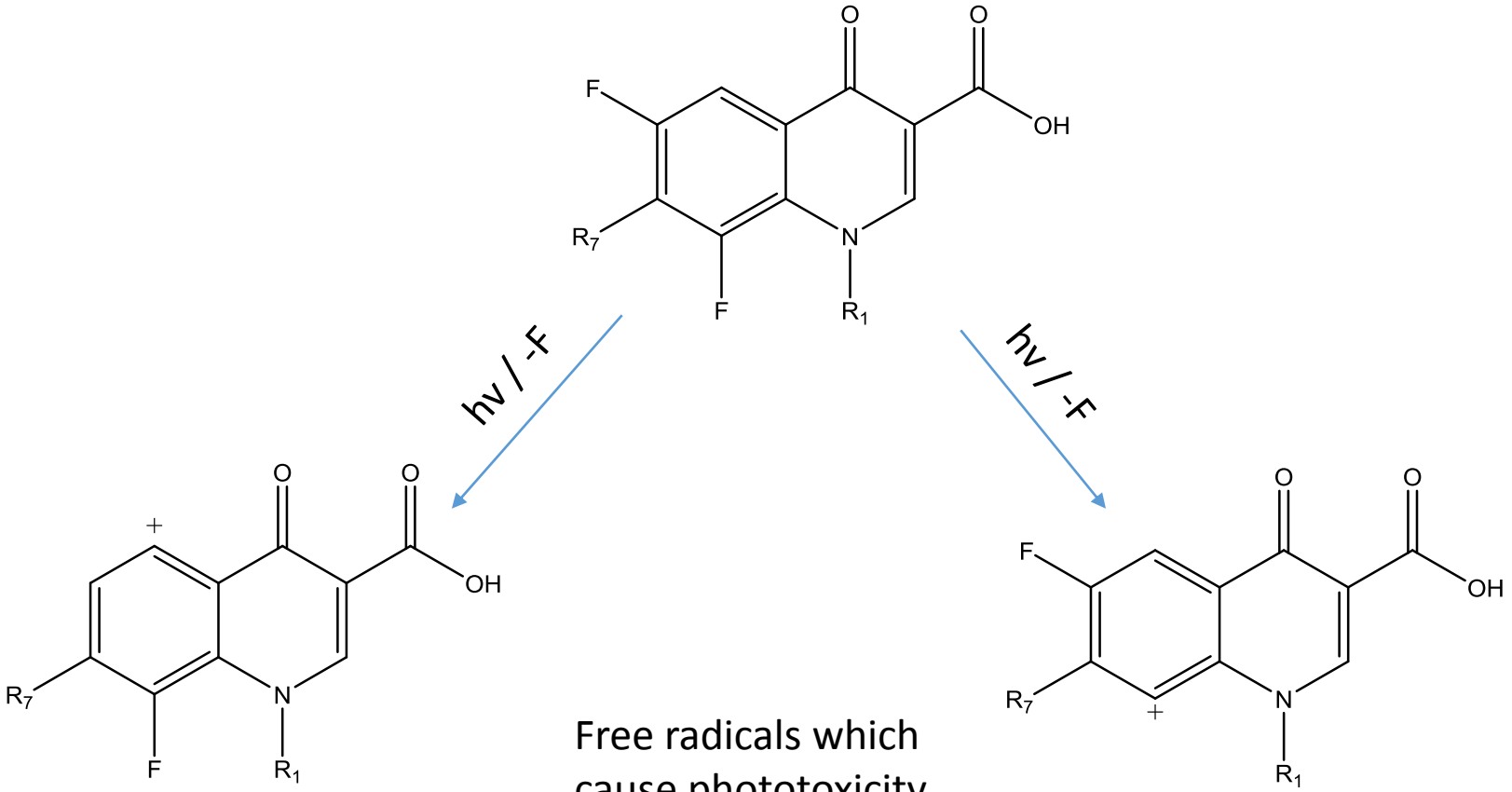
Fluroquinolones (Cont.)

- ***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.



Lomefloxacin

Phototoxicity of fluroquinolones

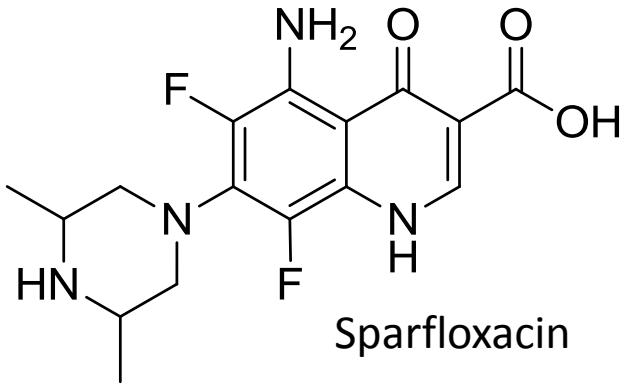


Free radicals which cause phototoxicity

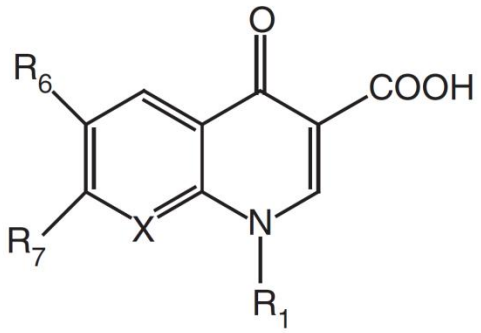
Fluroquinolones (Cont.)

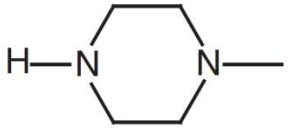
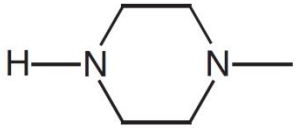
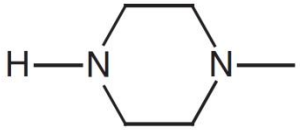
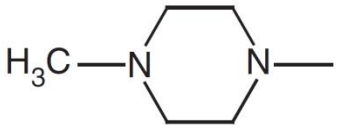
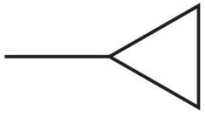
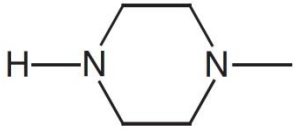
- ***Sparfloxacin***
- (*cis*)-5-amino-1-cyclopropyl-7-(3,5-dimethyl)-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid, is a newer fluoroquinolone
- Highly active against Gram+ve as well as Gram-ve bacteria. It is also active against anerobes.
- It has high tissue distribution 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.

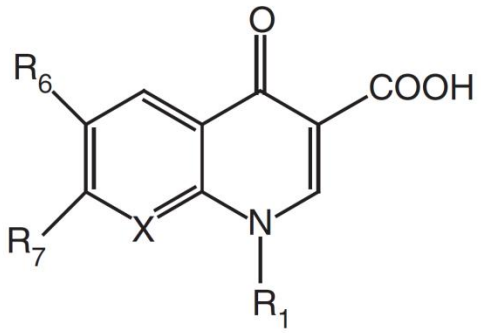
(5-amino or 5-OCH_3 → reduce phototoxicity of fluroquinolones)

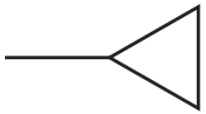
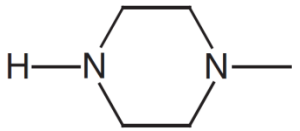
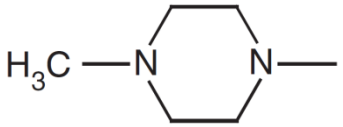
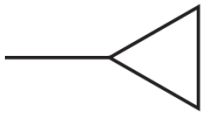
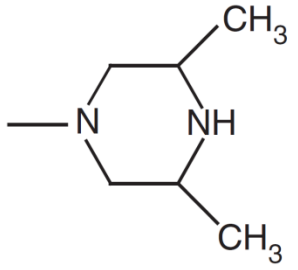
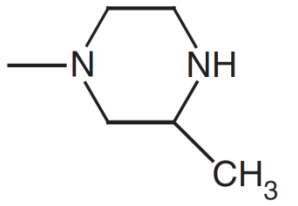


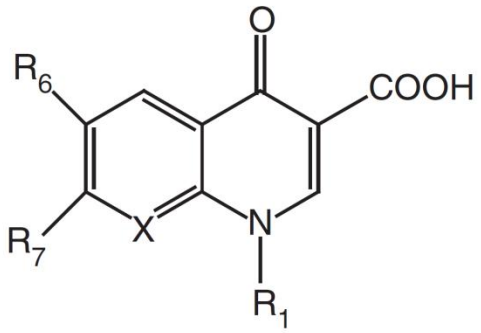
Sparfloxacin

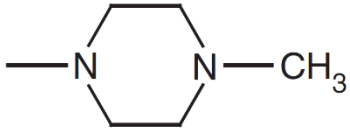
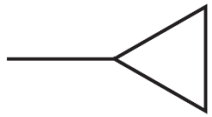
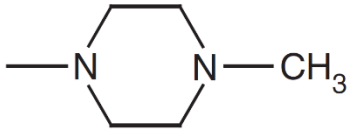
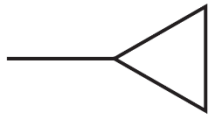
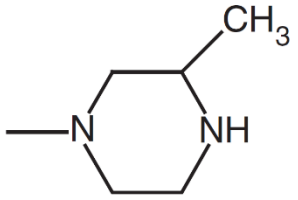


Name	X	R_6	R_1	R_7
Nalidixic acid	–N	–H	$-CH_2CH_3$	$-CH_3$
Enoxacin	–N	–F	$-CH_2CH_3$	
Pipemidic acid	–N	–H	$-CH_2CH_3$	
Norfloxacin	–CH	–F	$-CH_2CH_3$	
Pefloxacin	–CH	–F	$-CH_2CH_3$	
Ciprofloxacin	–CH	–F		



Name	X	R_6	R_1	R_7
Ciprofloxacin	–CH	–F		
Amifloxacin	–CH	–F	$-NHCH_3$	
Sparfloxacin	–CF	–F		
Lomefloxacin	–CF	–F	$-C_2H_5$	



Name	X	R_6	R_1	R_7
Fleroxacin	–CF	–F	$-CH_2CH_2F$	
Tefloxacin	–CH	–F		
Gatifloxacin	$-COCH_3$	–F		
Clinafloxacin	–CCl	–F