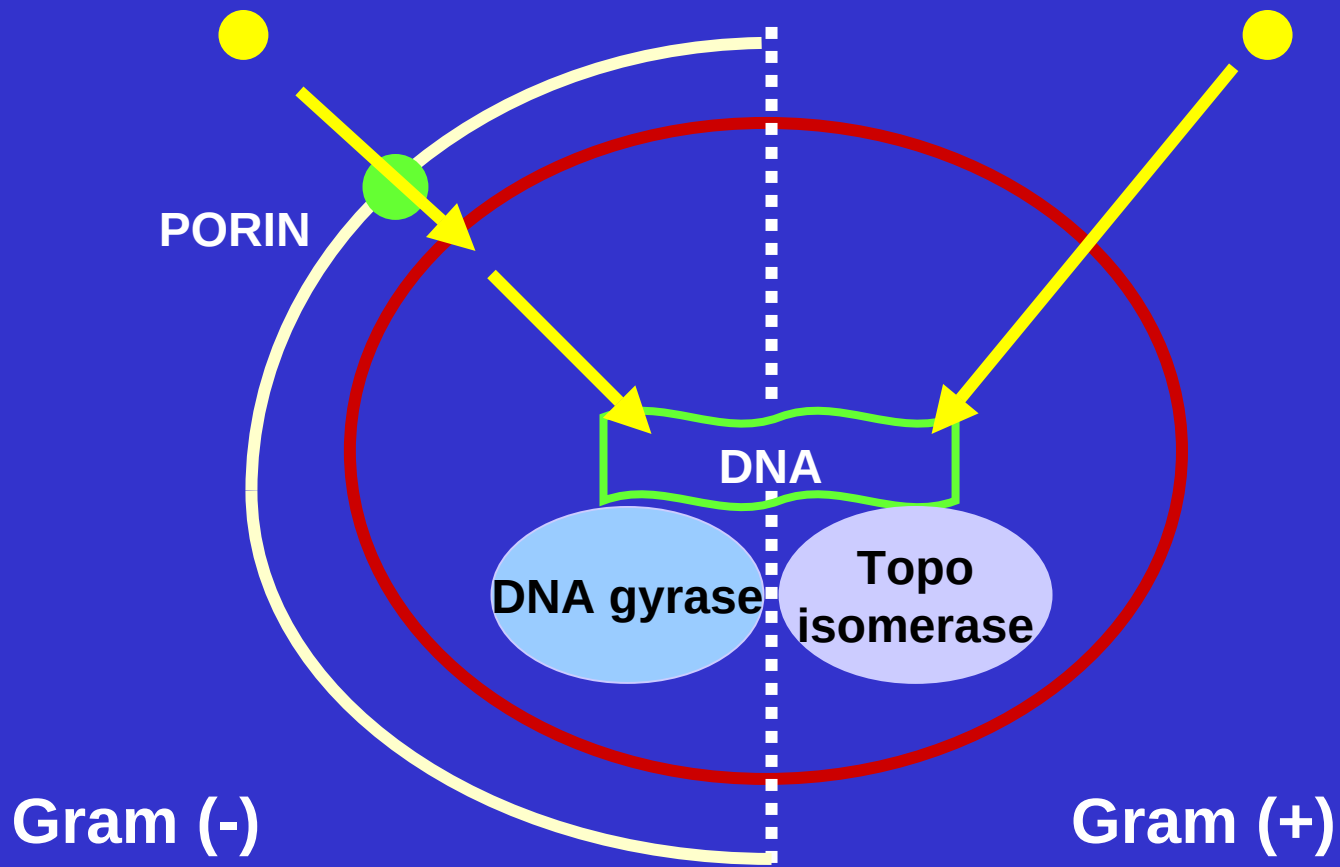
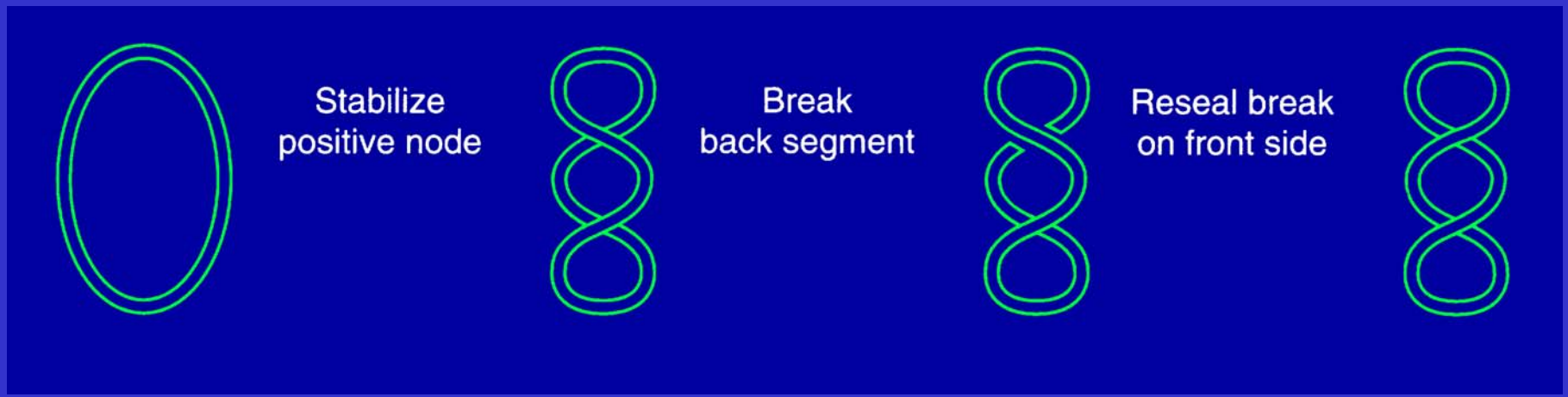
Mechanism of action of fluoroquinolones: the basics...



2 key enzymes in DNA replication:

DNA gyrase



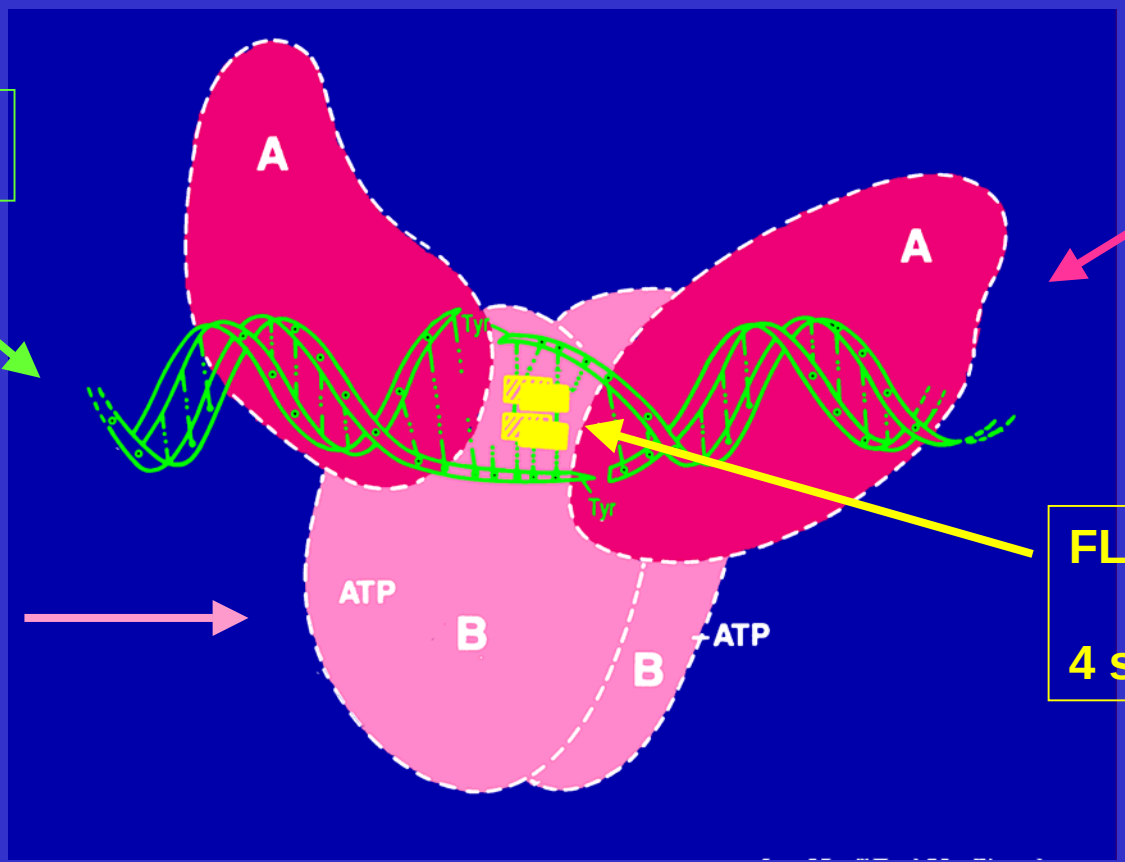
topoisomerase IV

bacterial DNA is supercoiled

Ternary complex
DNA - enzyme - fluoroquinolone

COVALENTLY CLOSED CIRCULAR DNA

DNA GYRASE catalytic subunits



DNA GYRASE ATP binding subunits

FLUOROQUINOLONES:

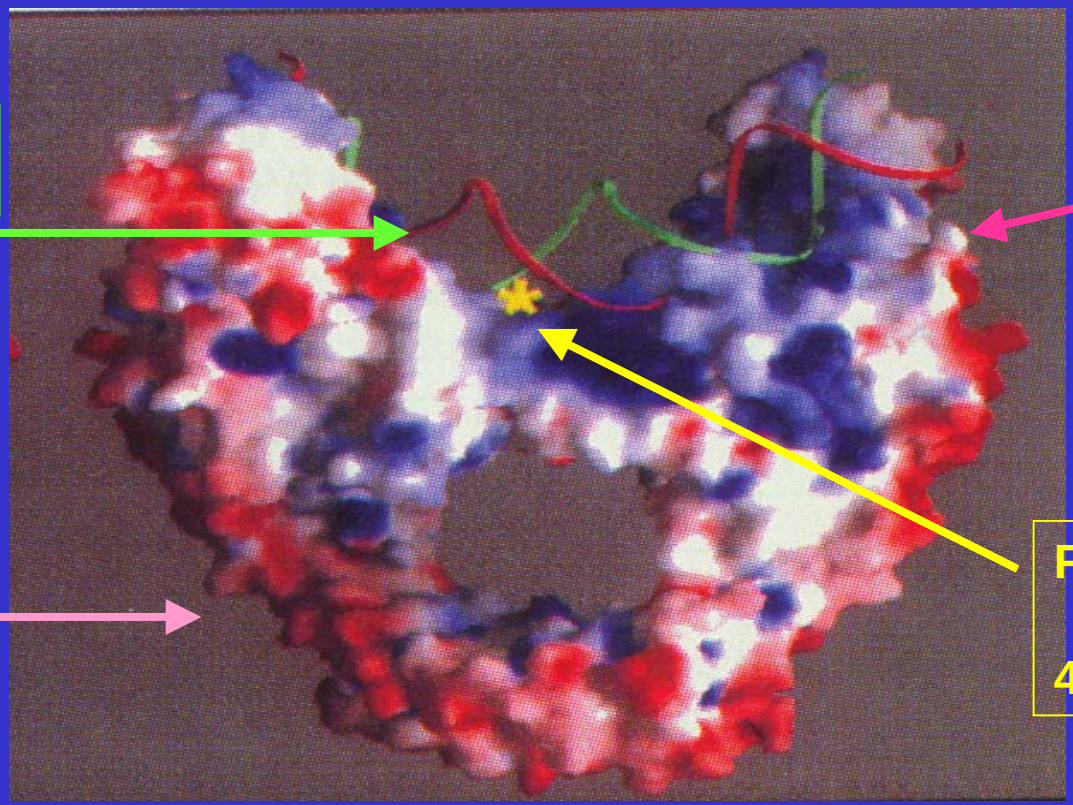
4 stacked molecules

(Shen, *in* Quinolone Antimicrobial Agents, 1993)

Ternary complex
DNA - enzyme - fluoroquinolone

COVALENTLY CLOSED
CIRCULAR DNA

DNA GYRASE
catalytic subunits



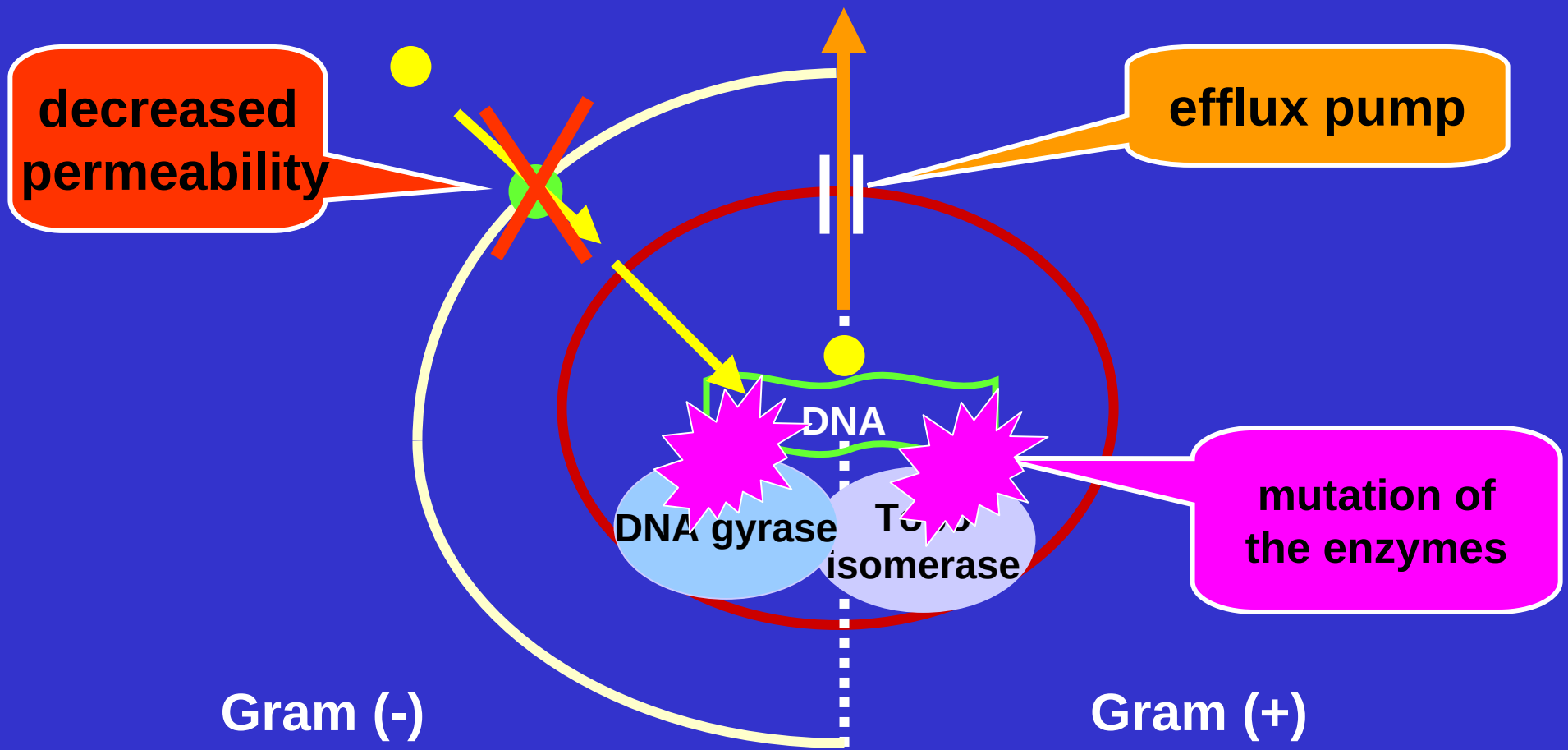
DNA GYRASE
ATP binding subunits

FLUOROQUINOLONES:

4 stacked molecules

Cabral *et al.*, Nature, 1997

Resistance to fluoroquinolones: the basics



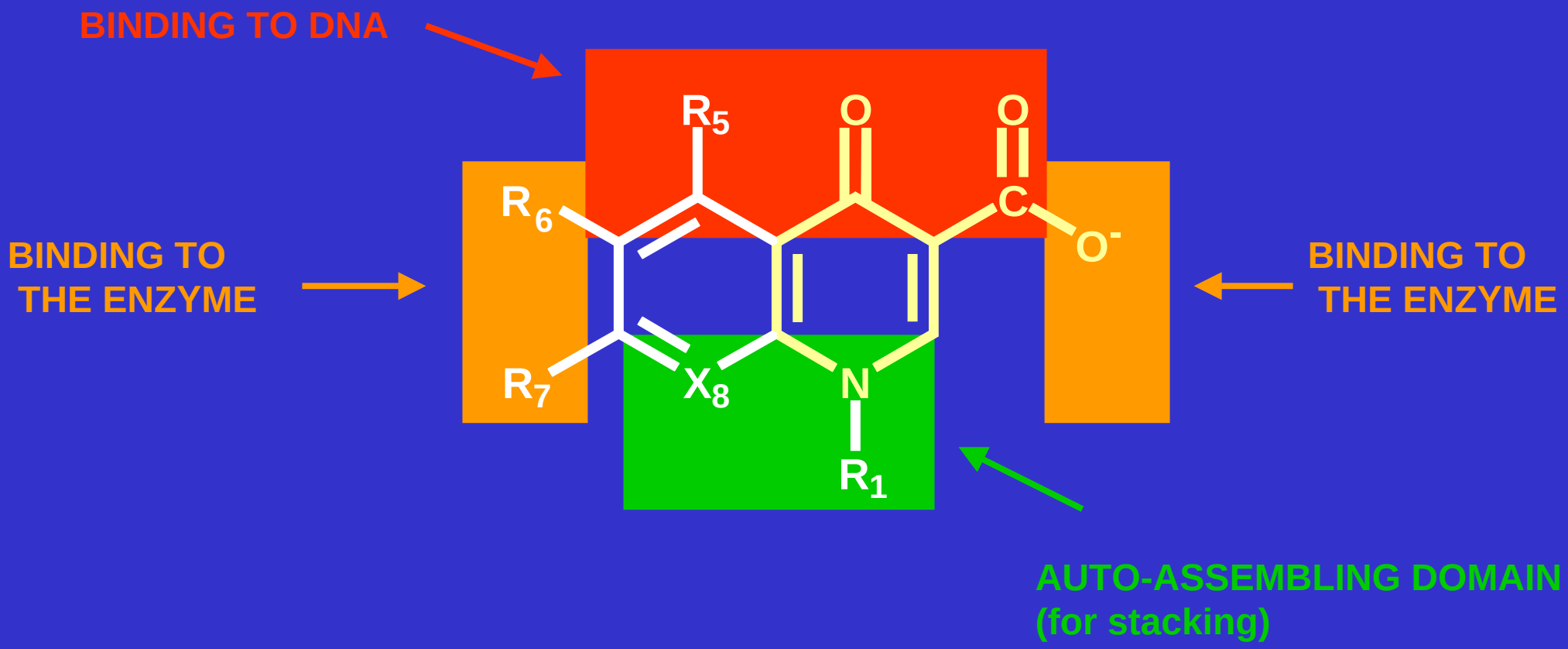
Fluoroquinolones are the first entirely man-made antibiotics: do we understand our molecule ?

Don’t panic, we will travel together….

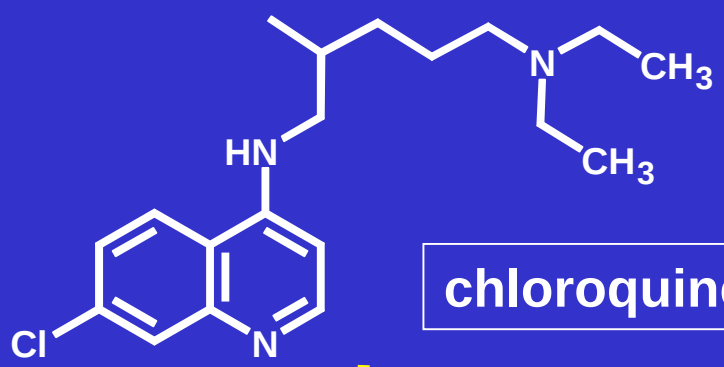
Chemistry and Activity

This is where all begins...

The pharmacophore common to all fluoroquinolones



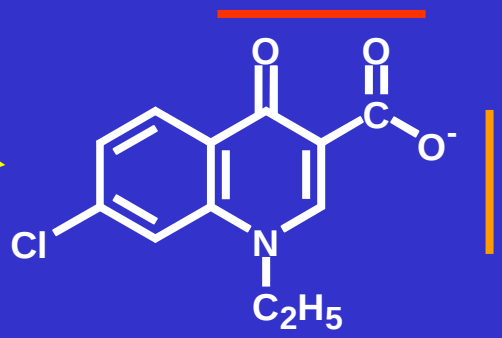
From chloroquine to nalidixic acid...



chloroquine

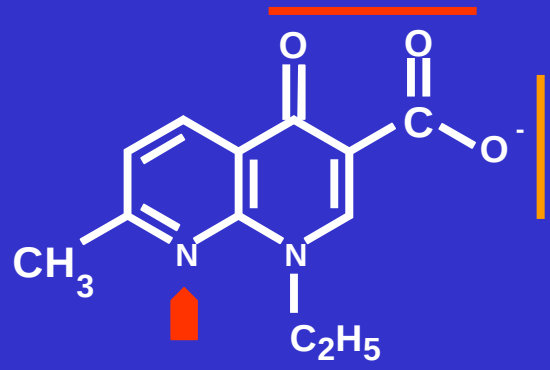
1939

1958



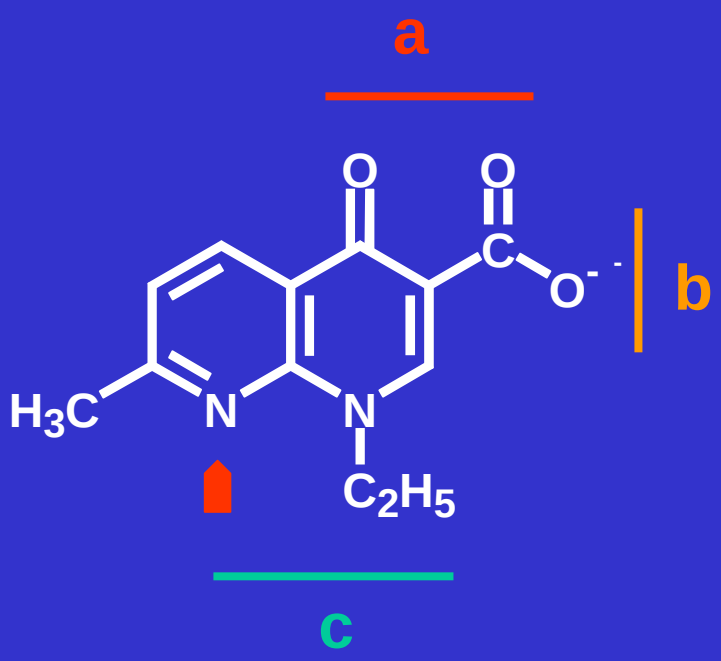
7-chloroquinoline
(synthesis intermediate found to display antibacterial activity)

nalidixic acid



1962

Nalidixic acid *



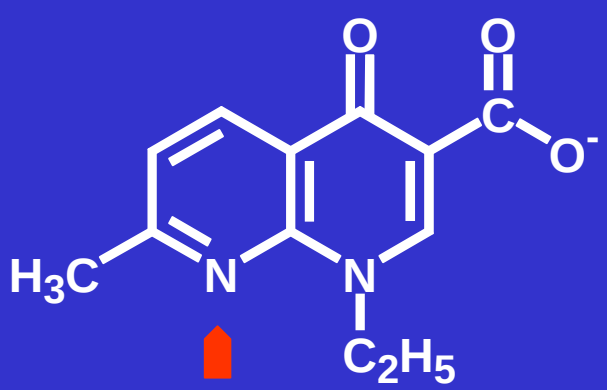
- typical chemical features of fluoroquinolones (a, b, c)
  BUT a naphthridone
  (N at position 8: ⬆)

- limited usefulness as drug
  - narrow antibacterial spectrum (*Enterobacteriaceae* only)
  - short half-life (1.5h)
  - high protein binding (90%)

* Belg. pat. 612,258 to Sterling Drugs, 1962

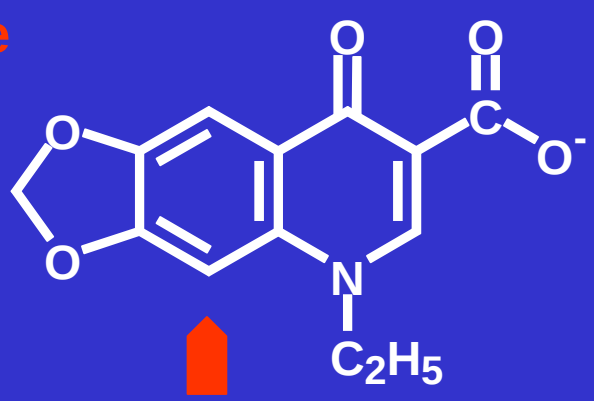
From nalidixic acid to the 1st fluoroquinolone (1 of 4)

nalidixic acid



1. modify naphthyridone into quinolone

oxolinic acid *



shows reduced protein binding...

* Ger. pat. to Warner Lambert, 1967

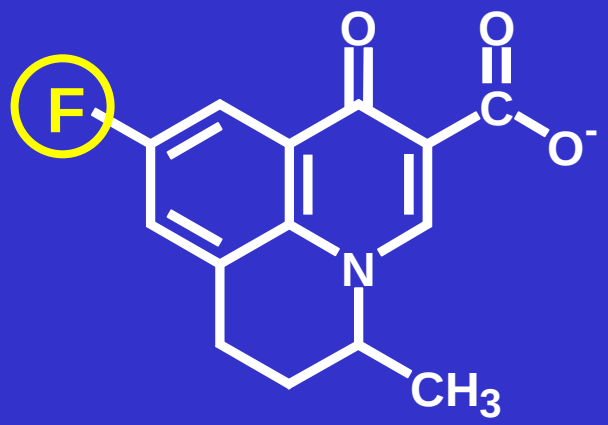
* quinoleine

From nalidixic acid to the 1st fluoroquinolone (1 of 4)

nalidixic acid

2. discovery of flumequine *

flumequine *



shows weak but broad Gram(-) activity

* Ger pat. to Rikker Labs, 1973

* benzo-quinolizine

From nalidixic acid to the 1st fluoroquinolone (1 of 4)

nalidixic acid

3. introduce a piperazine *

pipemidic acid *

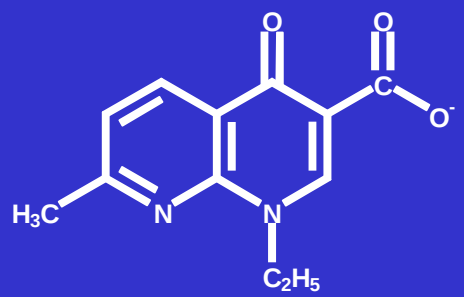
shows longer half-life...

* Ger. Pat. to Roger Bellon, 1974

* pyrido-2-3-pyrimidine

From nalidixic acid to the 1st fluoroquinolone (1 of 4)

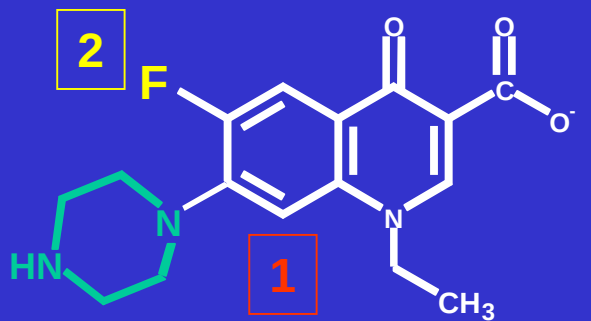
nalidixic acid



combine all 3 features *...

1978

norfloxacin *



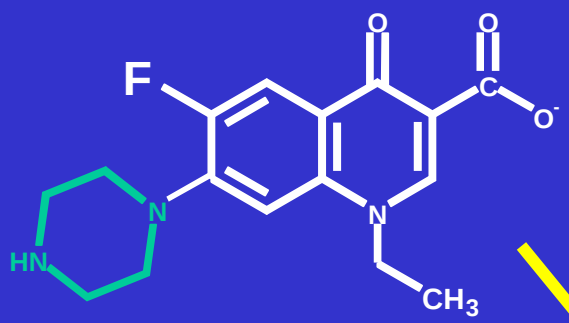
broader Gram(-) activity
less protein binding (50%)
longer half-life (3-4h)

* Belgian patent 863,429, 1978 to Kyorin

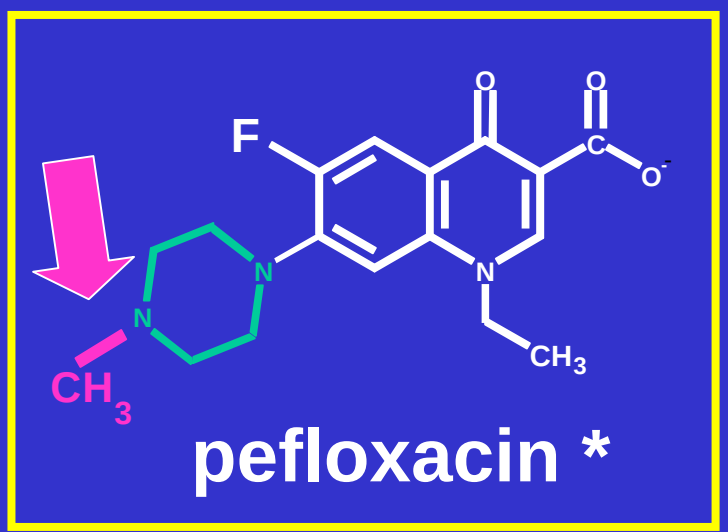
* 6-fluoro-7-pyrimidino-quinoleine

From norfloxacin to the other 1st generation fluoroquinolones: pefloxacin

norfloxacin



Add a methyl to still increase half-life

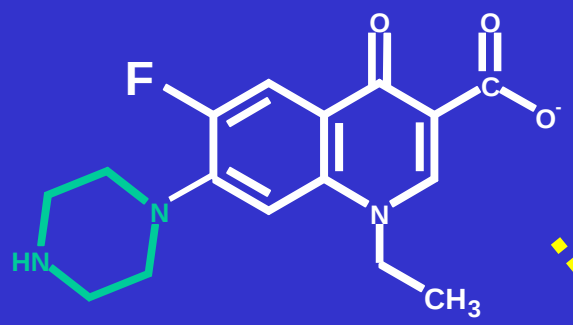


pefloxacin *

* Ger. pat. 2,840,910 to Roger Bellon/Dainippon,1979

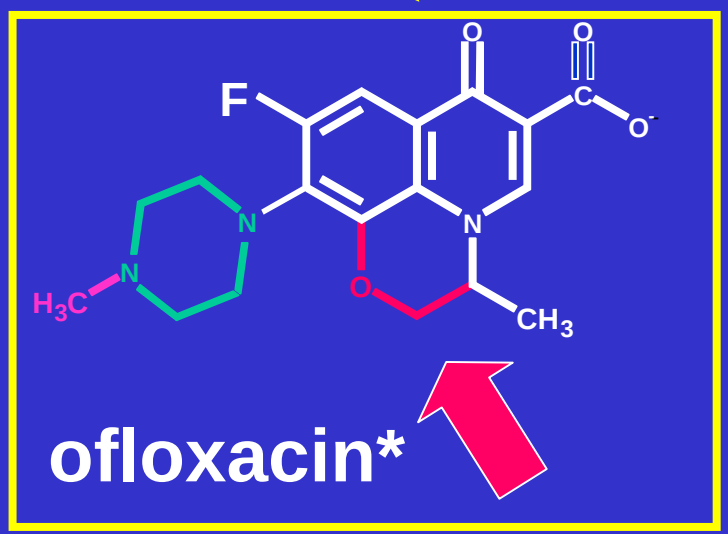
From norfloxacin to the other 1st generation fluoroquinolones: ofloxacin

norfloxacin



tricyclic compound
(as in flumequine but morpholine ring)

pefloxacin

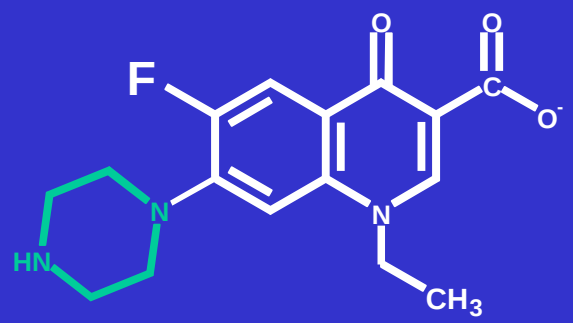


ofloxacin*

* Eur. pat. Appl. 47,005 to Daiichi, 1982

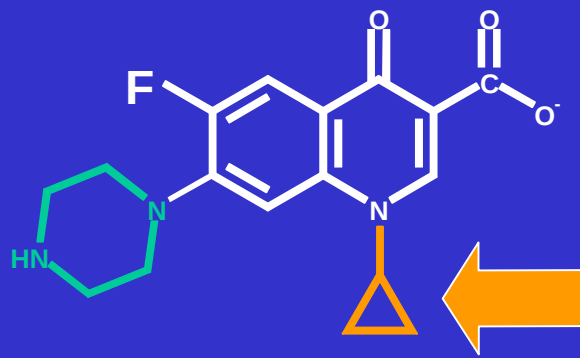
From norfloxacin to the other 1st generation fluoroquinolones: ciprofloxacin

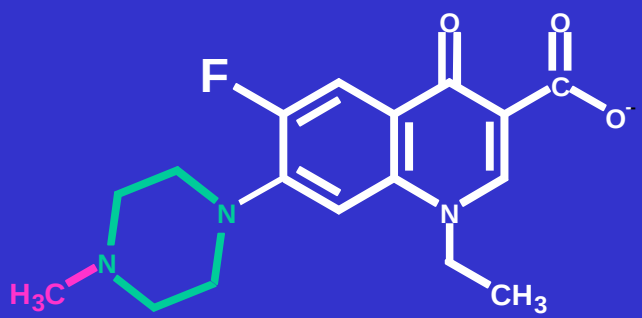
norfloxacin



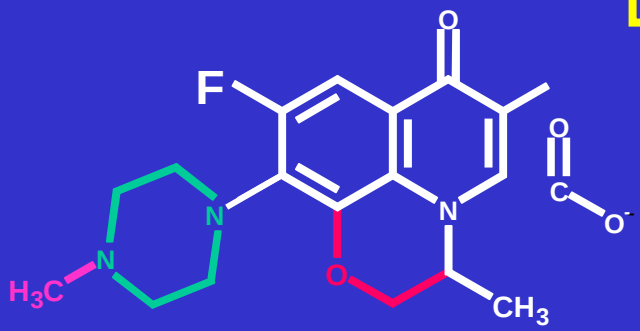
cyclopropyl to increase potency

ciprofloxacin *





pefloxacin

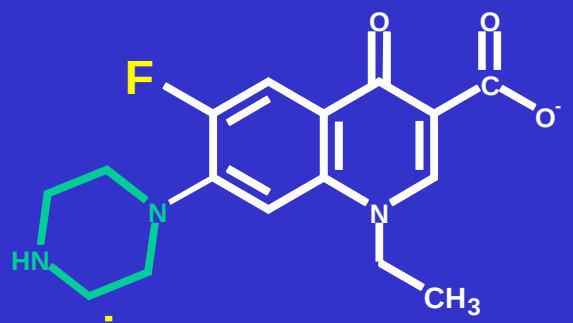


ofloxacin

* Ger. pat. 3,142,854 to Bayer AG, 1983

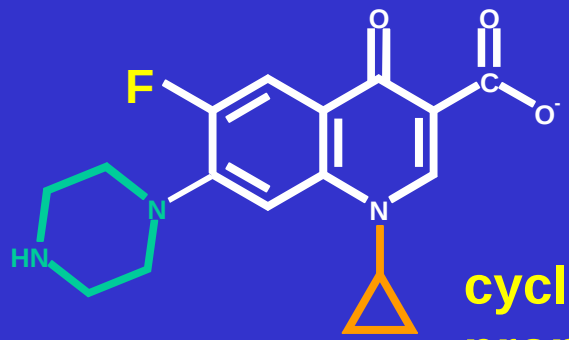
"1st generation" fluoroquinolones

norfloxacin



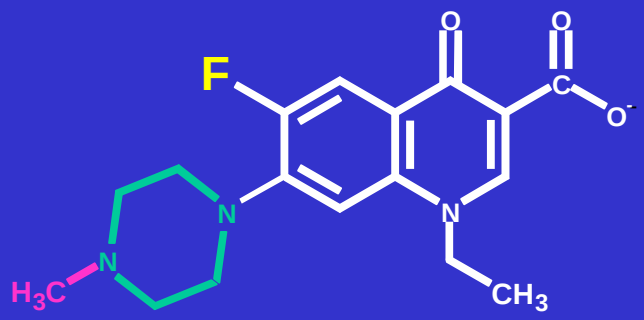
piperazine

ciprofloxacin

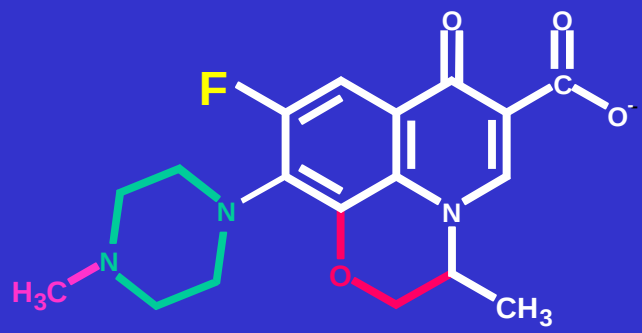


cyclo propyl

methyl



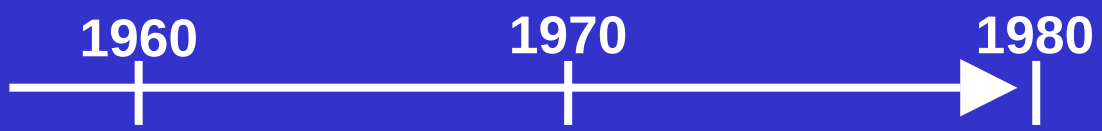
pefloxacin



ofloxacin

morpholine

The "first generation" of fluoroquinolones



- **Nalidixic acid**
- **Oxolinic acid**
- **Cinoxacin**
- **Pipemidic acid**

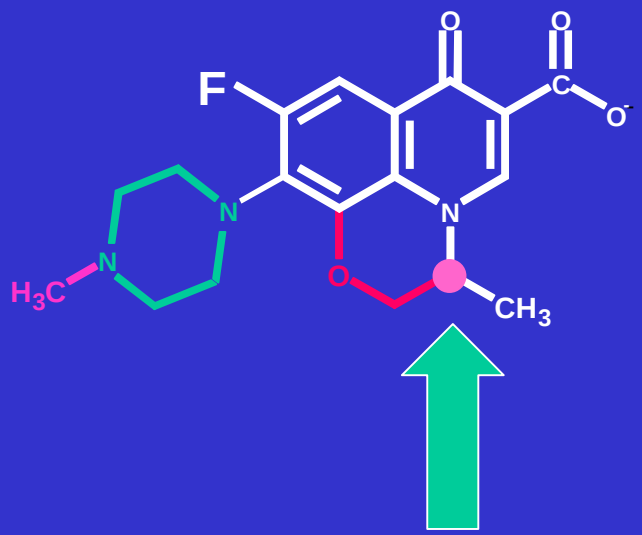
- **Norfloxacin**
- **Pefloxacin**
- **Ofloxacin**
- **Ciprofloxacin**
- **Fleroxacin**
- **Rufloxacin**

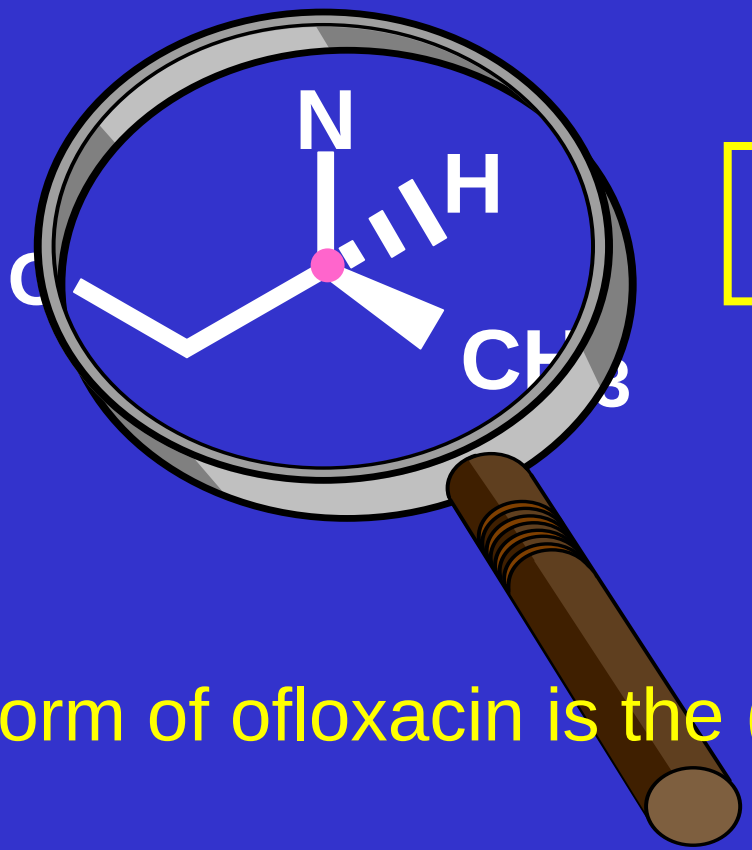
improved anti Gram (-) activity

$t_{1/2}$	activity
3-4 h	++
11 h	+
6 h	++
3-4 h	+++

From ofloxacin to levofloxacin...

Ofloxacin is a racemic mixture



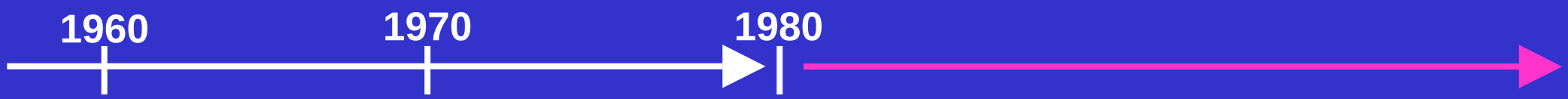


Levofloxacin is the pure (-) S isomer *

The active form of ofloxacin is the (-) S isomer

* Eur. pat. 206,283 to Daiichi, 1987

The present "first generation" of fluoroquinolones ...



- **Nalidixic acid**
- **Oxolinic acid**
- **Flumequine**
- **Pipemidic acid**

- **Norfloxacin**
- **Pefloxacin**
- **Ofloxacin**
- **Ciprofloxacin**
- **Fleroxacin**
- **Rufloxacin**

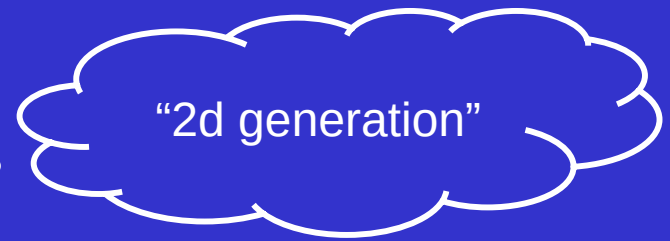
improved anti Gram (-) activity

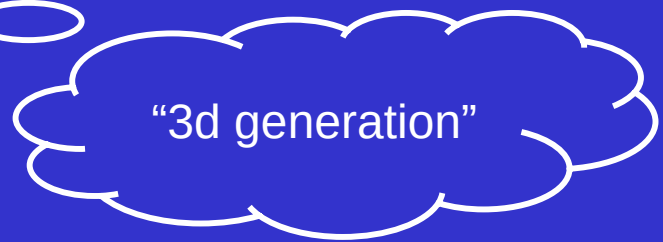
	$t_{1/2}$	activity
Norfloxacin	3-4 h	++
Pefloxacin	11 h	+
Ofloxacin	6 h	++
Ciprofloxacin	3-4 h	+++
Levofloxacin	6 h	++++

twice as active as ofloxacin **per g**

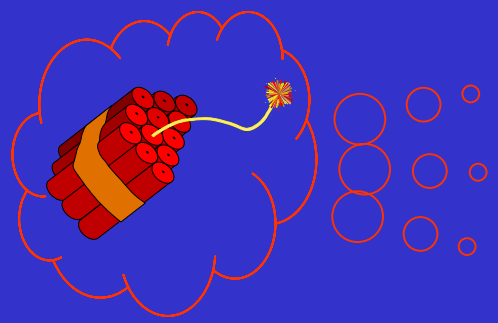
How to improve the chemotherapeutic usefulness of the "first generation" fluoroquinolones

1. Maintain broad Gram(-) activity
2. Improve Gram(+) activity
3. Acquire activity against anaerobes





The “second generation” fluoroquinolones



- Temafloxacin [a]
- Sparfloxacin [b]
- Grepafloxacin [c]
- Gatifloxacin [d]

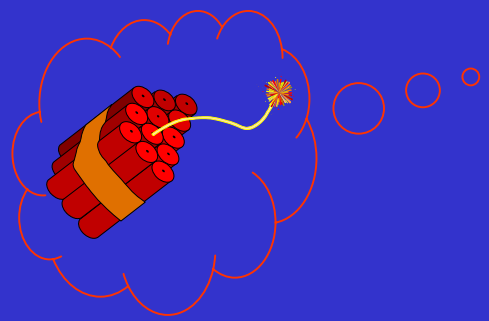
- Gram (-);
- improved Gram (+)

→ anti-anaerobe

a: Toyama, 1988 (?) ; b: Dainippon, 1985-1987; c: Otskuda, 1989; d: Kyorin, 1988

The “third generation” fluoroquinolones

1960 — 1970 — 1980 — 1990 — 2000



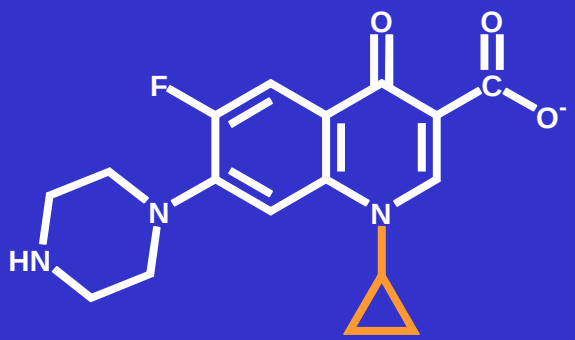
- Clinafloxacin [a]
- Trovafloxacin [b]
- Moxifloxacin [c]
- Gemifloxacin [d]

anti-Gram (-)
anti-Gram (+)
anti-anaerobe

a:Kyorin, 1987; b: Pfizer, 1993; c: Bayer, 1994; d: LG Chemical Ltd., S. Korea, 1994-98

Activity against *S. pneumoniae*

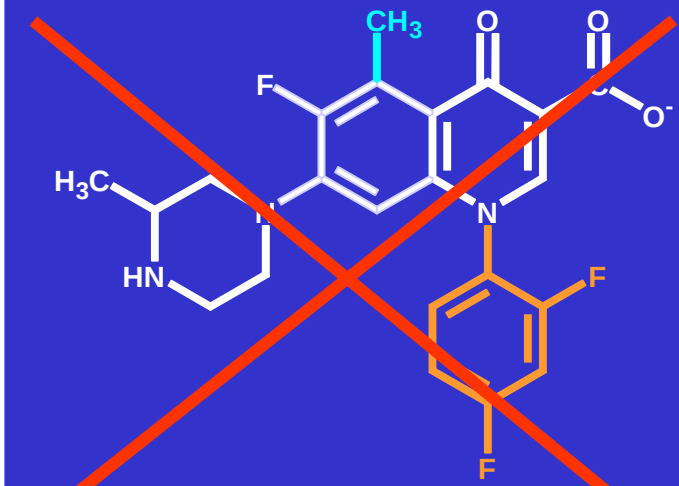
I



ciprofloxacin
0.5 - 2

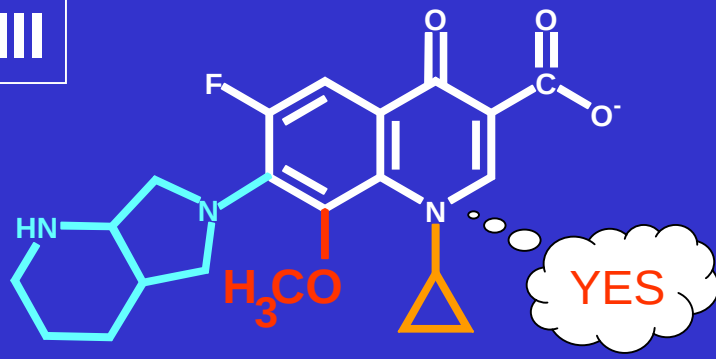
II

sparfloxacin
0.125 - 0.5



temafloxacin
0.5 - 1

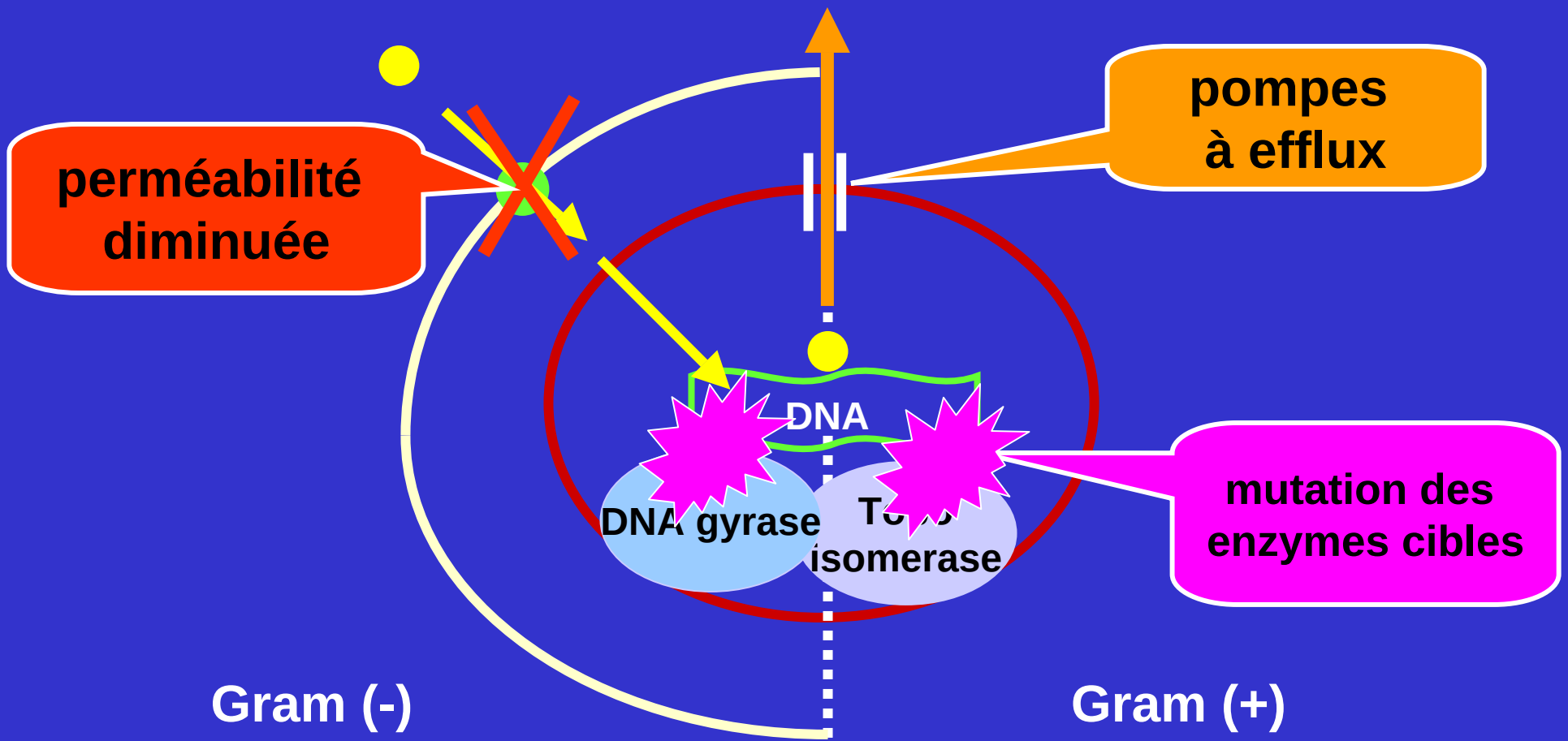
III



moxifloxacin
0.01 - 0.5

trovafloxacin
0.007 - 0.25

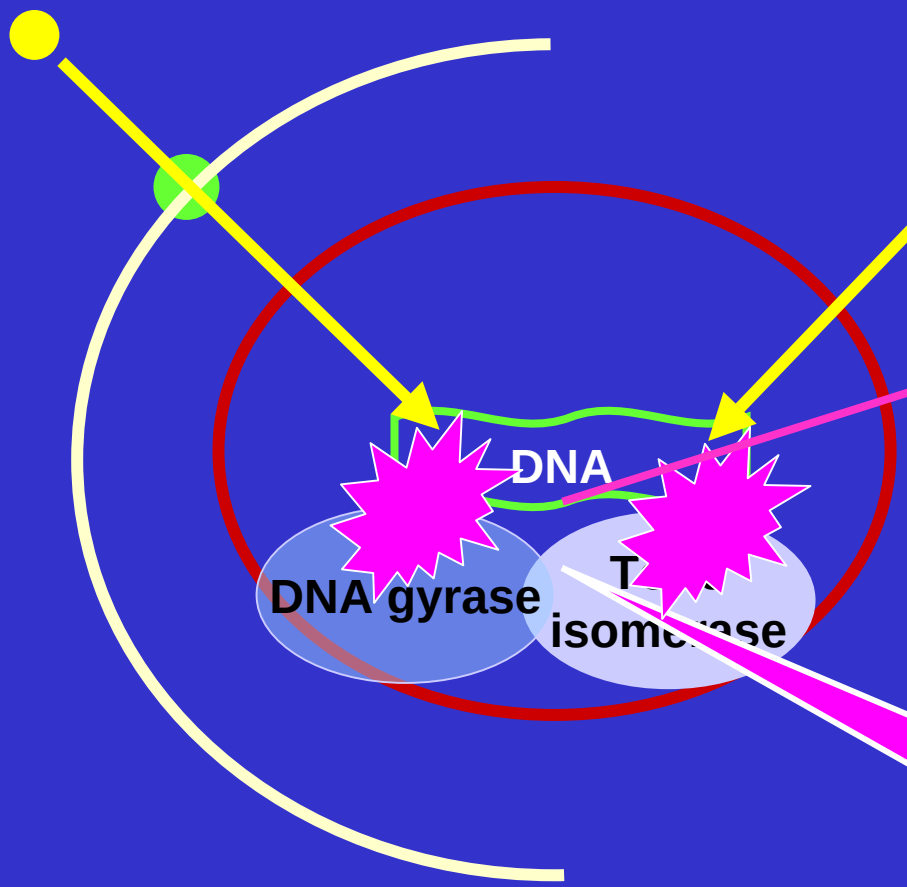
Resistance au fluoroquinolones : les mécanismes de base ...



Resistance au fluoroquinolones : rôle des mutations au niveau de la cible

Gram (-)

Gram (+)



Élévation des CMI de 3 à 5 dilutions (≈ 20 X) par mutation

mutation des enzymes cibles

Is there a SAR for emergence of resistance ?

The *"Mutant Prevention Concentration" **

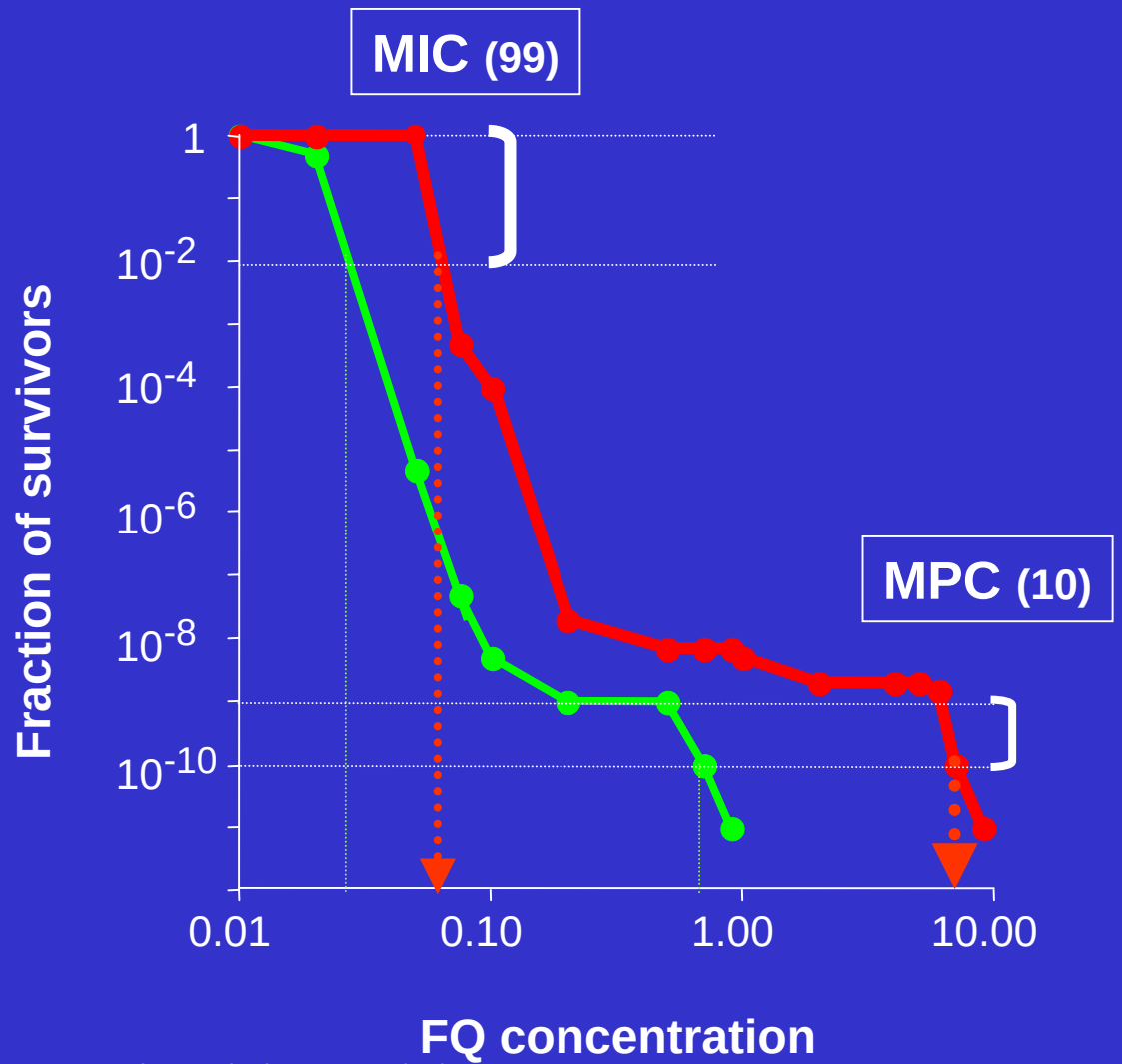
"When Mycobacterium bovis BCG and Staphylococcus aureus were plated on agar containing increasing concentrations of fluoroquinolone, colony numbers exhibited a sharp drop, followed by a plateau and a second sharp drop.

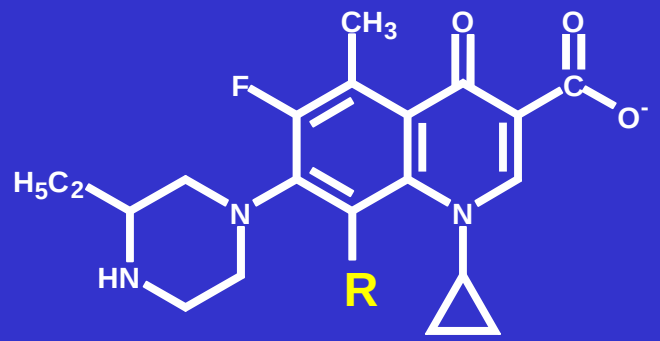
The plateau region correlated,vith the presence of first-step resistant mutants. Mutants were not recovered at concentrations above those required for the second sharp drop, thereby defining a **mutant prevention concentration (MPC)**.

A **C8-methoxy group** lowered the MPC for an N-1-cyclopropyl fluoroquinolone"

Is there a SAR for emergence of resistance ?

Bactericidal activity of FQs against *Mycobaterium bovis*



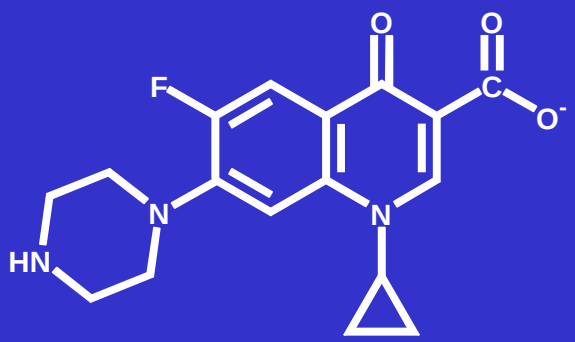


	PD160793	PD161148
	R = OCH_3	R = H
MIC 99	0.25	0.8
MPC 10	0.9	9
MPC/MIC	3.6	12

Dong *et al*; AAC 43:1756-1758

Fluoroquinolones with a C8-methoxy

I



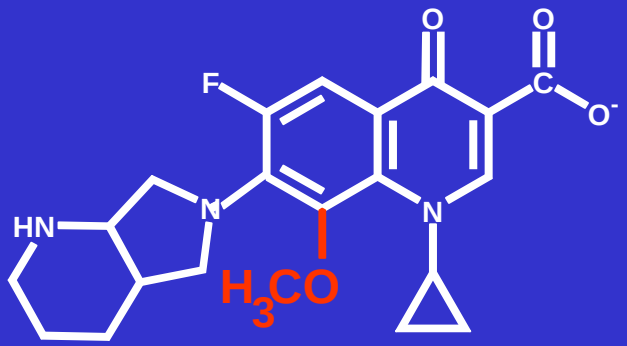
ciprofloxacin

II

gatifloxacin

Not in Belgium

III



moxifloxacin

Yes

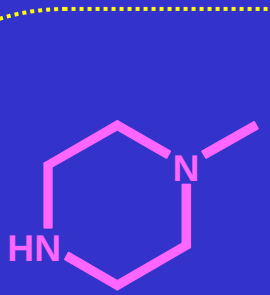
Toxicity

This is where all may fail...

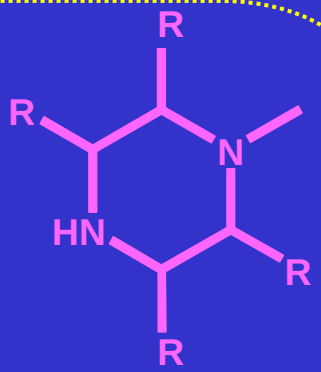
Frequent side effects of fluoroquinolones: is there a SAR ?

- COMPLEXATION WITH METALLIC IONS (Fe, Al, Mg, Ca)
- PHOTOTOXICITY
- DRUG INTERACTIONS: INHIBITION OF cyt P450 (1A2)
- CNS TOXICITY (BINDING TO GABA RECEPTOR)
- GASTRO-INTESTINAL DISCOMFORT
- CARTILAGE and MUSCULOSQUELETAL TOXICITY

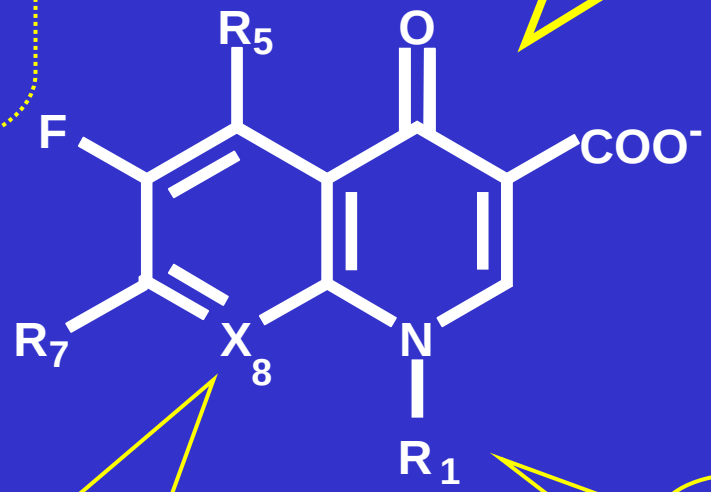
SAR of frequent side effects



Binding to GABA receptor

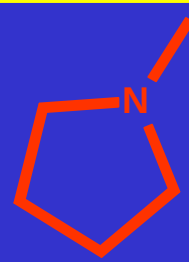


Penetration in CNS



Ca^{++}, Al^{+++}, Fe^{++} complexation

All FQs



Inhibition of P450

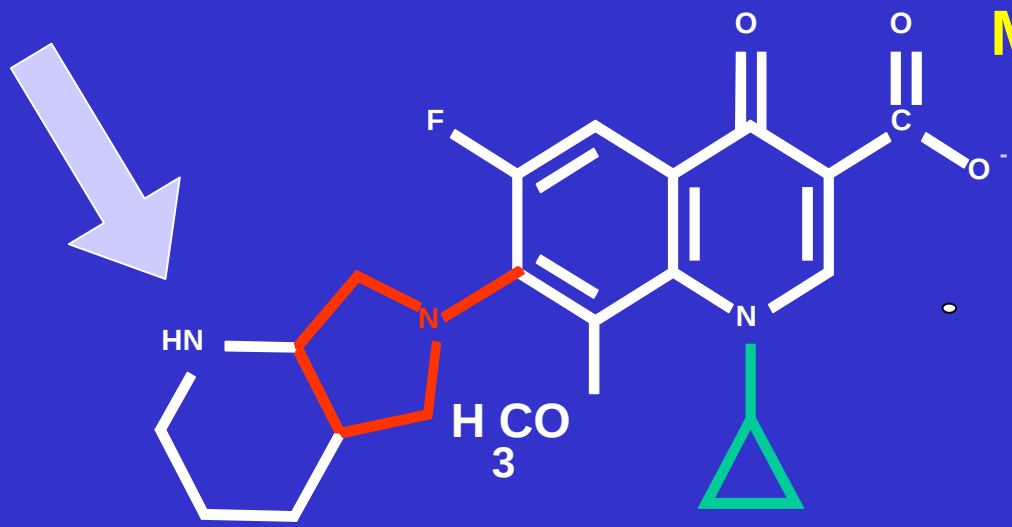
cipro, grepa ...

Inhibition of P450

sparflo, flero, lomeflo

Phototoxicity

Fluoroquinolone with low or no drug interactions..



Moxifloxacin

Yes

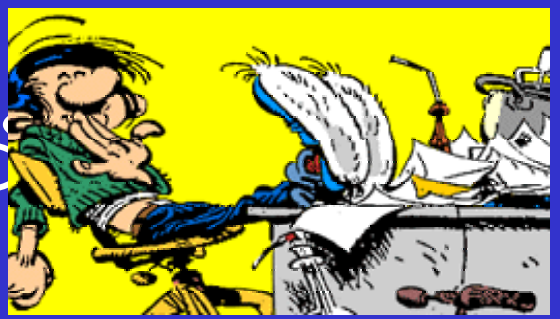
Rare side effects of fluoroquinolones:

RENAL TOXICITY
crystalluria, hematuria, interstitial nephritis, acute renal failure

CARDIAC TOXICITY (QT prolongation, *Torsades de pointe*)

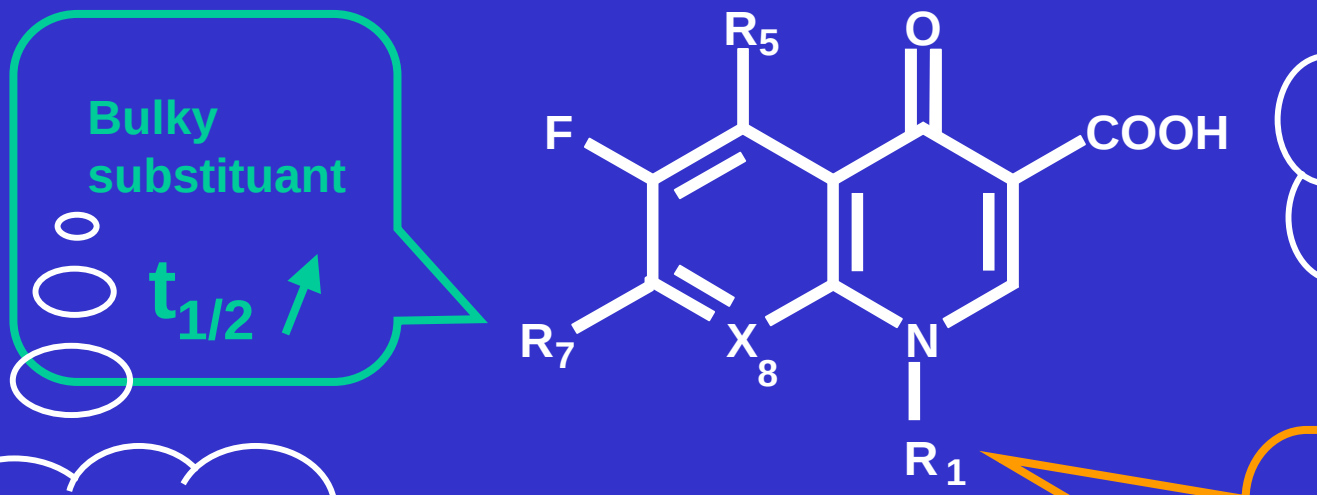
HEPATOTOXICITY
temafloxacin syndrome / trovafloxacin syndrome

Pharmacokinetics



This is where people start sleeping..

SAR of pharmacokinetic parameters



Bulky substituant

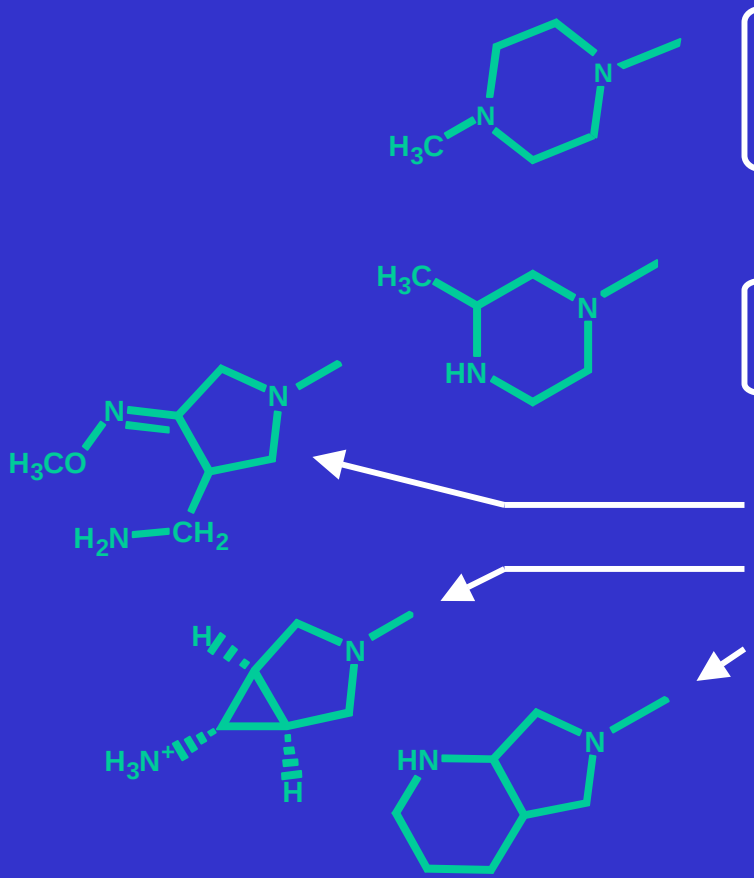
$t_{1/2}$ ↗

cipro
gati
moxi

peflo, oflo,
gati,
moxi

V_d ↗

SAR of main pharmacokinetic parameters:
how to get a long half life



	$t_{1/2}$ (h)	no. of daily administrations
oflo / lévo	5 - 7	2 x*
peflo	10	2 x*
flero	9 - 13	1 x
grepa	10 - 12	1 x
gati	13	1 x
gemi	8	1 x
trova	10	1 x
moxi	12	1 x
other FQ	3 - 6	2 x

* higher MIC...

Resistance: do not forget the correct dosing...

“Inadequate dosing of antibiotics is probably an important reason for misuse and subsequent risk of resistance. A recommendation on proper dosing regimens for different infections would be an important part of a comprehensive strategy. The possibility to produce such a dose recommendation based on pharmacokinetic and pharmacodynamic considerations will be further investigated in one of the CPMP working parties…”

European Agency of the Evaluation of Medicinal Products (London)

EMEA discussion paper
on Antimicrobial resistance
3 January 1999 EMEA/9880/99

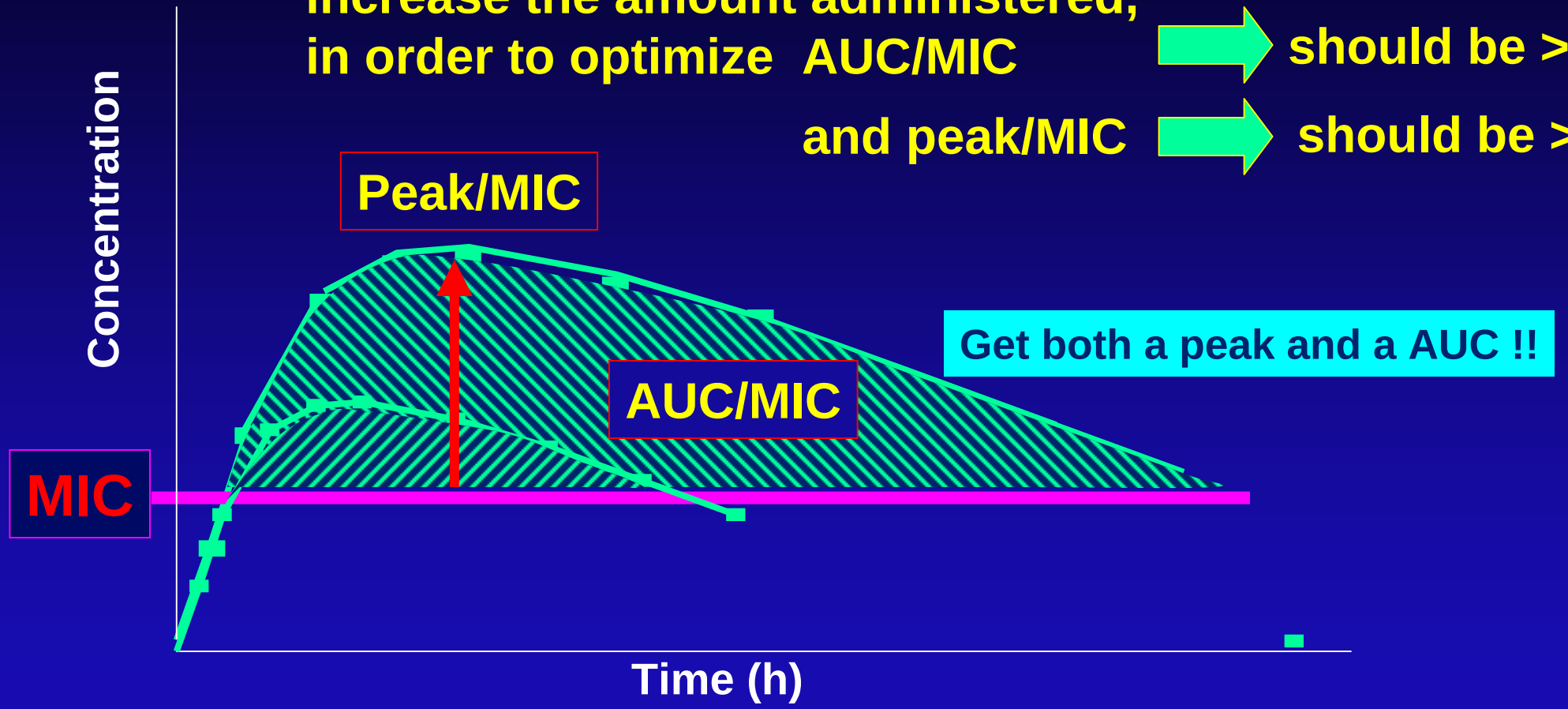
Pharmacokinetic parameters in relation with efficacy

	Dose (mg)	Cmax (mg/l)	MIC for pk/MIC=10	AUC (mg.h/l)	MIC for AUIC=125
norflo	400 (X2)	1.6	0.2	14	0.1
peflo	400 (X2)	4.6	0.4	108	1.0
cipro	500 (X2)	1.5	0.2	17	0.1
oflo	200 (X2)	3.1	0.4	66	0.4
levoflo	500	5.0	0.5	47	0.4
moxi	400	4.5	0.4	48	0.4

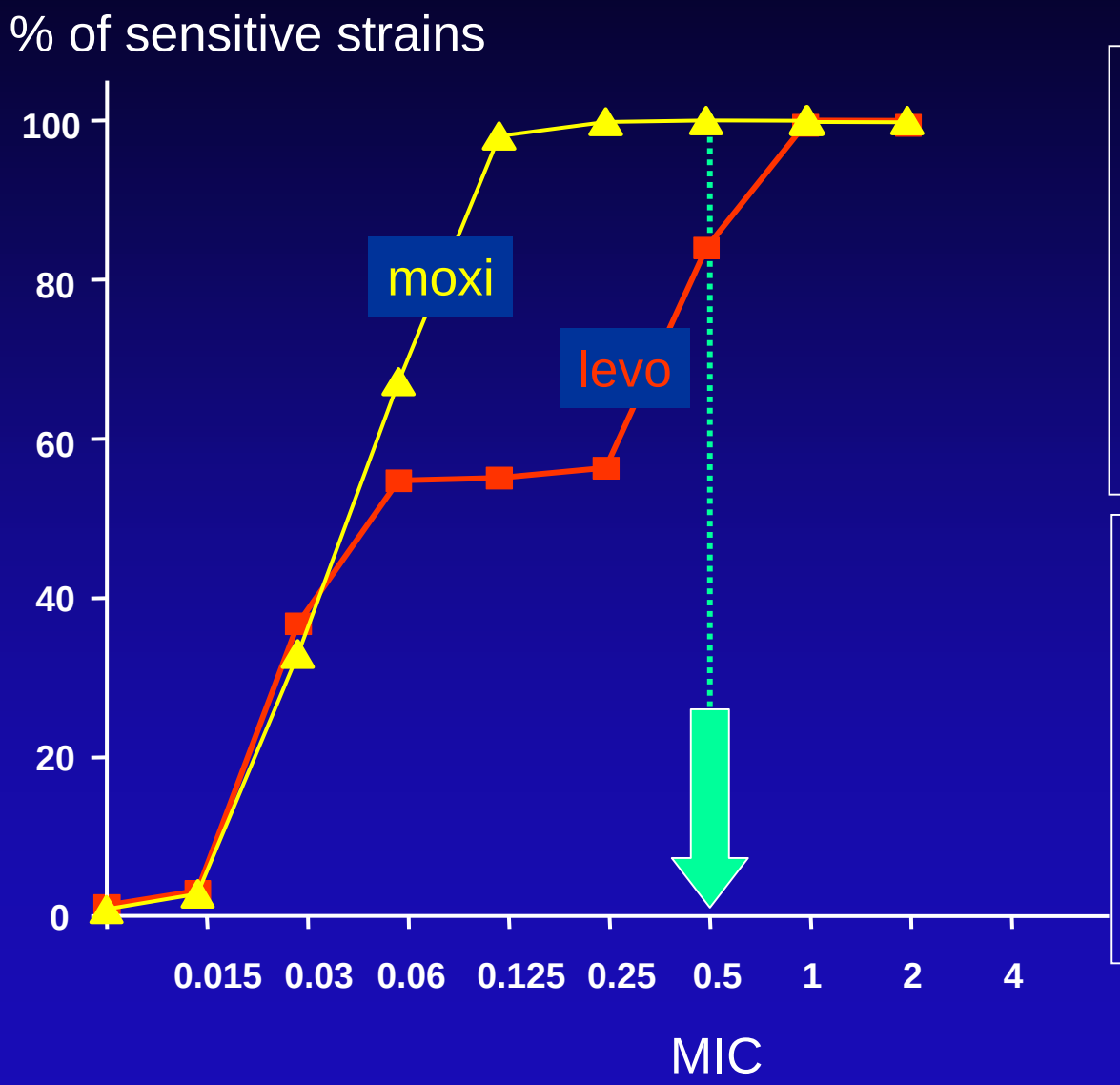
Optimizing dosage for fluoroquinolones

increase the amount administered, in order to optimize AUC/MIC ➡ **should be > 125**

and peak/MIC ➡ **should be > 10**



How to apply this ?



Levofloxacin 500 mg	1X /day	2X /day
AUC [(mg/l)xh]	47	94
• peak [mg/l]	5	5
✉ MIC_{max}	< 0.5	< 1

Moxifloxacin 400 mg	1X/day
• AUC [(mg/l)xh]	48
• peak [mg/l]	4.5
✉ MIC_{max}	< 0.5

MIC data: J. Verhaegen et al., 2001

Take home" message

- Dosage is key to success
- Dosage should match bacterial sensitivity
- peak, AUC/MIC are keys to success
- use a single, appropriate dose for long-life fluoroquinolones (moxifloxacin), or
- repeat the dose for short-lived fluoroquinolones (all others so far…)
- for fluoroquinolones, the limit is an **MIC of 0.5 µg/ml**