Antibacterial Agents

Structure Activity Relationships

André Bryskier MD

Antibacterial Agents

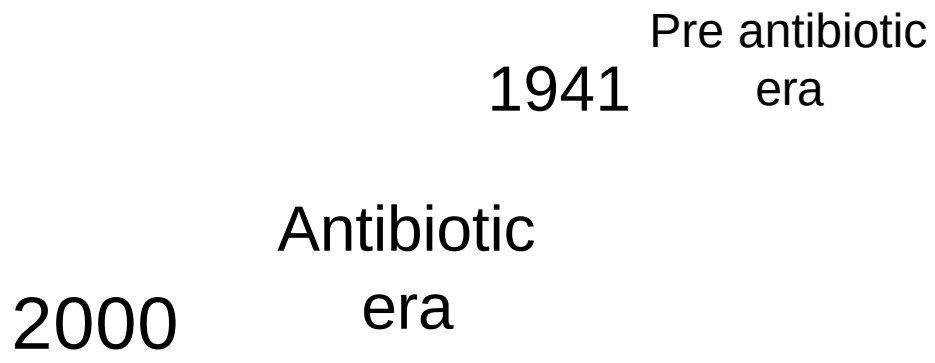
Natural compounds

- Beta-lactams
- Aminoglycosides
- Macrolides
- Streptogramin
- Lincosamines
- Peptides
- Mupirocin
- Ansamycins

Synthetic compounds

- Benzyl pyrimidines
- Sulphonamides
- Sulfones
- Furans
- 4-quinolones
- Oxazolidinones
- Nitroxoline
- Penem
- Fosfomycin
- Anti-TB

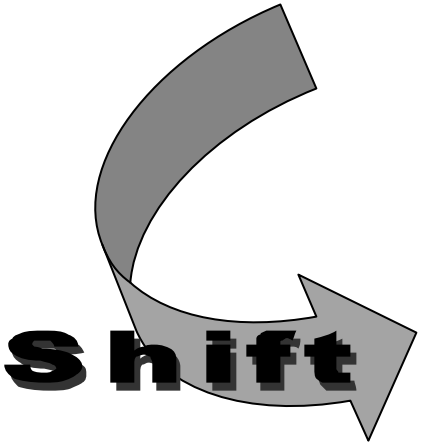
Antibiotic resistance



Research in anti-infectives

Research up mid 80s

- Enlarge the antibacterial spectrum
- Enhance the antibacterial activity (e.g. cefotaxime)
- Improve the pharmacokinetics
  (e.g. roxithromycin, clarithromycin, azithromycin)



Research after mid 80s

- Overcome bacterial resistance

Purification ⇒ e.g Penicillin G
Erythromycin A
Kanamycin

Semi synthetic

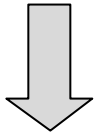
ß-lactams

Macrolides

Aminoglycosides

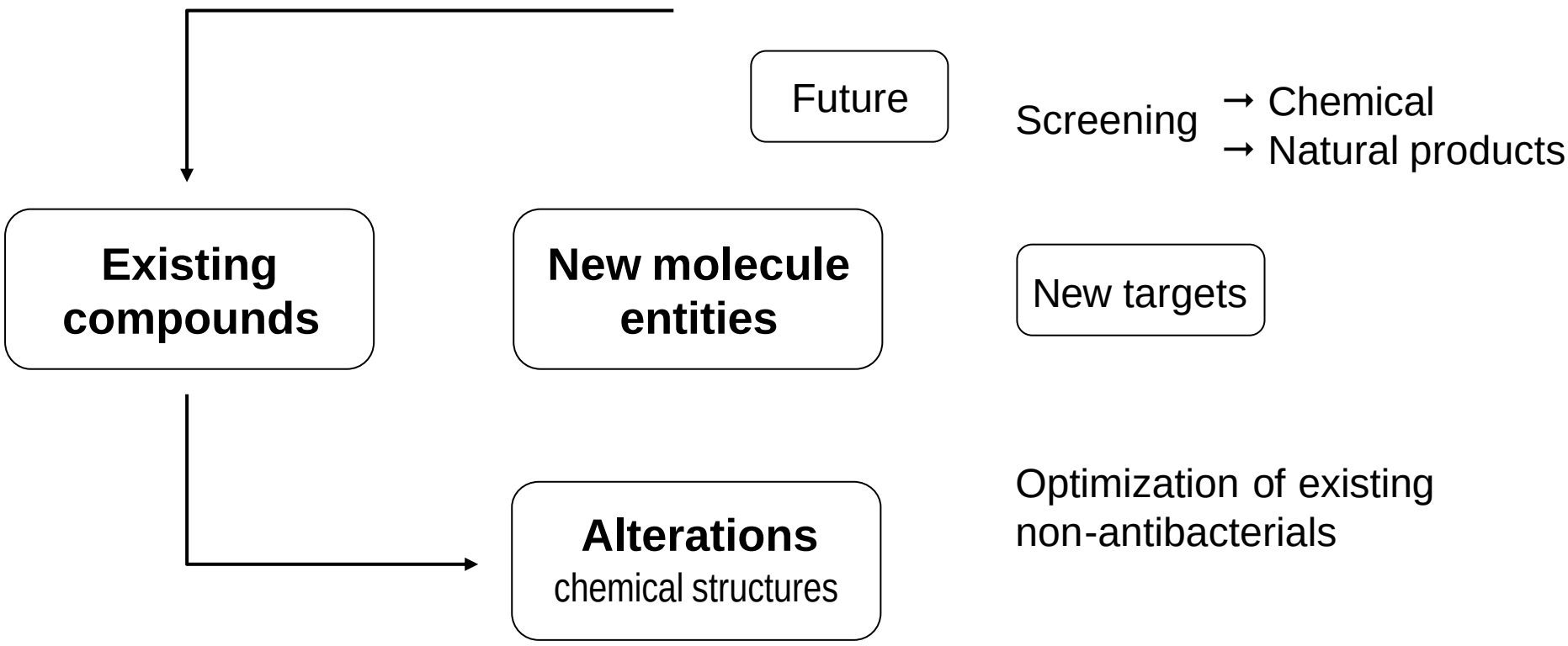
Structure-Activity-Relationships

WHY



- Antibacterial activity *in vitro* and *in vivo* (?)
- Bacterial resistance
- Bactericidal activity
- Toxicity-tolerance
- Optimisation of chemical strcutures
- Improvement of physicochemical properties
- Pharmacodynamics
- Pharmacokinetics.

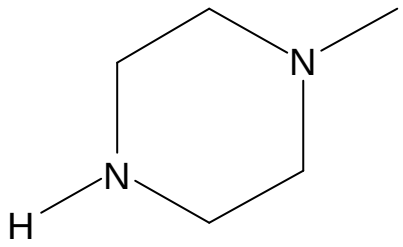
Antibacterial agents



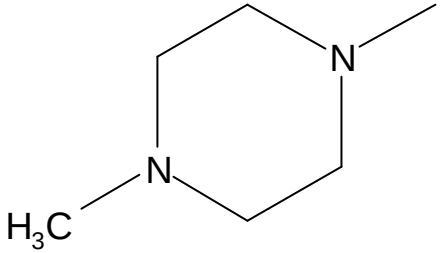
Improvement of physicochemical properties

Exemple : water solubility

(1)



Norfloxacin

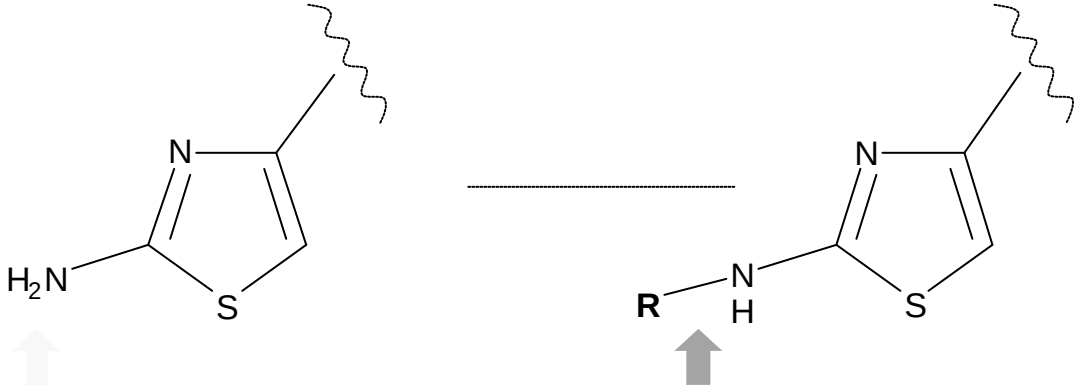


Pefloxacin (IV formulation)

Improvement of physicochemical properties

(2) Trovafloxacin —(Amino acid)→ Alatrovafloxacin (IV formulation)

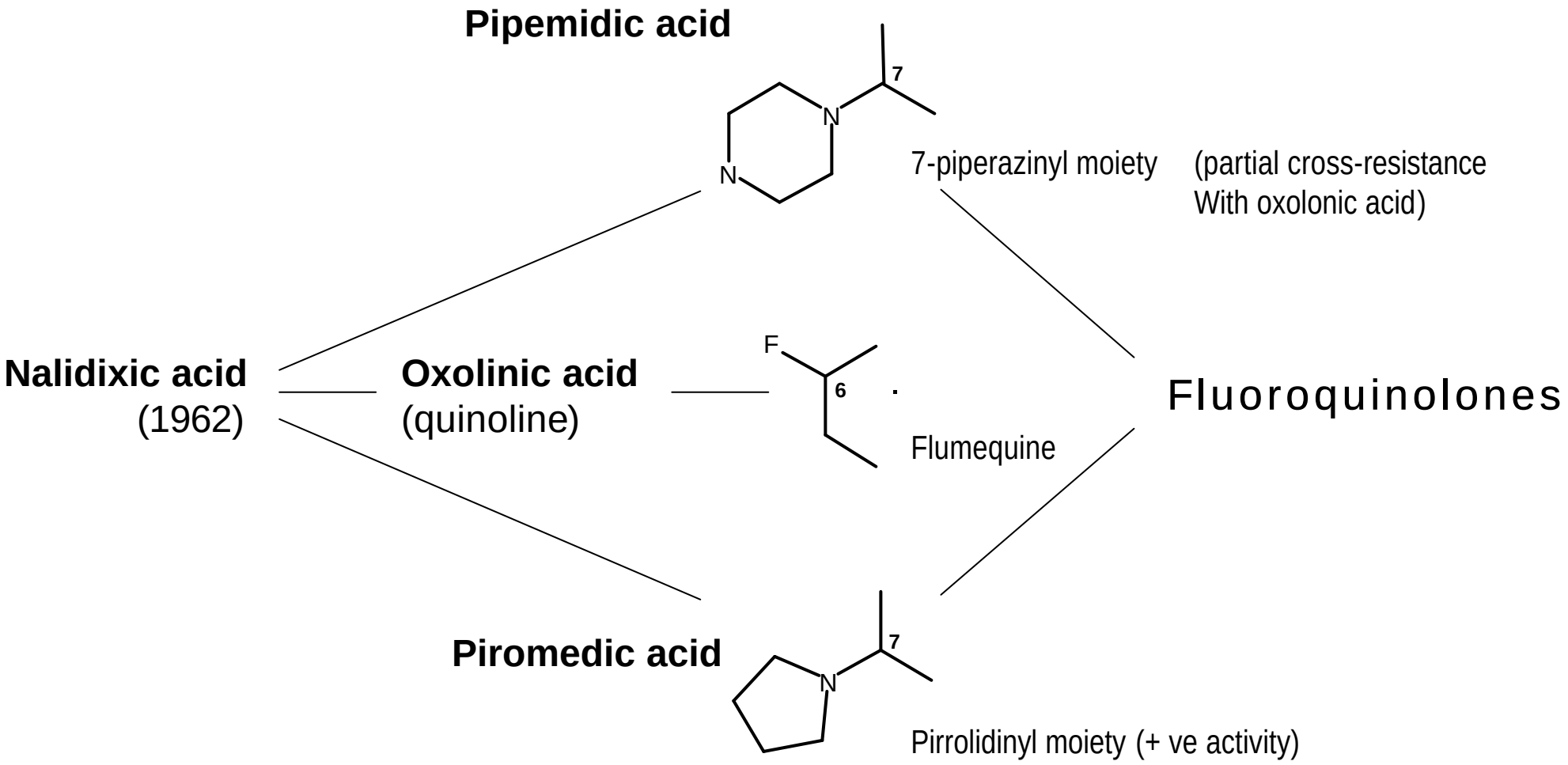
(3) Ceftizoxime —(Amino acid)→ Prodrug



Fluoroquinolones

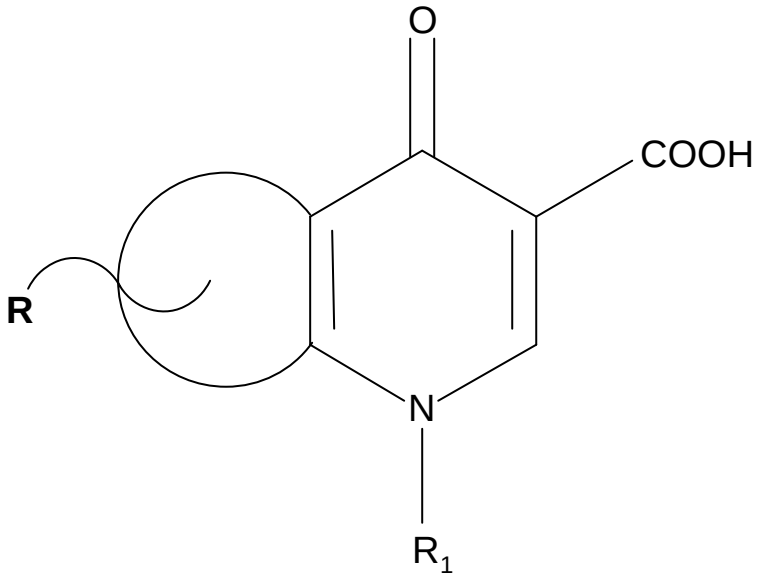
Fluoroquinolones

History of quinolones



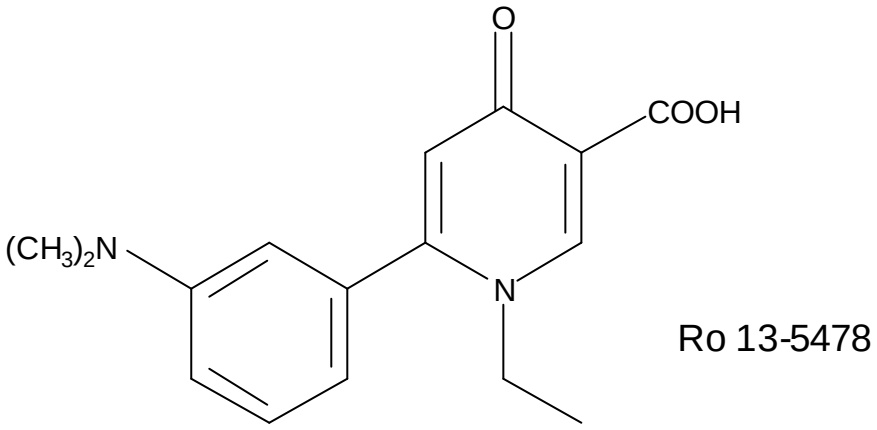
Fluoroquinolones

Definition



- Synthetic antibacterial agents
- Pharmacophore :
  pyrridone-ß-carboxylic acid
- Auxopharmacophore :
  fused aromatic ring
  appended substituents

Fluoroquinolones



Ro 13-5478

- Monocyclic derivative

Fluoroquinolones

Structure - activity relationships

- Classification
- Microbiology
- Pharmacokinetics
- Adverse events

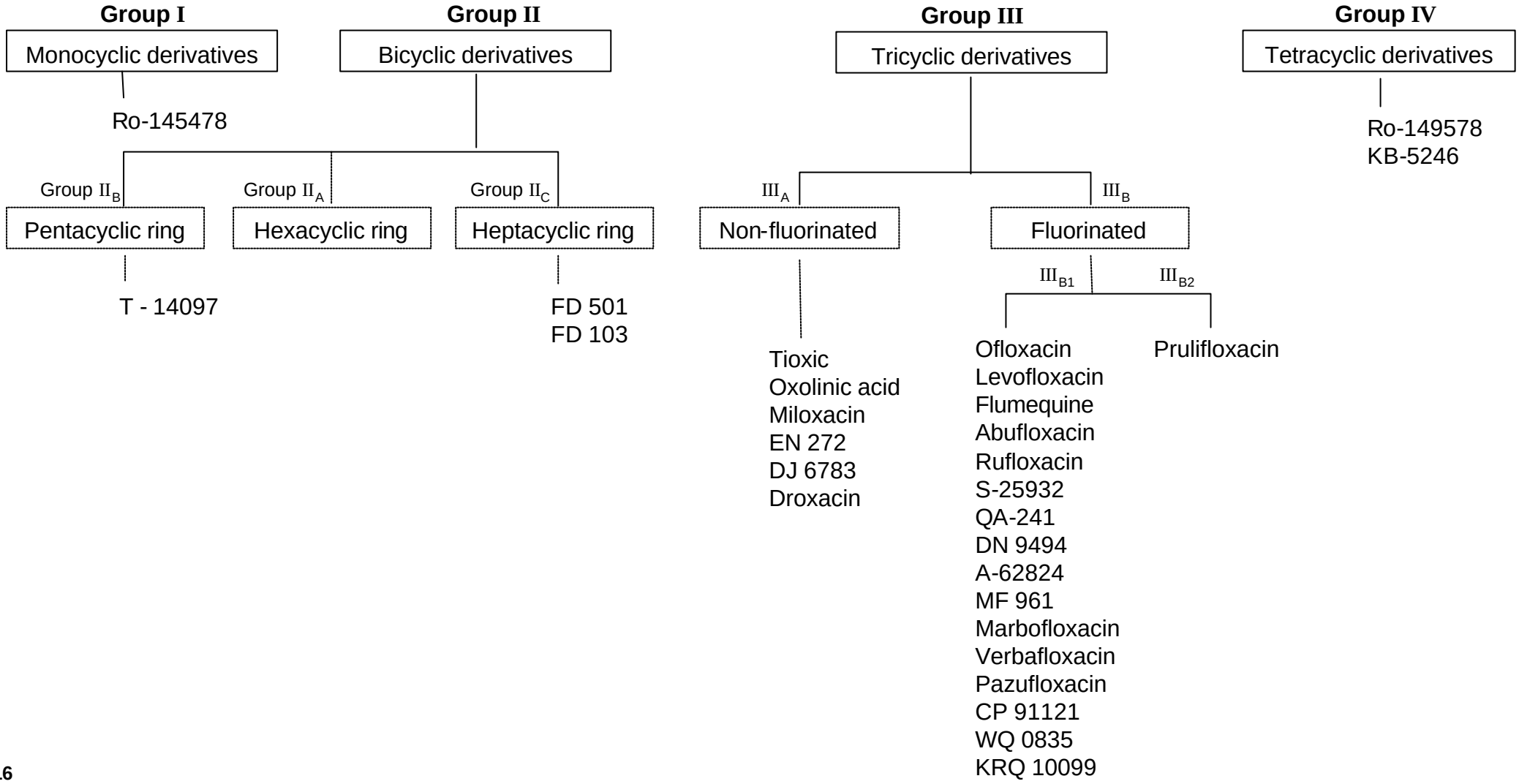
Fluoroquinolones

Classifications

- **Chemical** classification
- **Biological** classification

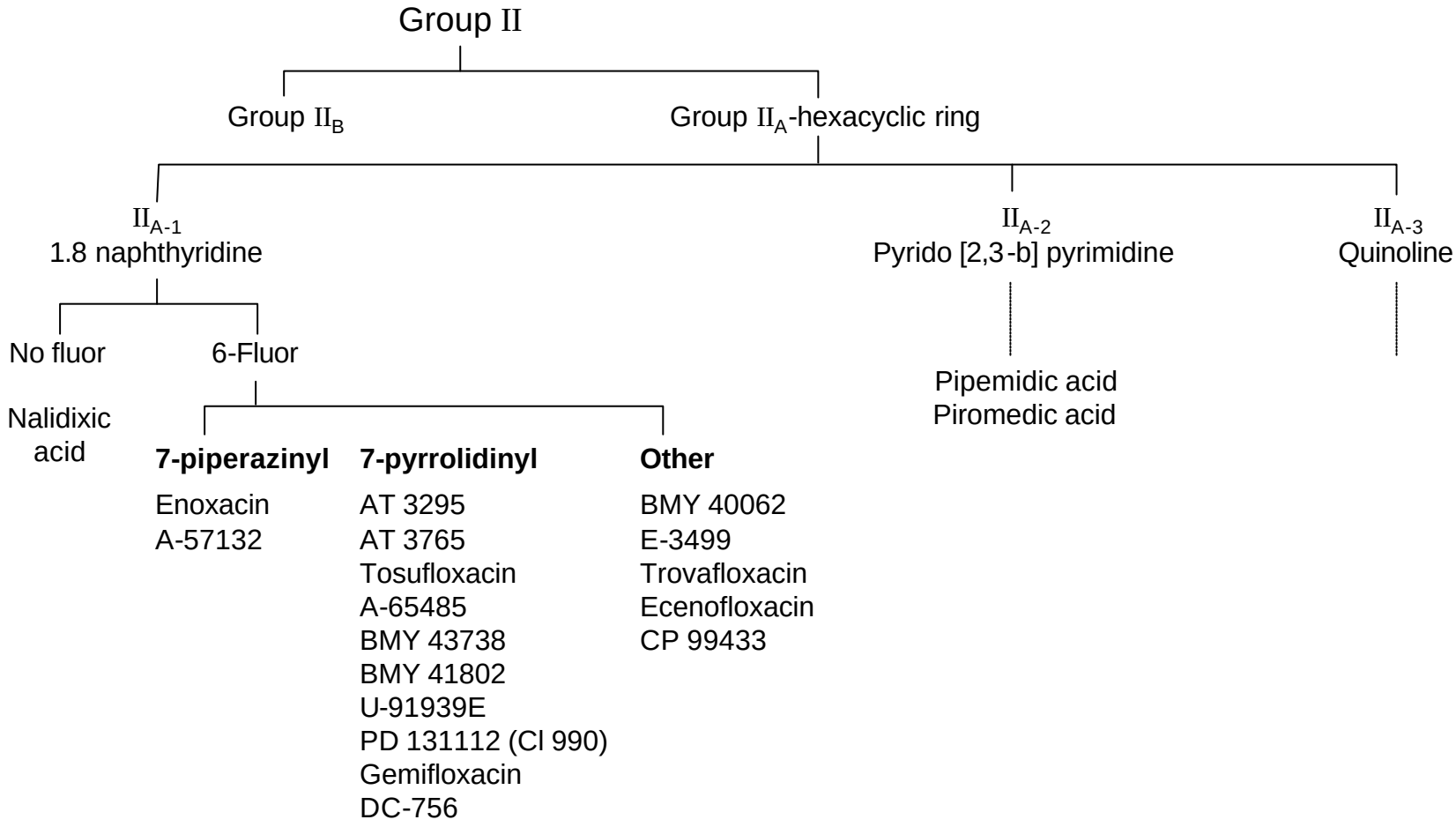
Fluoroquinolones

Chemical classification



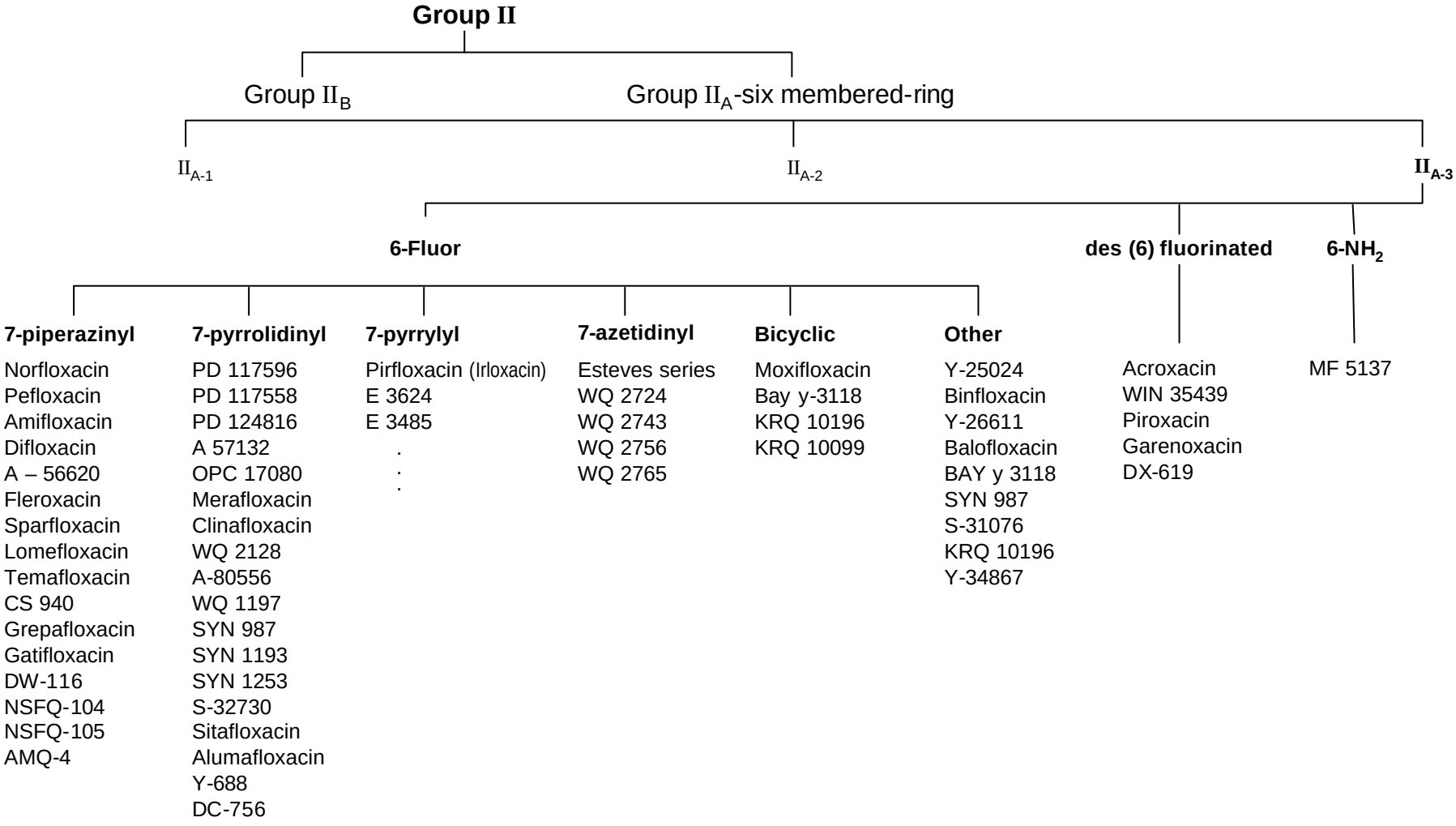
Fluoroquinolones

Chemical classification (bicyclic derivatives)



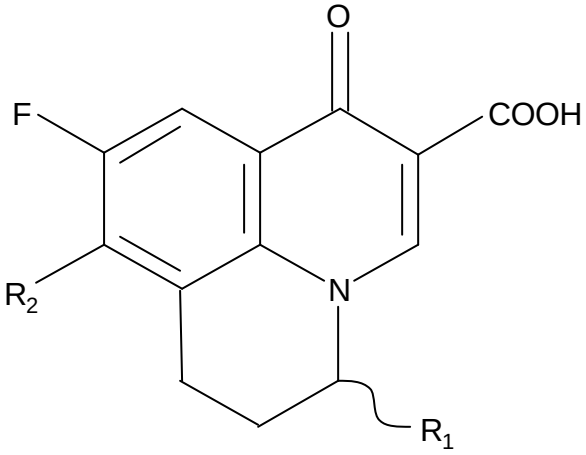
Fluoroquinolones

Chemical classification - Bicyclic derivatives

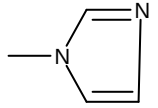
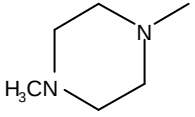


Fluoroquinolones

Tricyclic derivatives

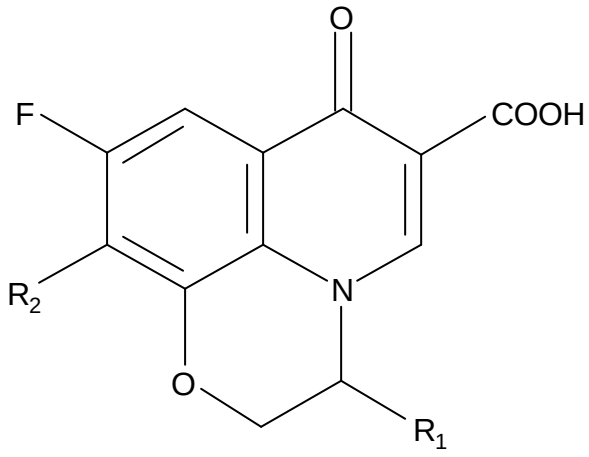


Benzoquinazoline derivatives

	R_1	R_2
Flumequine	CH_3	H
Methyl flumequine	CH_3	CH_3
Abufloxacin	CH_3	
Verbufloxacin	CH_3	

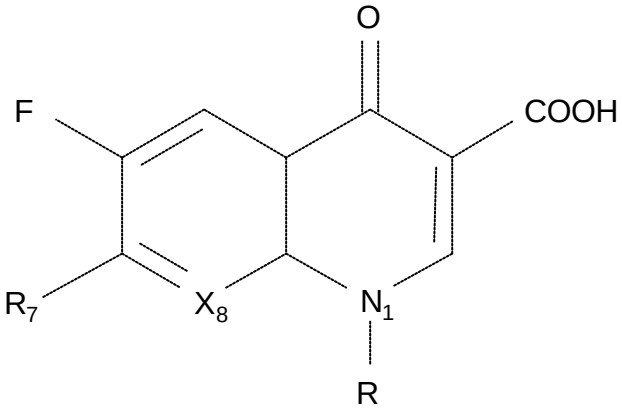
Fluoroquinolones

Tricyclic derivatives



	R_1	R_2
Ofloxacin		4’-methyl piperazinyl
Levofloxacin		4’-methyl piperazinyl
Neuquinoron		H_2N
CP 92121		Pyridine

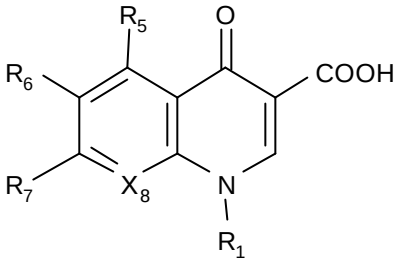
Fluoroquinolones



7-position

Bicyclic	Piperazinyl	Pyrrolidinyl	Azetidinyl	Pyrryl	Piperidinyl	Pyridinyl	Morpholine
Trovafloxacin	Ciprofloxacin	Clinafloxacin	E 4695	Irloxacin	Balofloxacin	WIN 52773	Y-26611
Moxifloxacin	Lomefloxacin	Nadifloxacin	E 4767				Y-25024
Danafloxacin	Norfloxacin	Sitafloxacin	E 4633				
Garenoxacin	Fleroxacin						
	Ofloxacin						
	Sparfloxacin						
	Grepafloxacin						
	Gatafloxacin						
	Levofloxacin						

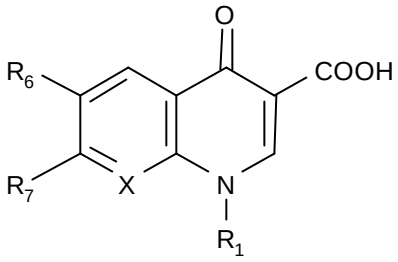
Fluoroquinolones



Substituents at position 8

C-F	CH_3	C-Cl	C-Br	N	$COCHF_2$	C-OCH_3	CH_2
Sparfloxacin	Alumofloxacin	Clinafloxacin	WQ 2743	Enoxacin	CS-940	Gatifloxacin	Ciprofloxacin
Lomefloxacin		Sitafloxacin		Tosufloxacin		Pazufloxacin	Temafloxacin
Fleroxacin		WQ 2724		CI 990		Y-688	Pefloxacin
KRQ 10196		WQ 3034		Gemifloxacin		S-32730	Norfloxacin
				Trovafloxacin		Balofloxacin	Grepafloxacin
				Ecenofloxacin		Moxifloxacin	
						Garenoxacin	
						DC-456	
						Y-34867	

Fluoroquinolones



Substituents at N-1

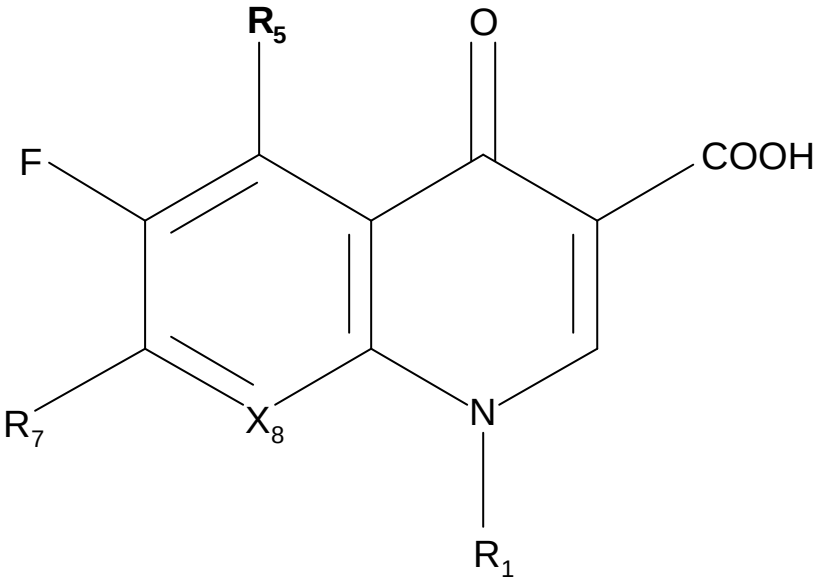
- Ethyl (C_2H_5)
  - Pefloxacin
  - Norfloxacin
  - Enoxacin
  - Lomefloxacin
- Fluoro ethyl (C_2H_4F)
  - Fleroxacin
- Cyclopropyl (*c*-C_3H_5)
  - Ciprofloxacin
  - Grepafloxacin
  - Y-688
  - S-32730
  - Alumofloxacin
  - Gemifloxacin
  - Moxifloxacin
  - FD 501
  - FD 103
  - Ecenofloxacin
  - CI 990
  - Balofloxacin
  - CS 940
  - T-3811
  - KRQ 10196
- Fluorophenyl (4'F-C_3H_5)
  - Difloxacin
- Difluorophenyl (2',4'-F-C_3H_5)
  - Temafloxacin
  - E-4868
  - Trovafloxacin
- Methyl amino (NH-CH_3)
  - Amifloxacin
- *t*-butyl
  - *t*-C_4H_6
  - 40062
- Oxetane
  - WQ 175
  - WQ 1197
  - WQ 1101

Fluorocyclopropyl
- Sitafloxacin
- DX-619

4'-F-pyridyl
- DW 116

5'-amino 2',4'F pyridinium
- WQ 3034
- WQ 2724
- WQ 2743

Fluoroquinolones

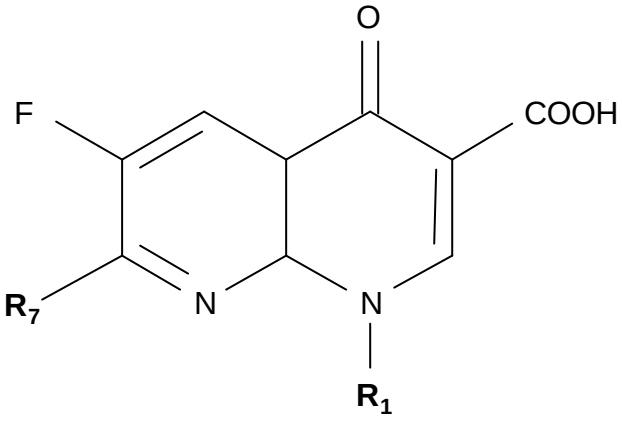


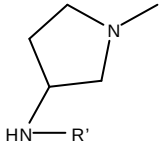
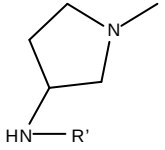
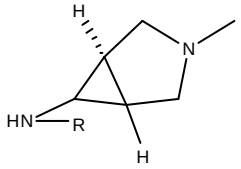
Substituents at C-5

NH_2	CH_3	OCH_3
Sparfloxacin	Grepafloxacin	SYN 1193
WQ 0175	BMY 43748	SYN 1253
PD 124816		
SYN 987		
FD 501		
FD 103		
KRQ 10196		

Fluoroquinolones

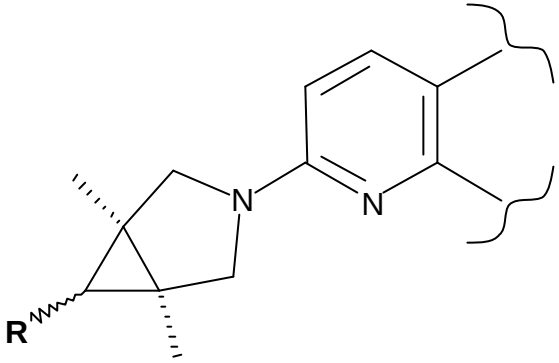
Prodrugs

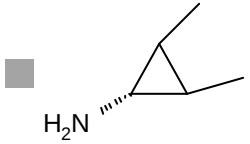
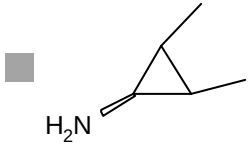


	R_1	R_7	R'-aminoacid
A 70826	2'4' difluorophenyl		L-norval-norval
PD 131628	Cyclopropyl		L-alanyl
Alatrovafloxacin	2'4' difluorophenyl		L-ala-L-ala

Fluoroquinolones

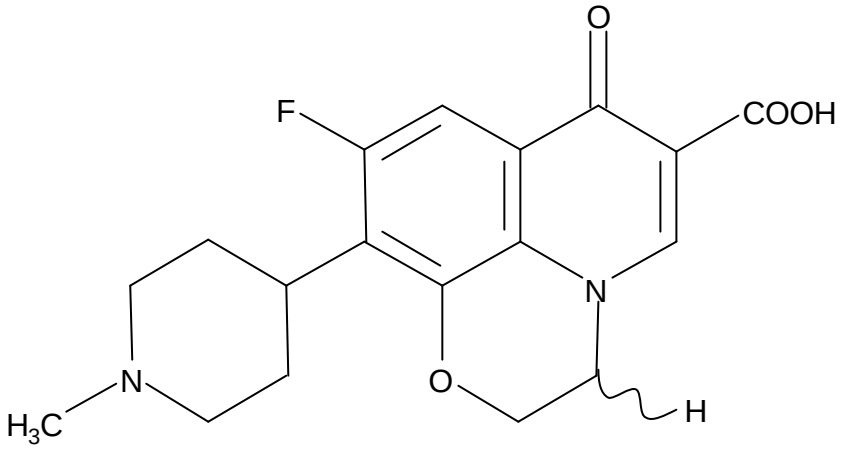
Trovafloxacin enantiomers



	In vitro activity	*In vivo* activity	Pharmacokinetics (animal)
	+++	+++	+++
	+++	+	+

Fluoroquinolones

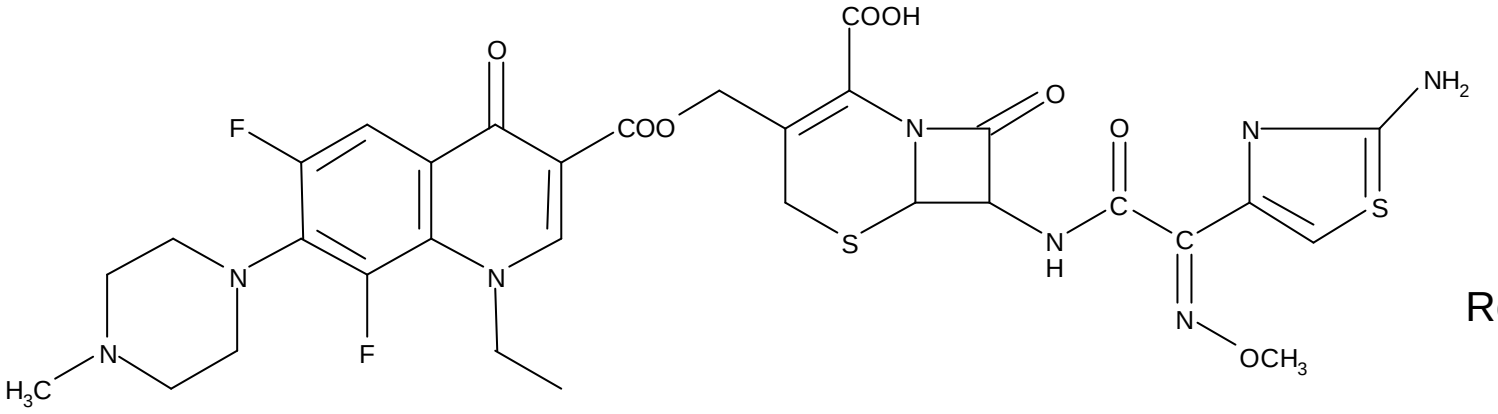
Enantiomers



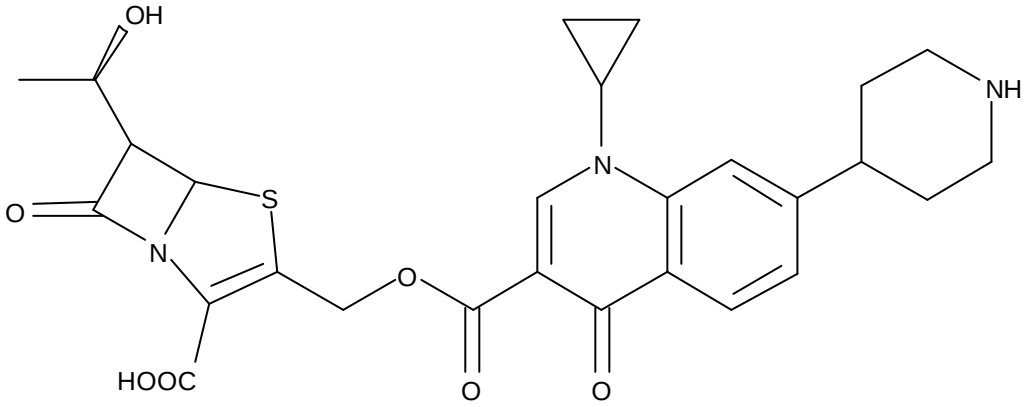
		In vitro activity	Pharmacokinetics
CH_3	Ofloxacin	Less active	No change
CH_3	Levofloxacin	2-4 x	No change
CH_3	*d*-ofloxacin	Inactive (MIC > 128 mg/l)	No change

Fluoroquinolones

Co drugs



Ro 23-9484

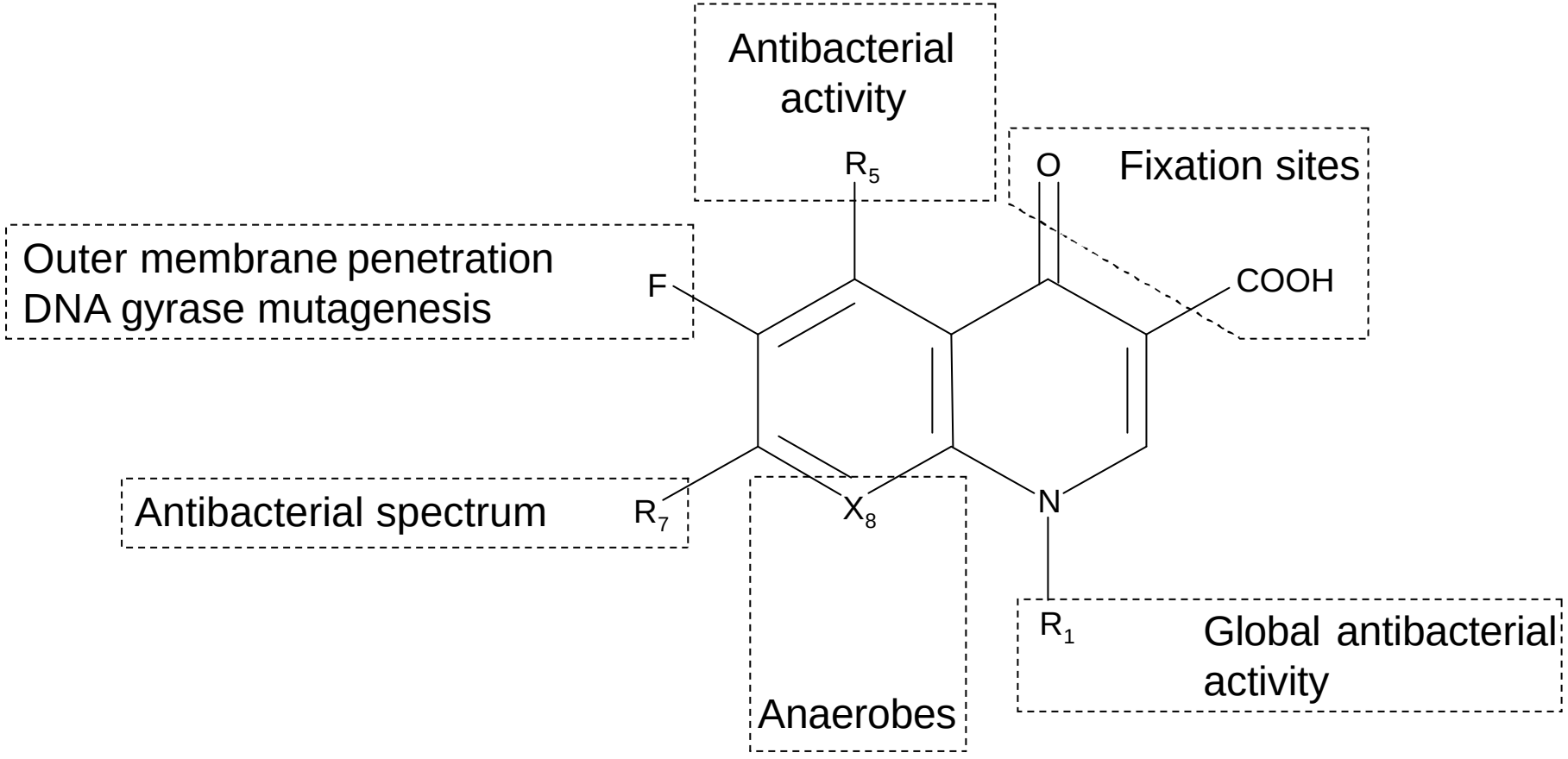


FCE 26600

- Antibacterial activity

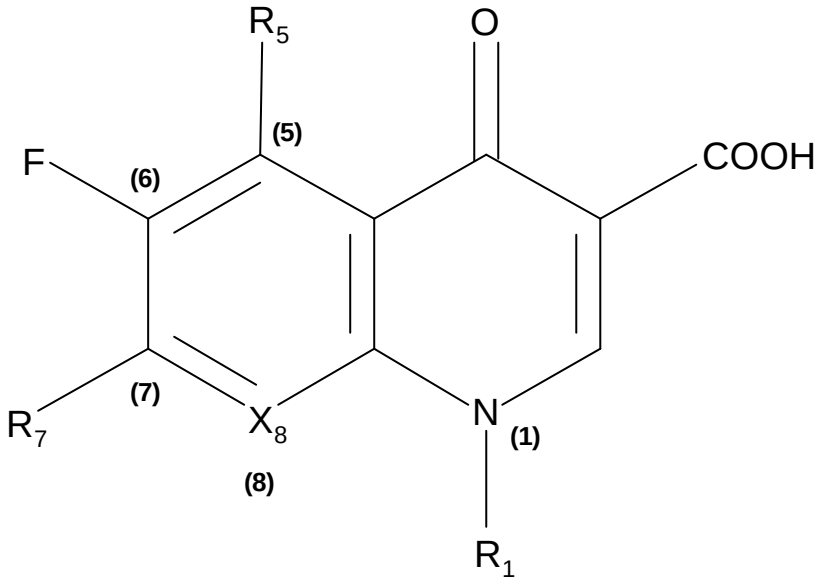
Fluoroquinolones

Antibacterial activity



Fluoroquinolones

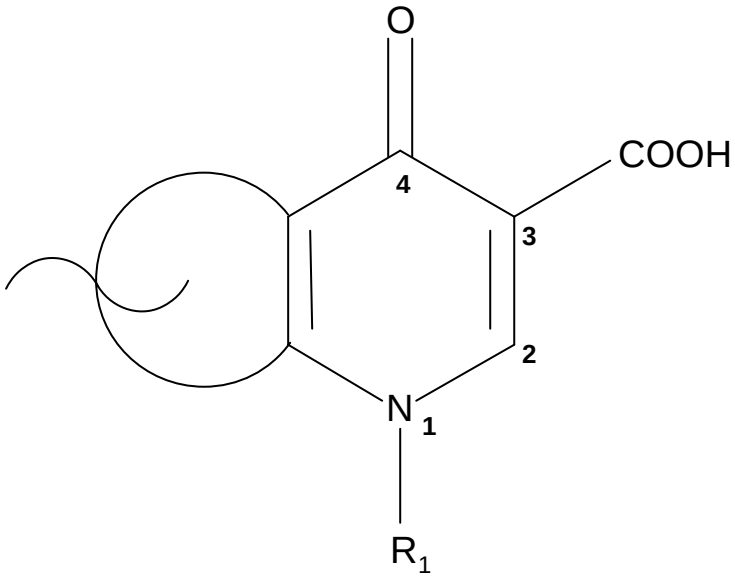
Antibacterial activity



- C-6 fluorine
- 7-substituent
- N-1 substituent
- C-5 substituent
- C-8 substituent

Fluoroquinolones

Antibacterial activity



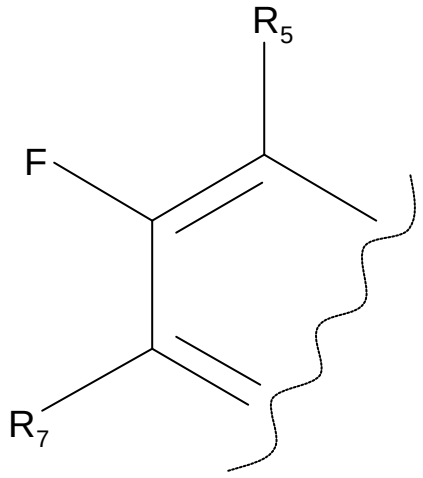
- Minimal requirement

  . double bond in 2-3 must be reduced
  . free ketone in position 4
  . free carboxylic group in position 3
  . N-1 has to be substituted

Fluoroquinolones

Antibacterial activity

C_6

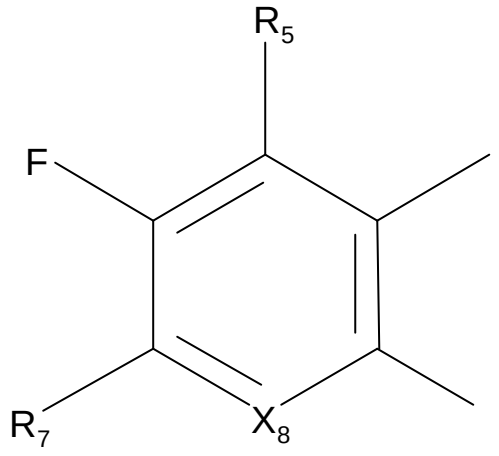


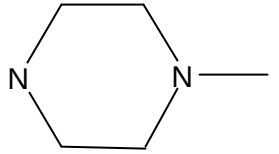
- C-6 fluorine enhances
  - → gyrase inhibition
  - → cell penetration.

Fluoroquinolones

Antibacterial activity

C_7

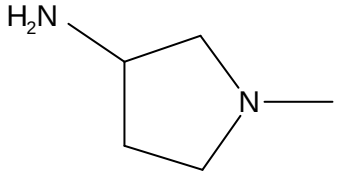




(piperazinyl)

R

- Best moiety against Gram-negative bacteria



(pyrrolidinyl)

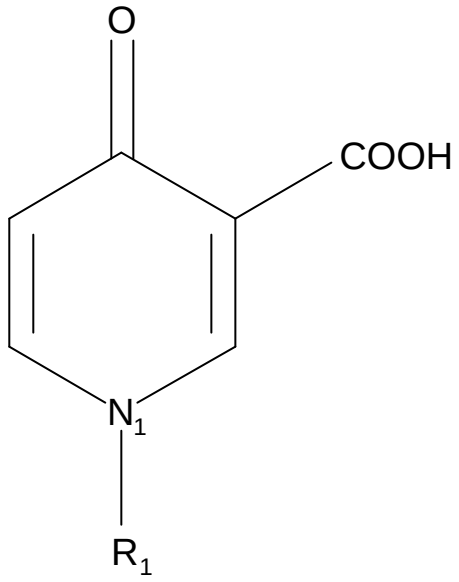
R

- Best moiety against Gram-positive cocci

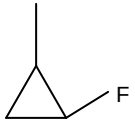
Fluoroquinolones

Antibacterial activity

N_1

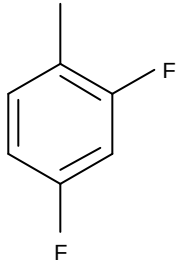


Fluorocyclopropyl Cyclopropyl > 2’4’ difluorophenyl > *t*-butyl > oxetane > butyl > ethyl

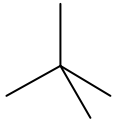


Sitafloxacin
DX-619

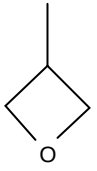
Ciprofloxacin



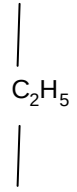
Temafloxacin



BMY 10062



WQ 1107

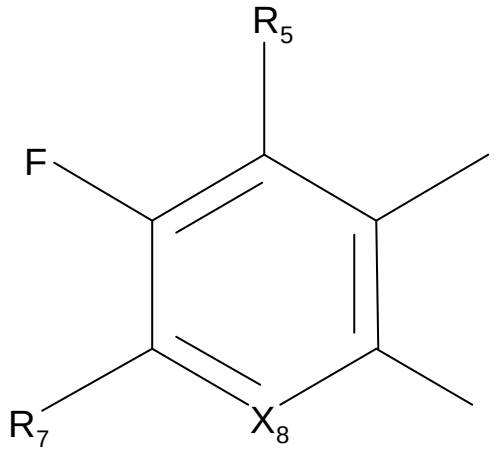


Norfloxacin

Fluoroquinolones

Antibacterial activity

C_5



- Additive activity against Gram-positive cocci

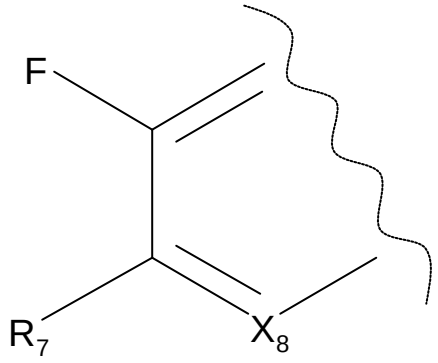
  NH_2 > OH > H

  e.g. : NH_2 ... sparfloxacin
  CH_3 ... grepafloxacin

Fluoroquinolones

Antibacterial activity

X_8



- Control anaerobe activity

  C-Cl = C-F = CO-CH_3 > CH > N

  e.g. : C-Cl ... clinafloxacin
  C-F ... sparfloxacin

Fluoroquinolones

Extend the antibacterial activity

Fluoroquinolones

Targeted indications mycobacteria

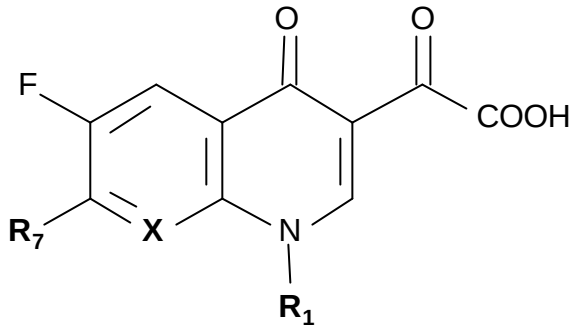
Mycobacteria

- Structure activity relationships have been extensively study
- Some fluoroquinolones are active *in vitro* and in clinical trials against
  - *M. tuberculosis*
  - *M. leprae*
- 1,8 naphthyridone are inactive.

Fluoroquinolones

Targeted indications

Mycobacteria



	R_1	X	R_7	MIC (mg/l) *M. fortuitum*	MIC (mg/l) *M. tuberculosis*
PD 163753	Cyclopropyl	C-Br	3'-methyl piperazinyl	≤ 0.03	0.76
PD 161144	Cyclopropyl	C-OCH_3	4'-ethyl	≤ 0.03	0.39
PD 163048	*tert* –butyl	N	3'-methyl piperazinyl	0.03	0.78
PD 163049	*tert* –butyl	N	3', 5' dimethyl piperazinyl	0.03	0.78
PD 161148	Cyclopropyl	C-OCH_3	3'-ethyl piperazinyl	0.03	0.10
Ciprofloxacin	Cyclopropyl	CH_2	piperazinyl	0.06	0.25
Sparfloxacin	Cyclopropyl	C-F	3', 5' dimethyl piperazinyl	0.06	0.06

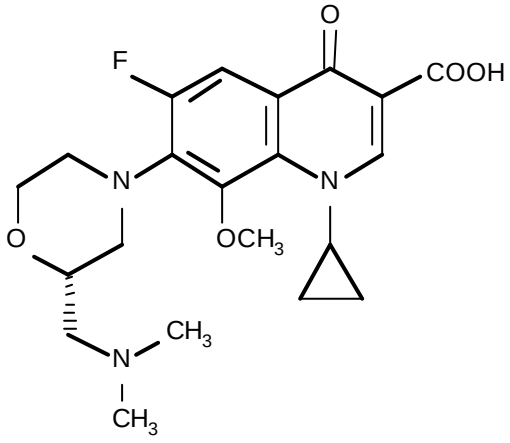
Fluoroquinolones

Targeted indications *H. pylori*

- **Two compounds**
  - → Natural compounds
  - → Y-34967

Fluoroquinolones

Targeted indications *H. pylori*



Y-34867

In vitro activity - *H. pylori*

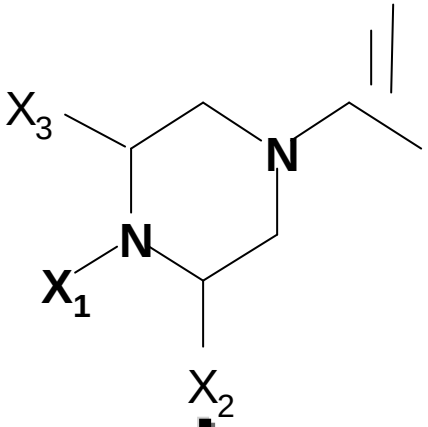
	MIC_{50} (mg/l)
Y-34867	0.025
Levofloxacin	0.39
Sparfloxacin	0.20
Amoxicillin	0.012
Clarithromycin	0.025

In vivo (murine infection - *H. pylori* 1907)

	MIC (mg/l)	Dose (mg/kg bid day 7)	Clearance (%)
Control	-	-	0
Y-34867	0.025	3	100
		10	100
Amoxicillin	0.39	30	100
Clarithromycin	0.05	30	0
		100	80

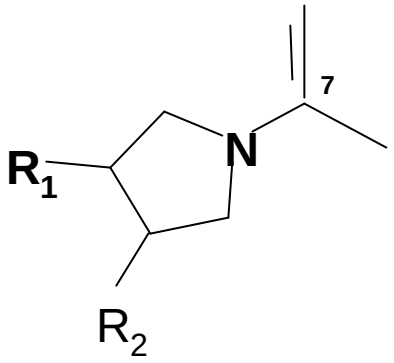
From Sukurai et al, 1998

Piperazinyl derivatives



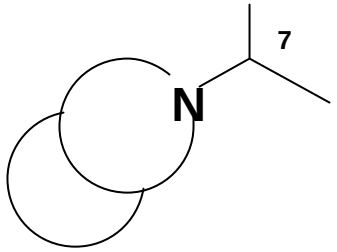
Temafloxacin
Sparfloxacin
Levofloxacin
Gatifloxacin
Grepafloxacin

Pyrrolidinyl derivatives



Tosufloxacin
Clinafloxacin

Bicyclic derivatives



Trovafloxacin
Moxifloxacin

S. pneumoniae

Respiratory Quinolones

S. pneumoniae

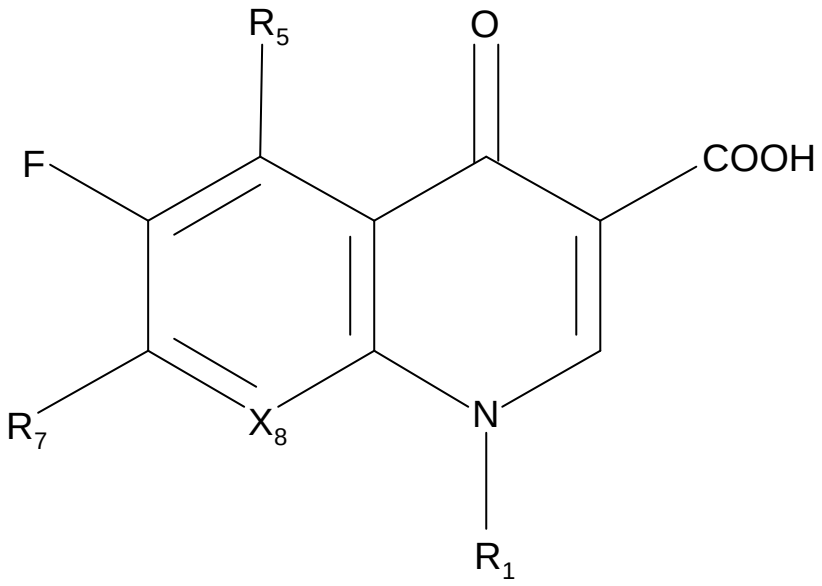
Ciprofloxacin
Norfloxacin
Lomefloxacin
Pefloxacin
Ofloxacin
Enoxacin
Fleroxacin

Levofloxacin
Moxifloxacin
Gatifloxacin
Sitafloxacin
Gemifloxacin
Garenofloxacin

Pharmacokinetics

Fluoroquinolones

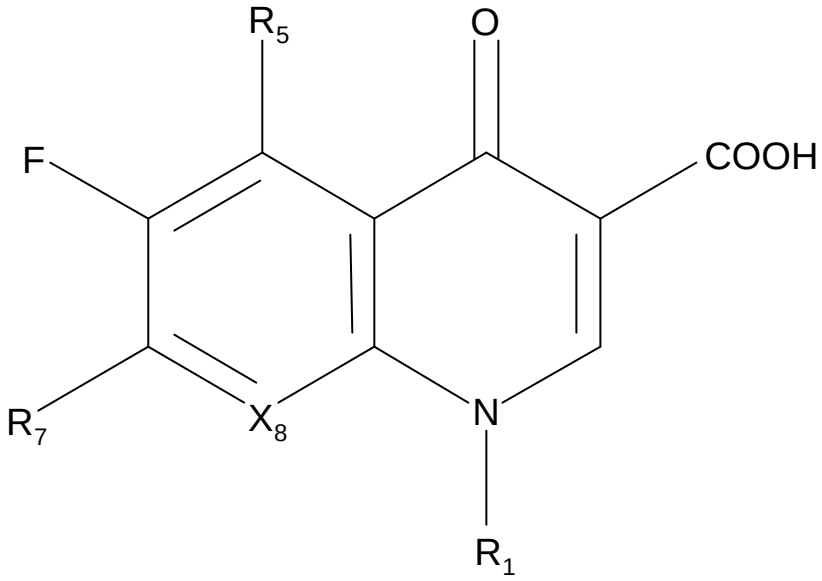
Pharmacokinetics



- C-8 substituent
- C-7 substituent

Fluoroquinolones

Pharmacokinetics

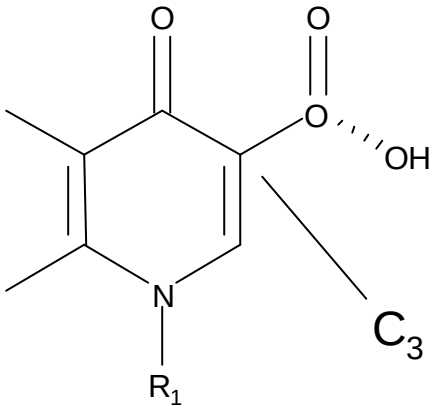


- C-8 substituent : oral absorption
- C-7 substituent : metabolism and oral absorption
- C-3 substituent : iron chelation

Fluoroquinolones

Pharmacokinetics

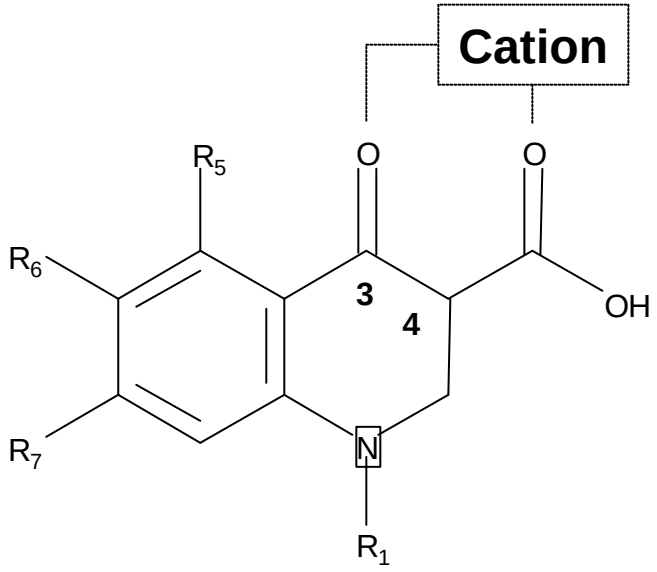
C_3



- Reduce oral absorption (interactions with antiacids, milk...) divalent cations : Ca^{++}, Fe^{++}, Zn^{++}

Metal cations, antacids, anti-ulcers

- Al^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+} and other cations formchelate complexes with fluoroquinolones.



- Reduce bioavailability of fluoroquinolones.

Fluoroquinolones

Pharmacokinetics

C_7

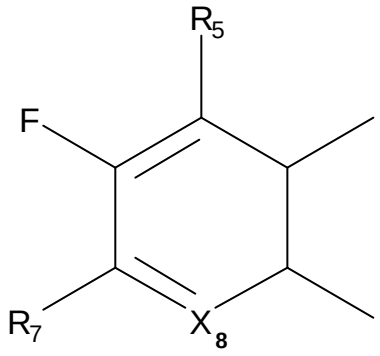
- Improve oral absorption and water solubility
  eg. norfloxacin *versus* pefloxacin

- Site of metabolism for C-7 piperazinyl derivative.

Fluoroquinolones

Pharmacokinetics

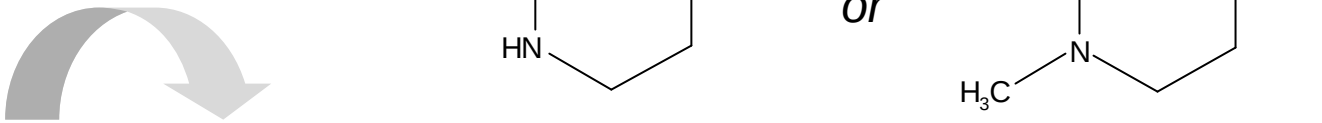
C_8



- Substituent at C-8 may improved oral absorption
  C-F, C-Cl > C-OCH_3, > CH

Fluoroquinolones

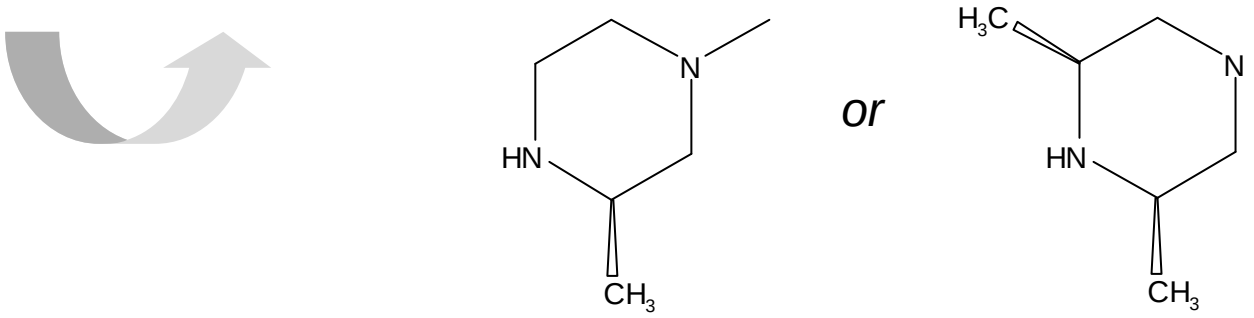
Pharmacokinetics



- Metabolism : 15-90% (nine metabolites)

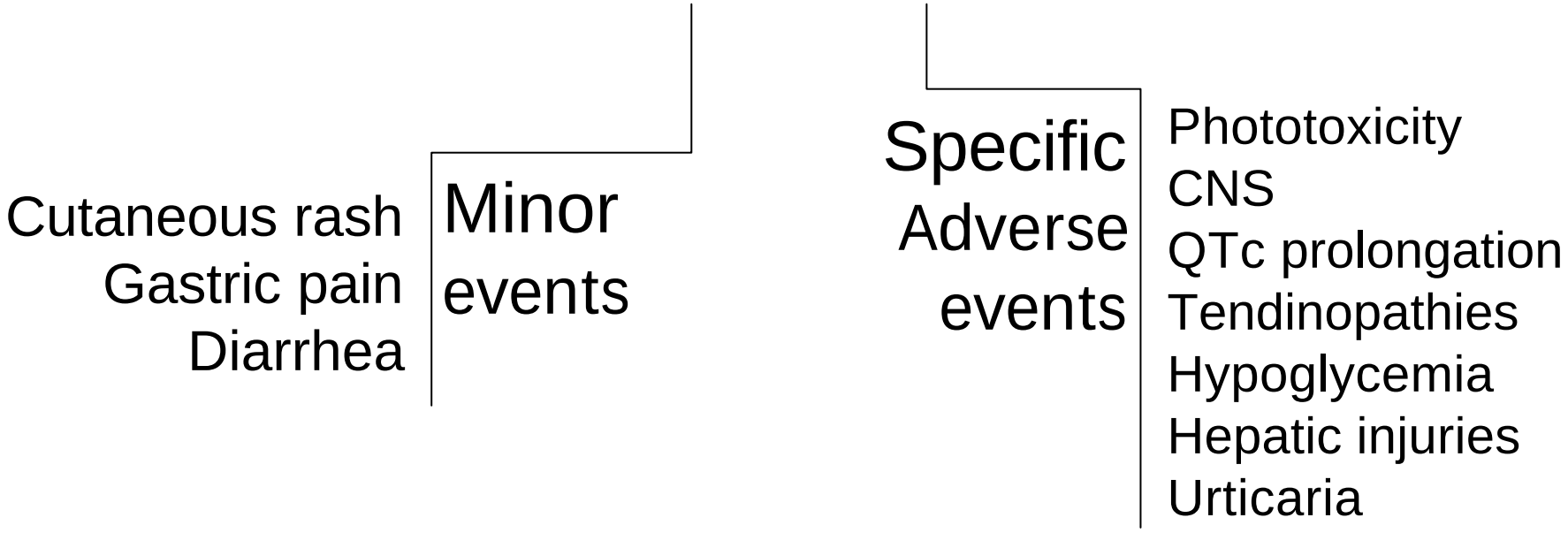
C_7 metabolism

- Metabolism : < 5% except grepafloxacin



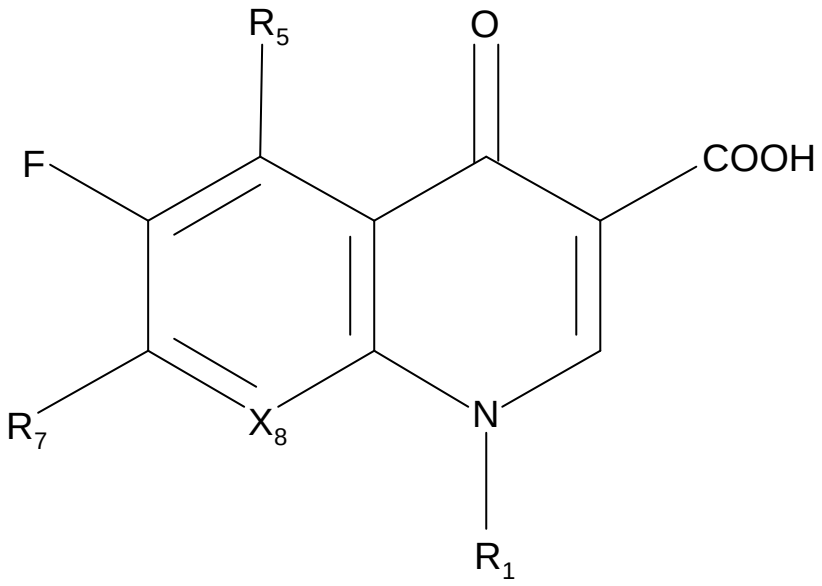
- Adverse events

Fluoroquinolones
Adverse events



Fluoroquinolones

Adverse events

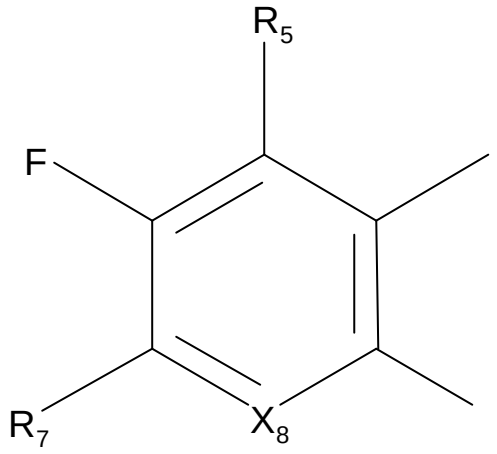


- C-7 substituent
- C-8 substituent
- N-1 substituent

Fluoroquinolones

Adverse events

R_7

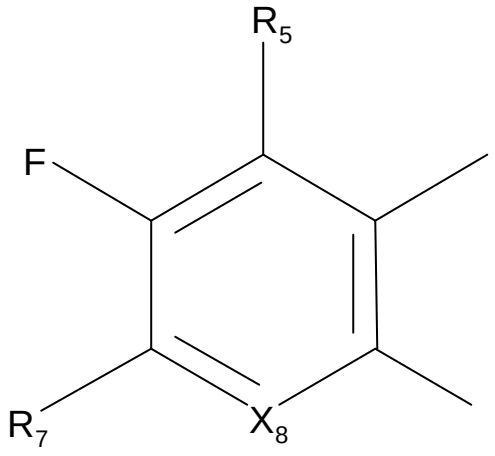


- GABA binding (CNS-tolerability)
  piperazine > pyrrole
- Theophylline interaction
  pyrrole > piperazine
- Genetic toxicity
  pyrrole > piperazine
- Solubility

Fluoroquinolones

Adverse events

X_8

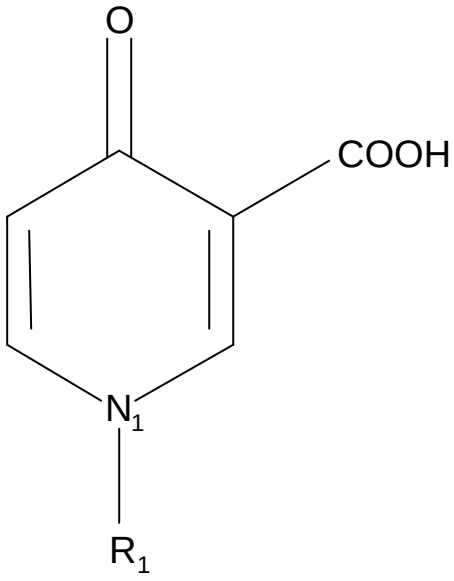


- Phototoxicity
  C-F > C-Cl > N > CH > C-OCH_3, C-CF
- Genetic toxicity
  C-F > C-Cl > C-OCH_3 > N > CH
- Water solubility

Fluoroquinolones

Adverse events

N_1



- Control theophylline
  cyclopropyl > ethyl > 2',4'-difluorophenyl > C_2H_4F
- Genetic toxicity
  cyclopropyl = *t*-butyl > 2.4'- difluorophenyl > ethyl

Fluoroquinolones

Adverse events

	Phototoxicity	Solubility	Genetic toxicity	Theophylline	CNS
N-1	-	-	+	+	-
C-5	+	-	+	-	-
C-6	-	-	-	-	-
C-7	-	++	++	+	++
X-8	++	++	++	-	-

Fluoroquinolones

Clastogenicity

	Concentration (mg/l) causing 50% cytotoxicity
Ofloxacin	= 500
Norfloxacin	= 500
Temafloxacin	= 500
Fleroxacin	= 500
Ciprofloxacin	330
Sparfloxacin	370
Tosufloxacin	120
Merafloxacin	190

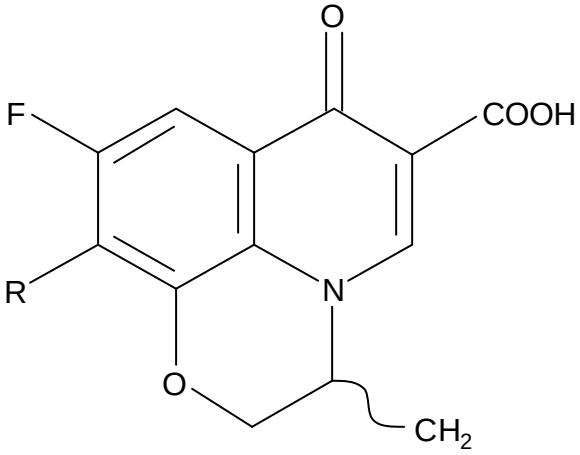
Fluoroquinolones

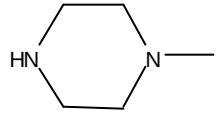
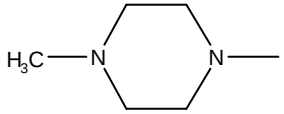
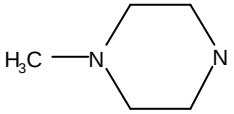
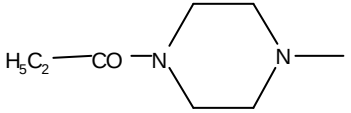
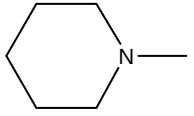
Topoisomerase II activity

Compound	ID_{50} (mg/l) DNA gyrase from *E. coli* KL-16	ID_{50} (mg/l) topoisomerase II from thymus
Ofloxacin	0.76	1870
Ciprofloxacin	0.13	155
Levofloxacin	0.78	280
Enoxacin	1.72	93
Merafloxacin	3.55	64
Nalidixic acid	23.00	325

Fluoroquinolones

Affinity for GABA receptors



R	IC_{50} (M)
H	$> 10^{-3}$
	1.8×10^{-5}
	1.0×10^{-3}
	$> 10^{-3}$
	$> 10^{-3}$
	$> 10^{-3}$

GABA receptors

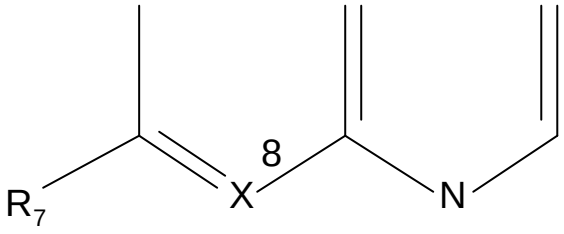
	IC_{50} (M)	
	Without NSAID	4-biphenylacetate
Norfloxacin	1.4×10^{-5}	$< 10^{-8}$
Enoxacin	1.4×10^{-4}	1.1×10^{-7}
Ofloxacin	1.0×10^{-3}	8.3×10^{-7}
Ciprofloxacin	7.6×10^{-5}	3.0×10^{-8}
Tosufloxacin	5.7×10^{-4}	1.2×10^{-4}
Fleroxacin	7.6×10^{-4}	1.0×10^{-4}
Sparfloxacin	9.1×10^{-4}	5.2×10^{-5}
Levofloxacin	$> 10^{-3}$	3.5×10^{-4}
Sitafloxacin	1.0×10^{-3}	3.6×10^{-4}
BAY y 3118	$> 10^{-3}$	2.2×10^{-4}

Affinity for the GABA receptor of fluoroquinolones

Adapted from HORI

Fluoroquinolones

Phototoxicity



Chemical structure

Substituents	Phototoxicity
C-F	+
C-Cl	
N	
C-H	
C-F	
$C\text{-}OCH_3$	-

Adapted from Domagala

Fluoroquinolones

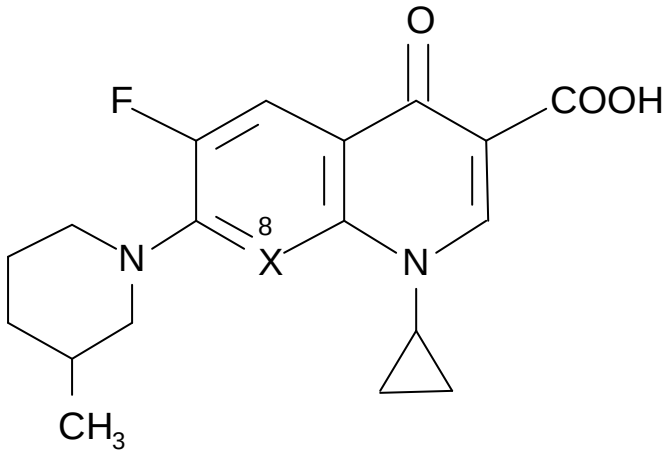
Photosensibility

Highest no effect phototoxic dosage (mg/kg)

Ciprofloxacin	> 300	Cl 990	> 300
Ofloxacin	> 300	Fleroxacin	> 300
Norfloxacin	> 300	Clinafloxacin	> 300
Tosufloxacin	> 100	Sparfloxacin	> 100
Neuroquino??	> 300	Lomefloxacin	> 300
Temafloxacin	300	Cl 938	300
Fleroxacin	172	Bay y 3118	172
Enrofloxacin	100	Murafloxacin	100

Phototoxicity

? Method :
ear swelling of mice after UV-A irradiation and quinolone administration



X	Dose (mg/kg)	N	Inflammation
O-CH_3	200	6	0/6
	800	6	0/6
8-F	3.1	6	0/6
	12.5	6	3/6
	50	6	5/6
-H 8	50	6	0/6
	200	6	6/6
	800	6	6/6

From Maratuni et al, 1993

Fluoroquinolones

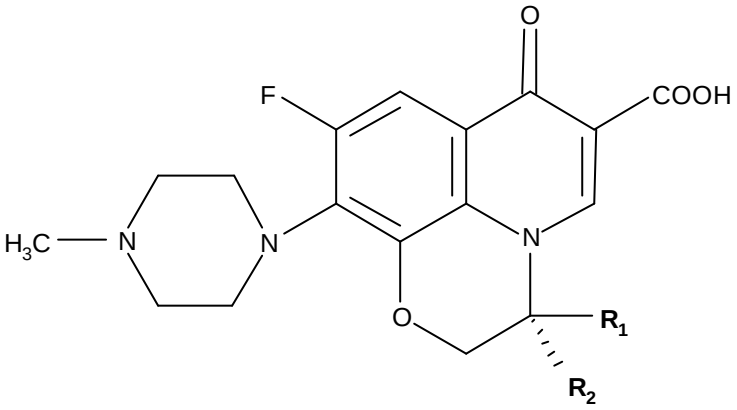
Photocarcino-genotoxicity

Mice SKH-1 (hairless) - 1.5 hours / day of 25 J/cm^2 of UV-A for 78 weeks

	T_{50} % (weeks)	Carcinoma Tumors
8-methoxy psoralen	4	++
Lomefloxacin	16	+++
Fleroxacin	28	(+)
Ofloxacin	> 50	-
Ciprofloxacin	> 50	-
Nalidixic acid	> 50	-

Fluoroquinolones

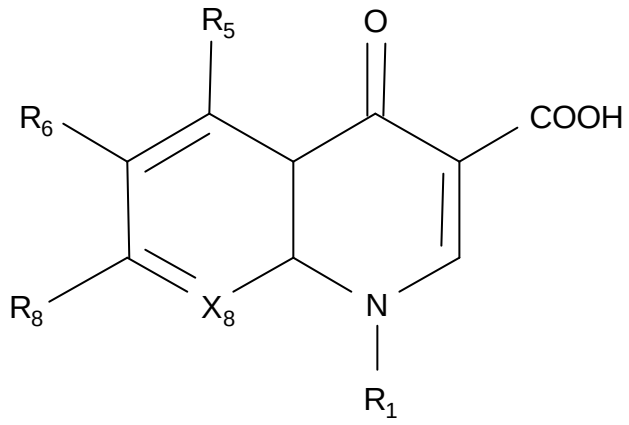
Mutagenicity



Compound	R_1	R_2	MIC (μg/ml)	ID_{50} (mg/l) DNA gyrase	ID_{50} (mg/l) topoisomerase II
Levofloxacin	CH_3	H	0.025	0.38	1380
DR-3354	H	CH_3	1.56	4.70	2550
Ofloxacin	CH_3		0.05	0.75	1870
DN-9494	$=CH_2$		0.05	0.70	64
DL-8165	H	H	0.10	3.10	178

Fluoroquinolones

Toxicity-tolerance : cardiotoxicity



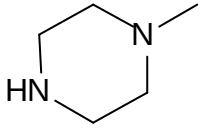
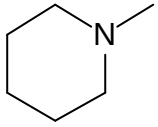
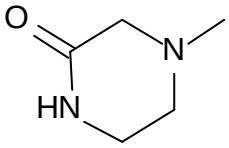
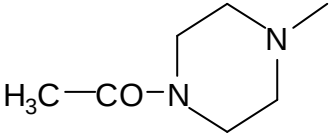
- Bulky substituents at C 5seem to be responsible for cardiotoxicity (e.g : sparfloxacin : $C_5 = NH_2$)

Fluoroquinolones and QTc effect in humans

Agent	Route of administration	QTc Prolongation (mean ± sd - msec)
Sparfloxacin	PO	10.3 ± 27.6
Grepafloxacin	PO	8
Gatifloxacin	PO	6 ± 26
	IV	12.1
Gemifloxacin	PO / IV	2.9 ± 16.5
	PO	5 ± 25.6
Levofloxacin	PO	4.6 ± 2.3

Owens, RC Jr. *Pharmacotherapy.* 2001 ; 21 : 301 - 319.

Theophylline (rat)

R_7	% of inhibition of 1,3 DMU
	47
	< 1
	2
	6

Conclusion

Fluoroquinolones

- Difficult to predict
  - Increased difficulties in synthesis
  - Tolerance
- Medical need
  - Overcome ciprofloxacin resistance
  - (*S. aureus, P. aeruginosa*....)
- New concept
  - Targeted clinical indication : e.g ....
  - (*Helicobacter pylori*, mycobacteria)

Fluoroquinolones

Expand the clinical indications

Intra abdominal infections

Lower respiratory tract intections
Upper respiratory tract infections

Fluoroquinolones

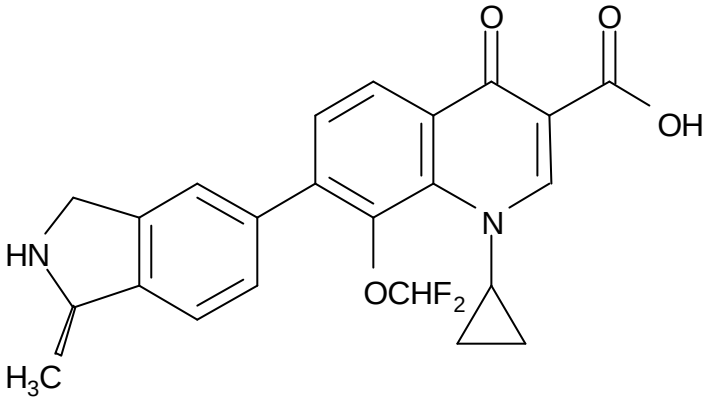
Future - New avenues

- Development of new chemical structures
- Improvement *in vitro* activity correlated with clinical outcome
- Increased problems in the field of side effects.

- New classifications of quinolones
- Extend the antibacterial activity
- Expand the clinical indications
- Overcome ciprofloxacin resistance.

Fluoroquinolones

Garenoxacin



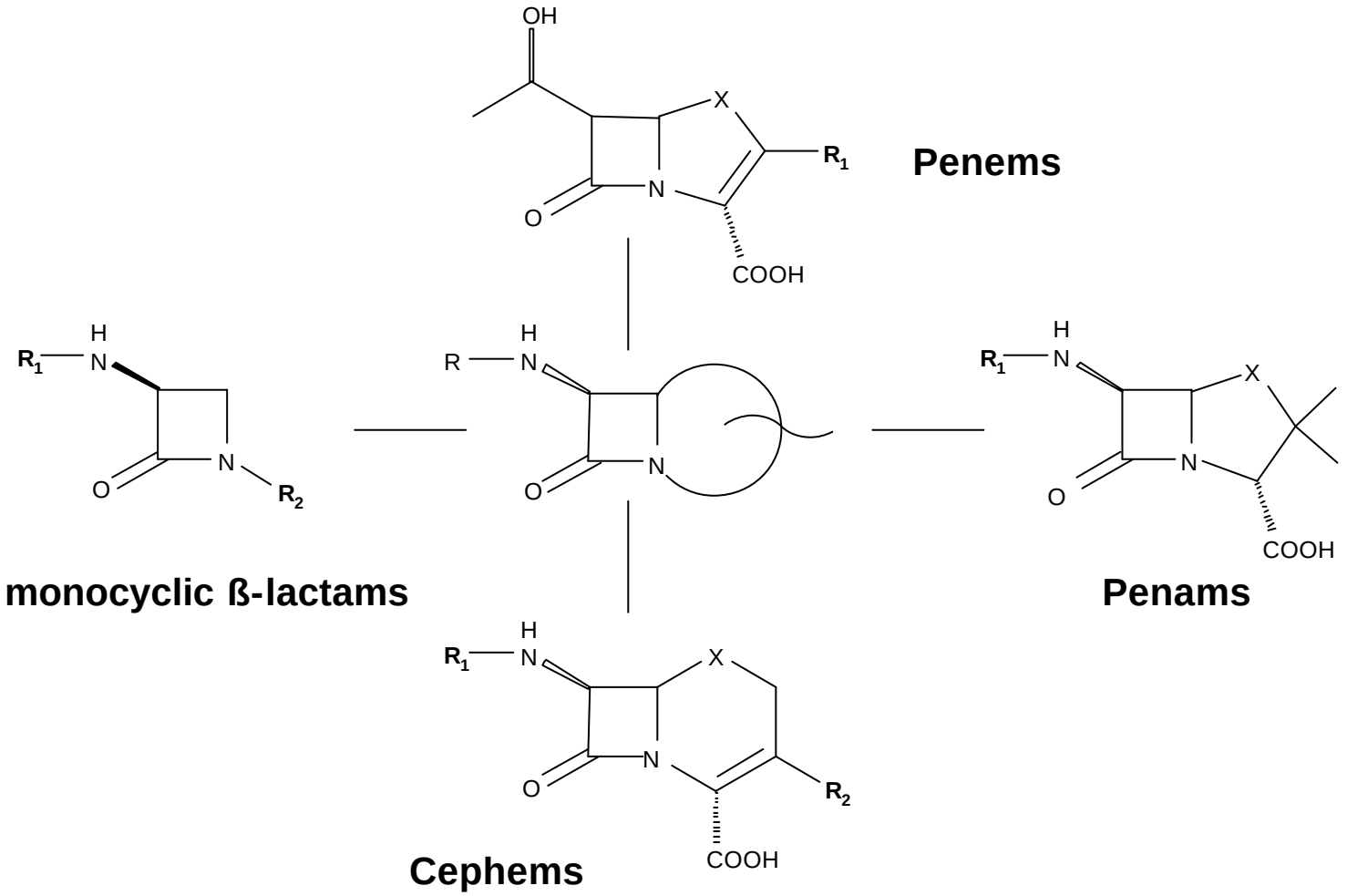
- No 6-fluorine
- 7-dihydro iso indanyl
- Less activity on cartilage than other fluoroquinolones

	GAR	TRO	CIP
S. pneumoniae	0.03	0.12	1.0
E. coli	0.03	0.03	0.01
B. fragilis	0.12	0.12	2.0

ß-lactams

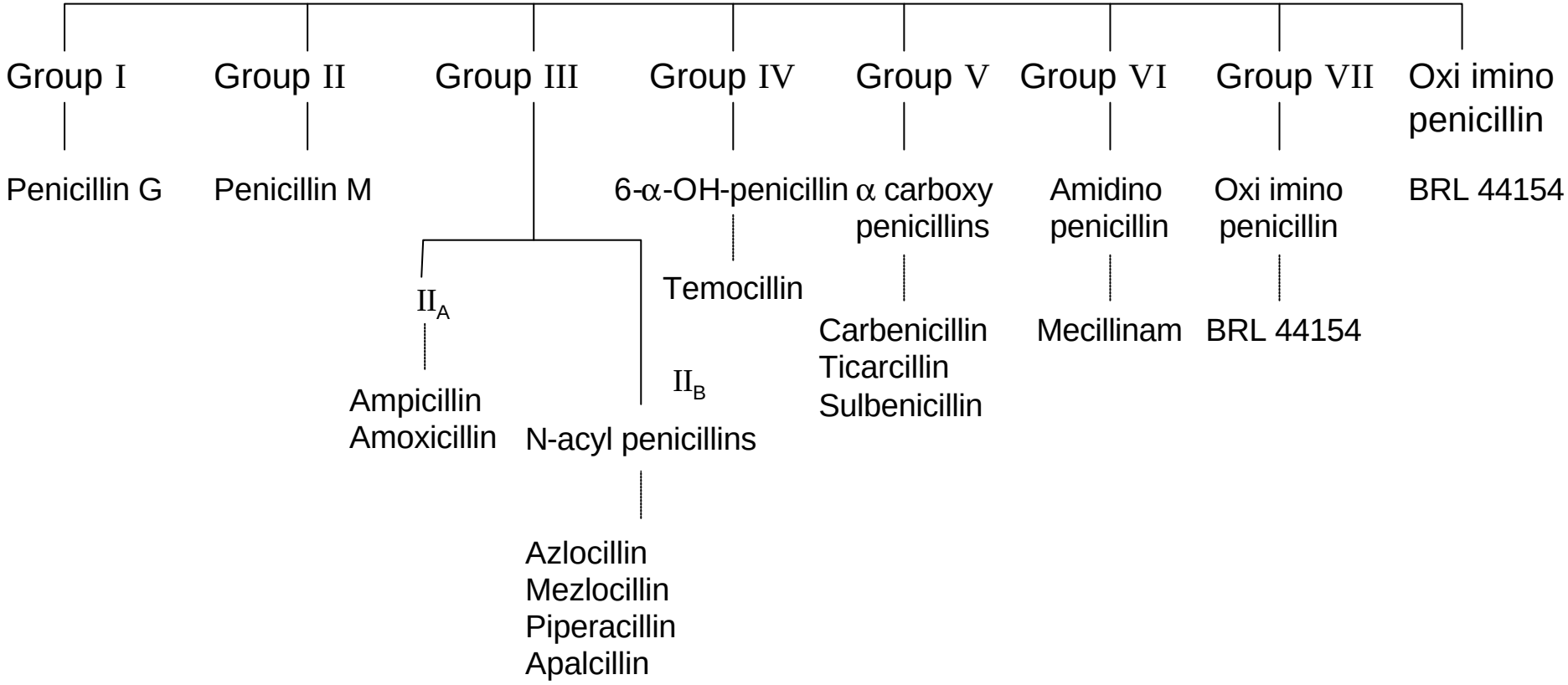
ß-lactam

Classification



ß-lactam

Penams



Cephems

Cephalosporin C

Discovered in 1953 (Newton & Abraham)

Isolated from *Cephalosporium acremonium* (Brotzu, 1945)

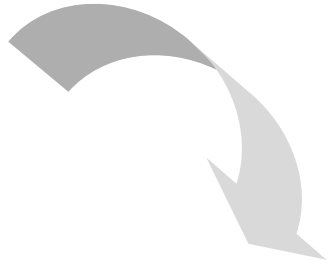
Chemical structure was elucidated in 1959

1969 : 7 amino cephalosporinic acid (7-ACA)

Cephems

Wave of parenteral cephems

- 7-ACA (1960)

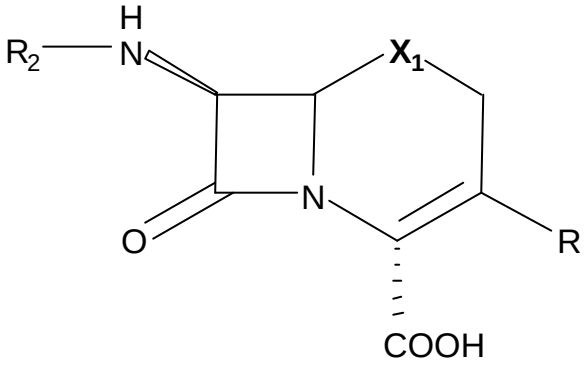


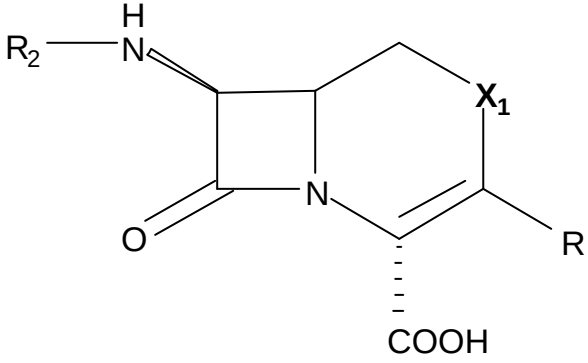
Cephalothin
Cephaloridine
(1964)

Cephems

Classification

Chemical classification (1) - Modification of the ring





	X_1
Cephalosporin	S
Oxacephems	O
Carbacephems	CH_3

	X_1
-iso-2 cephalosporin	S
-iso-3 oxacephems	O
	CH_3

Cephems

Discovery of cephalosporin

- Mould from *C. acremonium* (1945)

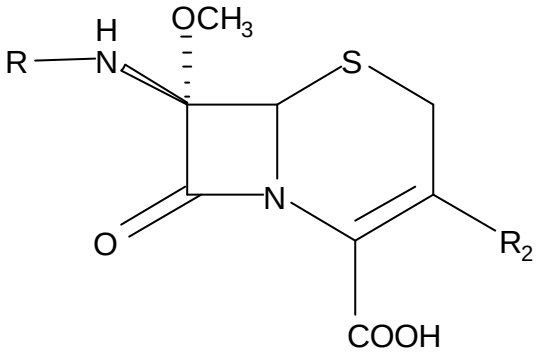
- Cephalosporin C (1953)

- 7-amino cephalosporinic acid (7-ACA) (1960)

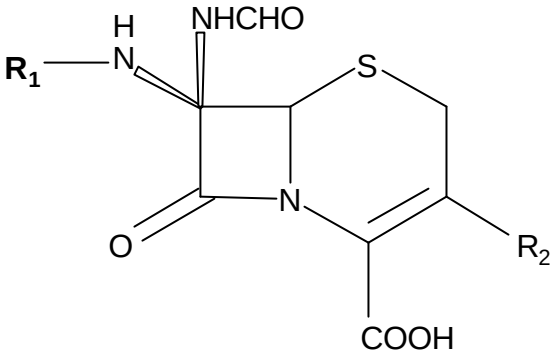
Cephems

Classification

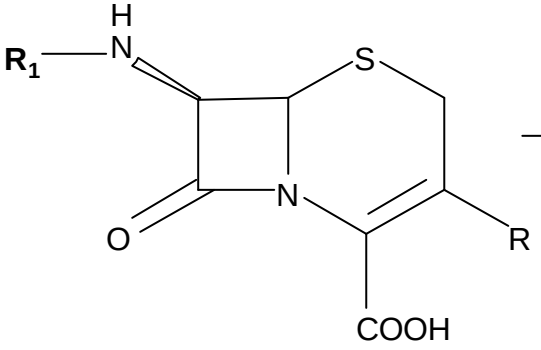
Chemical classification (2) - Modification substituents



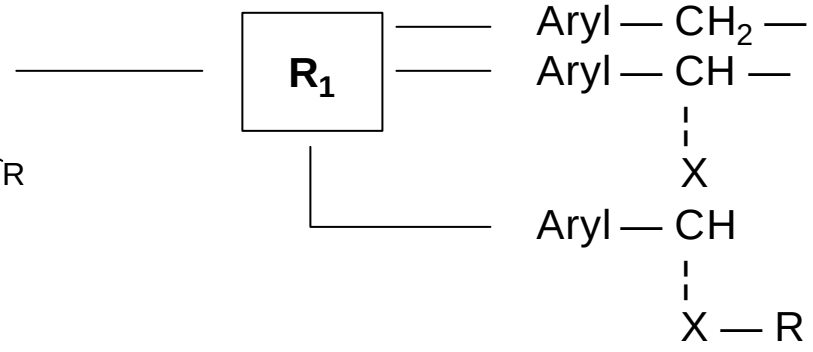
Cephamycins



Cephabicins



Cephalosporins



Cephems

Microbiological classification

- Cephems could be divided according to their antibacterial spectrum in three groups

  limited spectrum : I and II

  Broad spectrum : III, IV and V

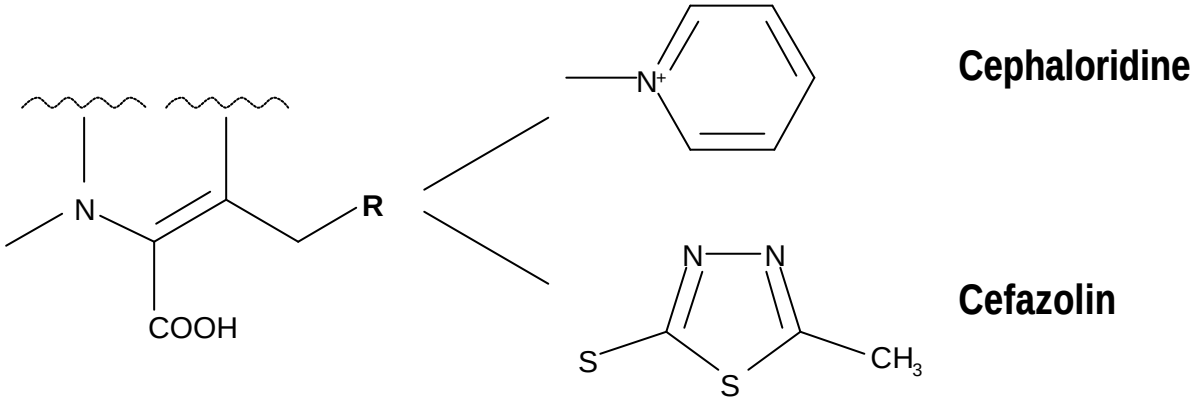
  Narrow spectrum: VI and VII

Group Ⅰ : Cephalothin, cephaloridine

- Active against penicillinase producing *S. aureus*
- Other cephems from group Ⅰ show marginal antibacterial activities
- Group Ⅰ cephems show moderate anti Gram negative activities.

Group I : Cephems

- Designed to overcome *S. aureus* resistant in penicillin G
- First cephalosporins to bear at C-3 an heterocycle moiety



Cephaloridine

Cefazolin

- Activity against Gram-negative bacilli (Enterobacteriaceae) and stability to ß-lactamase hydrolysis - equivalent to that of ampicillin.

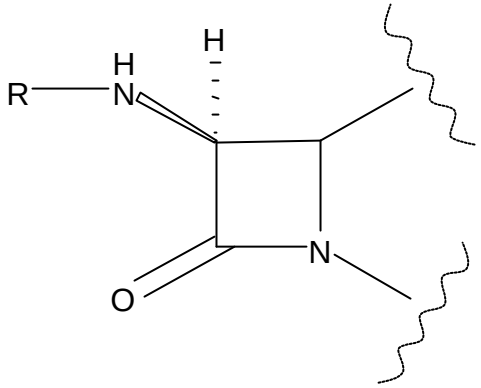
Cephems

Group II

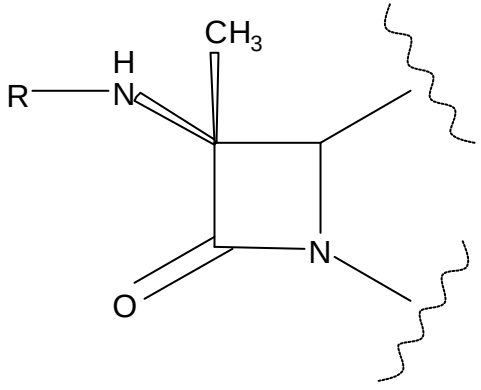
- Designed to increase the antibacterial activity against Gram negative bacilli (enterobacteriaceae).
- Increase stability to ß lactamase hydrolysis.

Cephems - Group II

- Cephalosporins



- Cephamycins

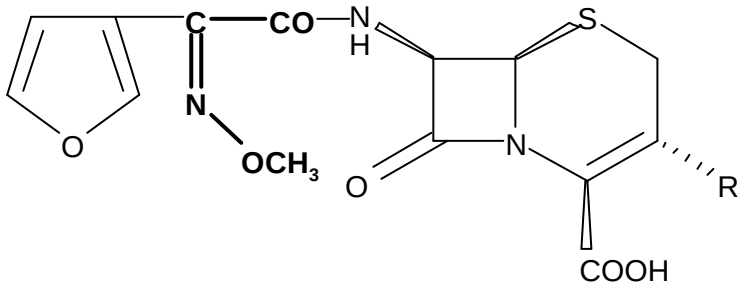


Group II-Cephems

- Limited spectrum cephems, stable to broad spectrum ß-lactamases
- Less active against *S. aureus* (penicillinase-producing strains) than group I compounds
- More active against Enterobacteriaceae than group I cephems.

- Cephem

  Cefuroxime was the first derivative with an oxime side-chain.



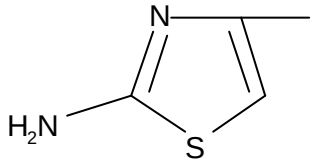
Cephems - Group II

In vitro activity

	MIC (mg/l)			
	Cefuroxime	Cefamandole	Cefoxitin	
E. coli	1.0	1.0	4.0	(TEM-1)
K. pneumoniae	2.0	0.5	4.0	(CEZ-R)
S. marcescens	64.0	> 64.0	16.0	
H. influenzae	0.5	1.0	2.0	
S. aureus peni-R	1.0	1.0	1.0	
S. pneumoniae	0.12	0.25	2.0	

Group III - cephems

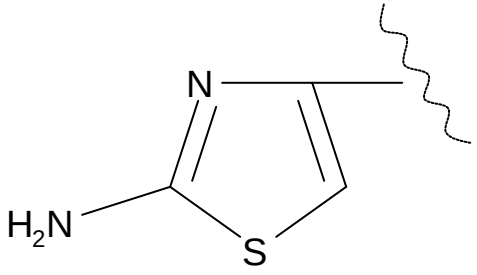
- Cephems which belong to group III have two or more of the following characteristics
  - . 2-amino-5-thiazolyl ring



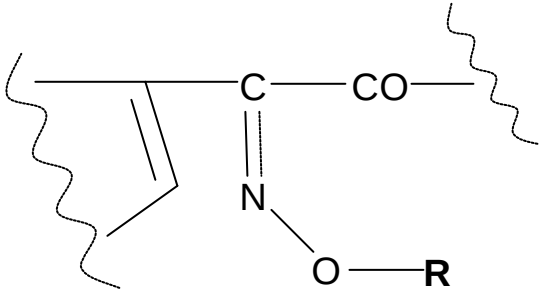
  - . broad antibacterial spectrum
  - . MIC_{50} values = 1.0 mg/l for *H. influenzae*, *Neisseria* spp, *S. pneumoniae*, *S. pyogenes*, Enterobacteriaceae (non producing class I ß-lactamases or ESBL)
  - . good stability to hydrolysis by plasmid mediated broad spectrum ß-lactamases
  - . good antipseudomonal activity.

Group III - cephems

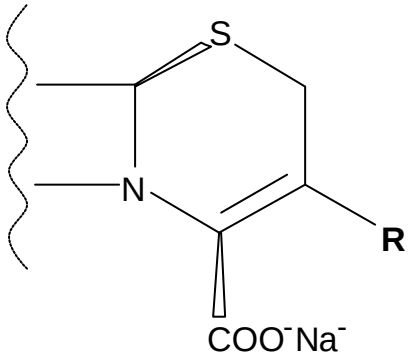
- Group III is the most important group
- All the molecules are chemically related to cefotaxime
- All have a 2-amino-5-thiazolyl ring.



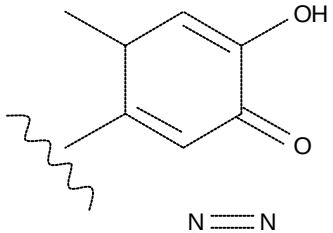
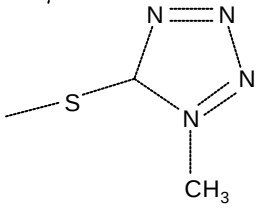
Alkoxy amino side chain at C-7

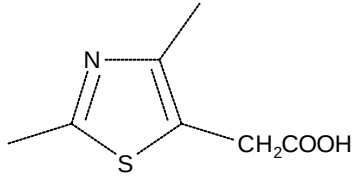
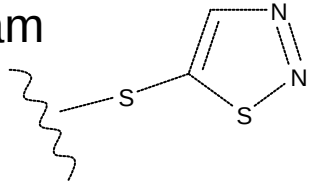


Group III - cephems



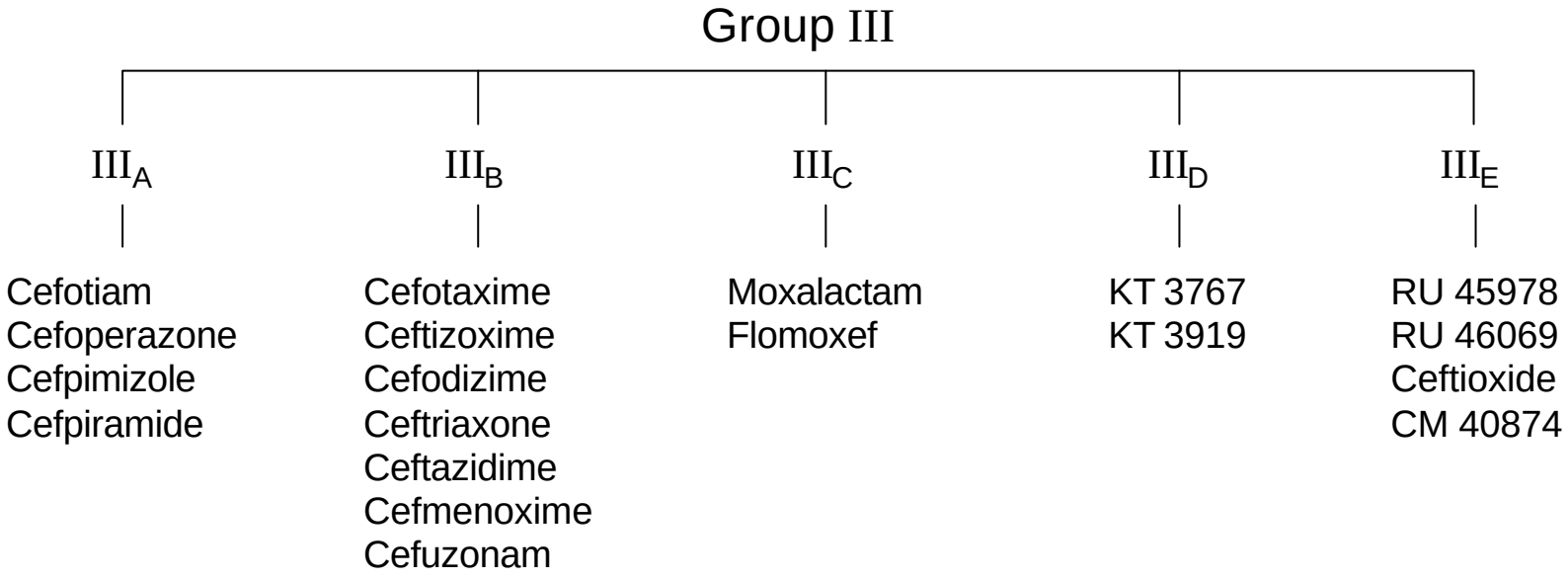
C_3 Side chain

	R
Ceftizoxime	-H
Cefotaxime	$-CH_3OCOCH_3$
Ceftriaxone	
Cefmenoxime	

	R
Cefodizime	
Cefuzonam	

Cephems

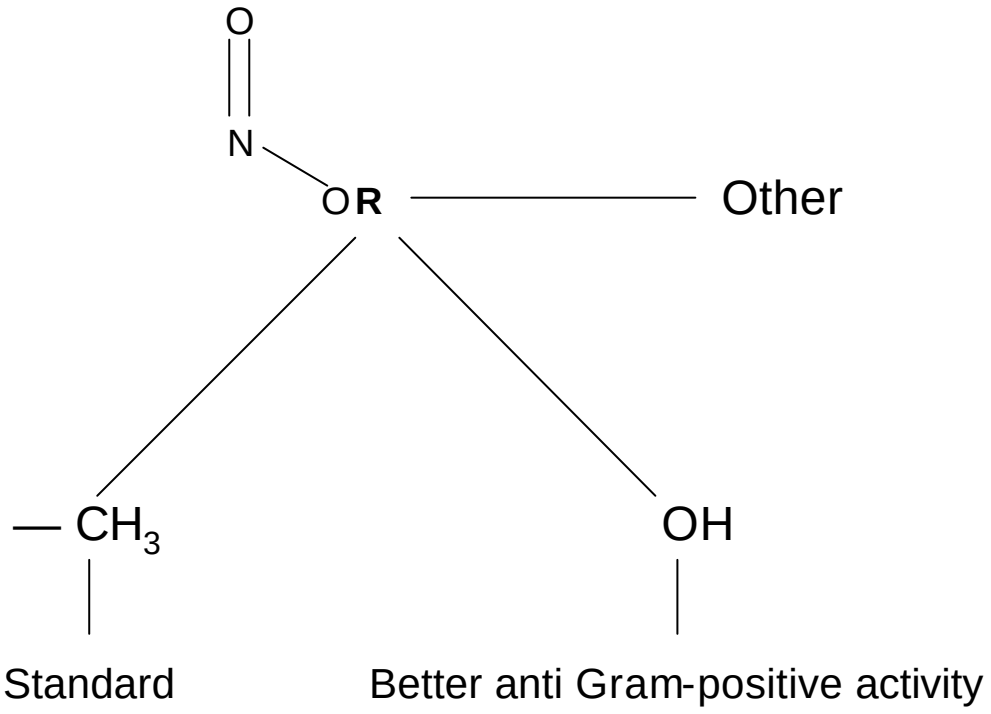
Group III - Five subgroups A to E



Cephems - Group III

Chemical modifications

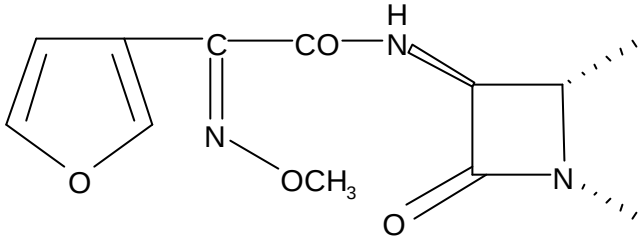
C-7 oxime side-chain



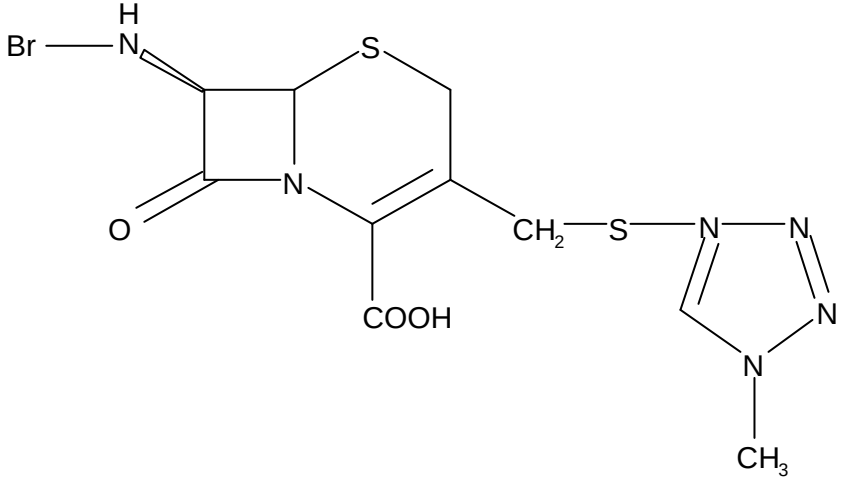
Cephems - Group III

Chemical modifications

- C-7 : methoxyimino side-chain (cefuroxime)



- C-3 : N-methyl tetrazol thio moiety (cefamandole)

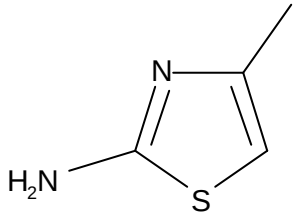


Cephems - Group III

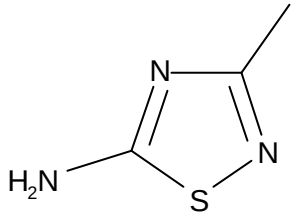
Chemical innovation

C 7moiety

2-amino 5-thiazolyl ring



5-amino 2-thiadiazolyl ring



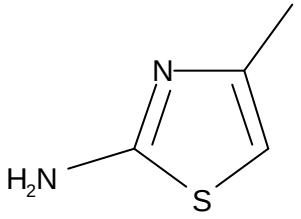
- Improve antipneumococcal activity
- Decrease anti Enterobacteriaceae activity

Cephems - Group III

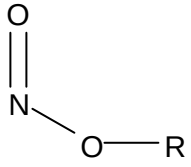
Chemical structure

- Innovation

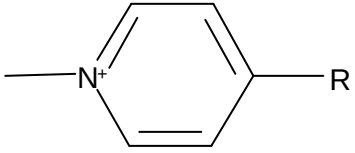
  2-amino 5-thiazolyl ring



+ Oxime side-chain



- Discovery



Antipseudomonas activity

Group III - cephems

Evolution

- Strep 1 : to improve the antibacterial activity extend the antibacterial spectrum
  → Cefotaxime
- Strep 2 : to improve the pharmacokinetic profile
  → Ceftriaxone
- Strep 3 : acquisition of new properties : immunorestoration
  → Cefodizime

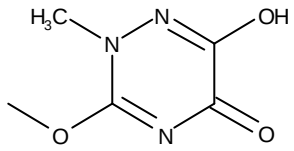
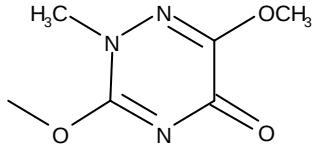
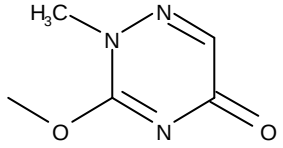
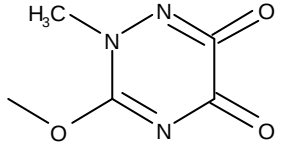
Group III - cephems

Improve the antibacterial activity

	MIC (mg/l)	
	Cefamandole	Cefotaxime
S. pneumoniae	0.25	0.12
E. coli Ampi-S	1.00	0.03
E. coli Ampi-R	16.00	0.12
K. pneumoniae cefazolin-S	0.50	0.12
K. pneumoniae cefazolin-R	> 128.00	0.12
Enterobacter spp	32.00	0.03
C. freundii	8.00	0.12
Indd + *Proteus*	8.00	0.12
S. marcescens	> 64.00	0.12
H. influenzae ß-	1.00	0.03
H. influenzae ß+	8.00	0.06

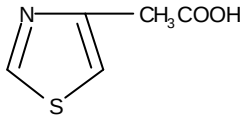
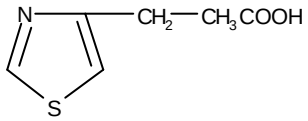
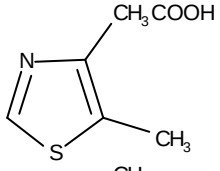
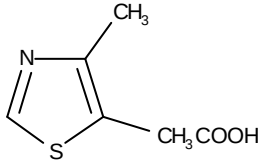
Group III - cephems

Long-acting cephem : ceftriaxone

	Elimination half-life (min) (rats)
	35
	12
	10
	10

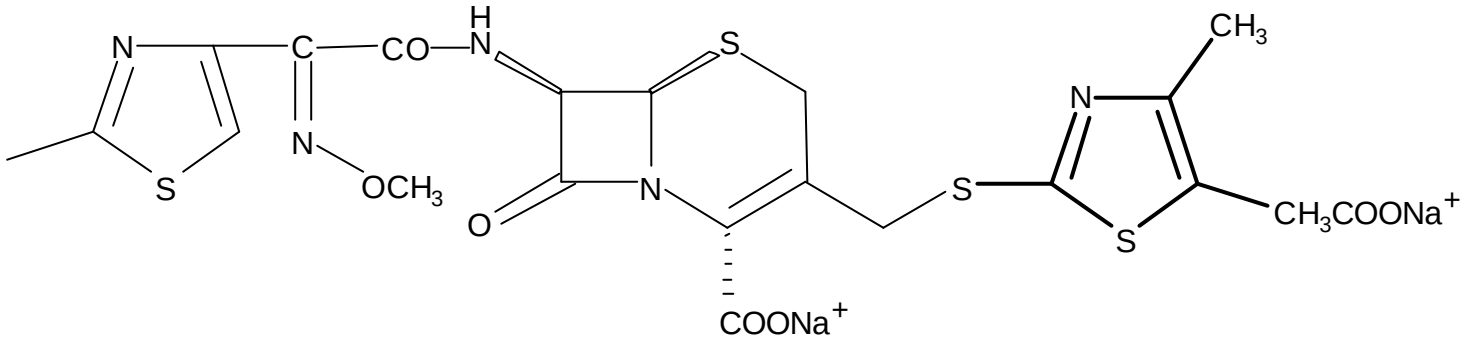
Cefodizime

Structure-activity-relationships (pharmacokinetics)

	MIC (mg/l)	
	T ½ (h)	AUC (mg.h/l)
	0.27	8.5
	0.31	1.8
	0.52	15.2
 Cefodizime	1.28	40.7
Cefotaxime	0.30	21.4

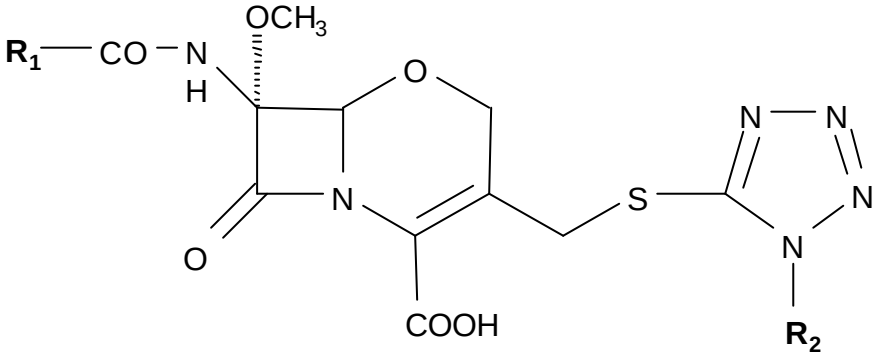
Group III - cephems

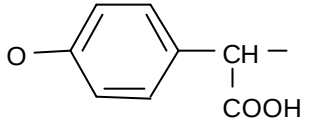
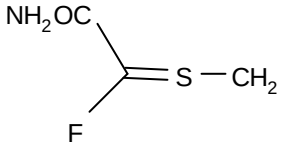
Cephalosporin BRM : cefodizime



Cephems

Group III - oxa-1-cephem



	R_1	R_2
Latamoxef		$-CH_3$
Flomoxef	$F_2-CH-S-CH_2-$	$-CH_2-CH_2OH$
2355-S		$-CH_2-CH_2OH$

Cephems

Group III - oxa-1-cephem

- Flomoxef is more active than latamoxef against Gram-positive cocci

	MIC_{50} (mg/l)		
	Latamoxef	Flomoxef	Cefotaxime
S. aureus	6.25	0.39	1.56
S. epidernidis	25.00	1.56	1.56
S. pneumoniae	1.56	0.10	= 0.025

- Latamoxef and flomoxef share tho same *in vitro* activity against Gram-negative bacilli
- Latamoxef is responsible of disuliram-like syndrome and hyprothrombinemia (N-methyl substituent) and bleeding (α carboxylic group at C-7).

Cephems - Group IV

- These compounds have been designed overcome class I producing strains within Enterobacteriaceae.

Cephems

Group IV - Definition

- Group III definition
- C3 quaternary ammonium moiety
- Activity against Enterobacteriaceae producing class 1 ß lactamase.

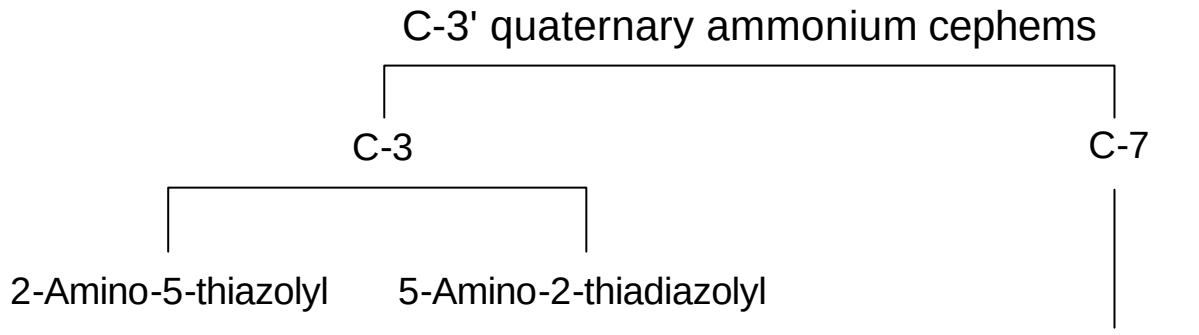
Cephems

Group IV - Antibacterial activity

- Enhance activity against Enterobacteriaceae producing class I ß lactamase (Amp C)
- Mechanism of action
- Some compound retain good anti Gram positive activity (cefpirome, cefozopran)
- Hydrolysis by ESBL.

Cephems

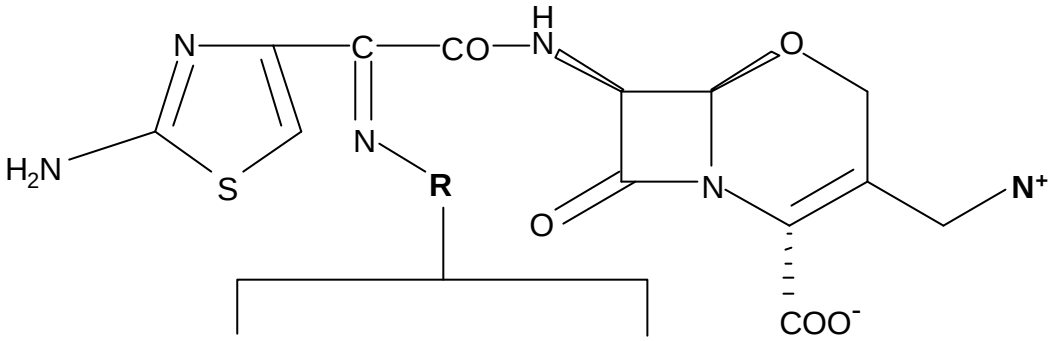
Group IV - Classification



	Ceftazidime	Cefclidin	L-640876
	Cefpirome	Cefozopran	L-642946
	Cefepime	Cefluprenam	L-652813
	Cefquinone	FK 518	
	Cefoselis	YM 40220	
	DQ-2556		
DN 9550	CS-461		
ICI 194008	ME-1228		
ICI 193428	MT-520		
TOC-39	MT-382		
TOC-50	ME-1221		
CP 6679	ME-1220		

Cephems - Group VI

- C-3 quaternary ammonium cephem are zwitterionic compounds



Negative charge
(SO^- or COO^-)

Negative charge
(OCH_3)

Dianionic cephems
Ceftazidime
Cefsulodin

Zwitterionic cephems
Cefpirome
Cefepime
Cefoselis
Cefclidin
Cefluprenam

Cephems - Group IV

Mechanism of action

Velocity through outer membrane + **Poor affinity to ß-lactamase** + **Strong affinity to PBPs**

Cephems

Group IV - Weaknesses

- Hydrolysis by ESBL
- Variable activity against *P. aeruginosa*
- Short elimination half- life (≈ 2 hours).

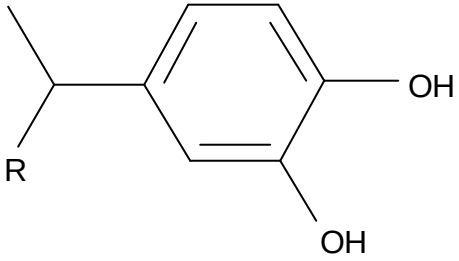
Cephems

Group V

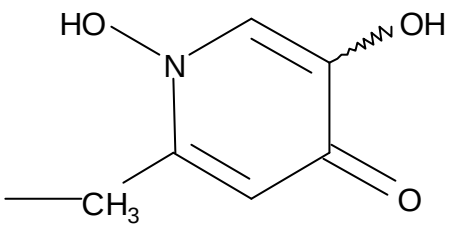
- Designed to overcome resistance due to ESBL, producing strains
- Increase *in vitro* activity against *P. aeruginosa*

Cephems - Group V

Chemical modification



Catechol moiety
fixes on the oxime chain

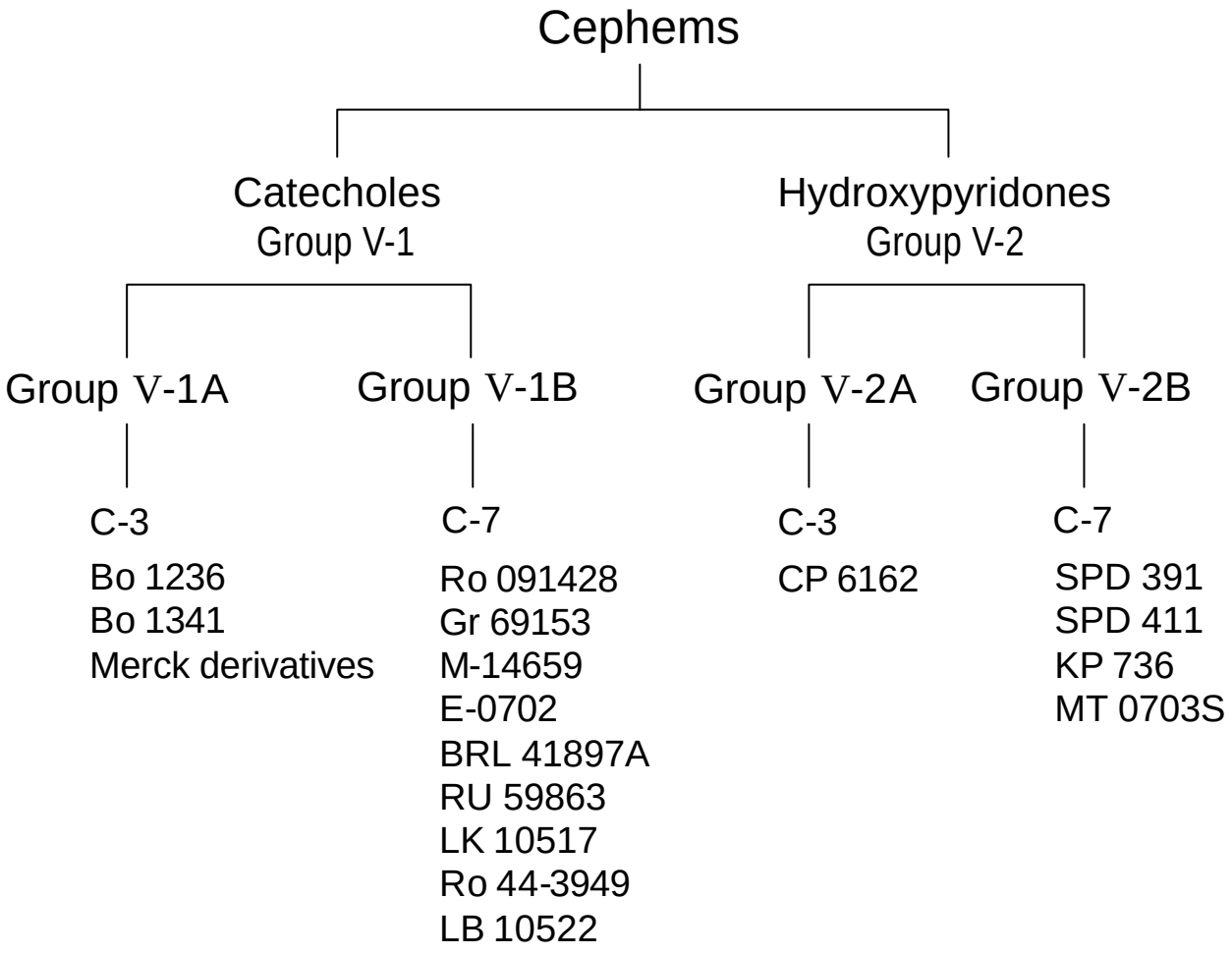


Pyrridone fixes

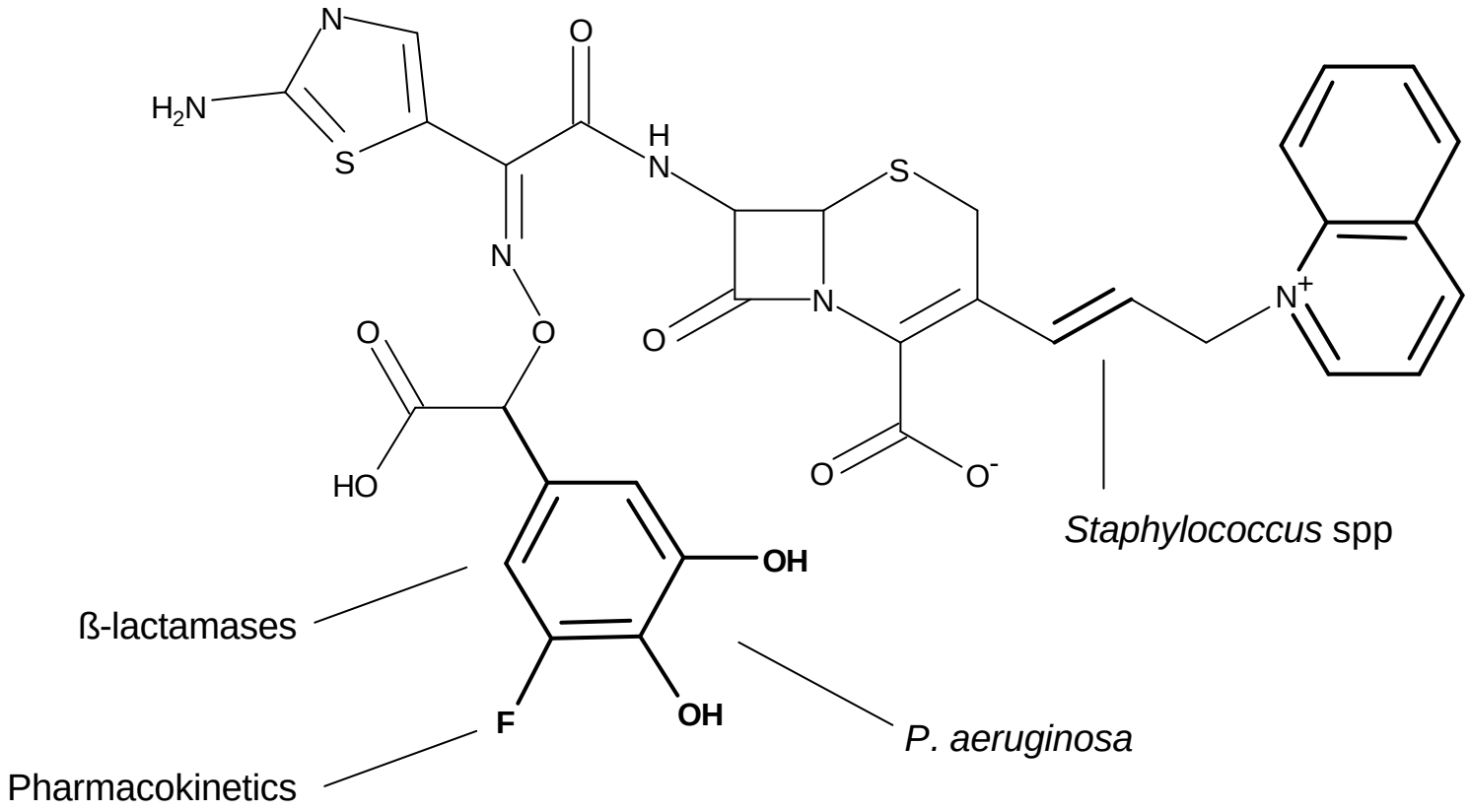
on the oxime chain

Cephems

Group V - Classification



RU 59863


ß-lactamases
Pharmacokinetics
Staphylococcus spp
P. aeruginosa

Cephems - Group V

- Antibacterial activity
  - . overcome ESBL
  - . original additional mechanism of action : Fe^{2+} chelation.
- Wearknesses
  - . metabolism
  - . tolerance (?)
  - . cost of production.

Cephems - Group VI

Investigations

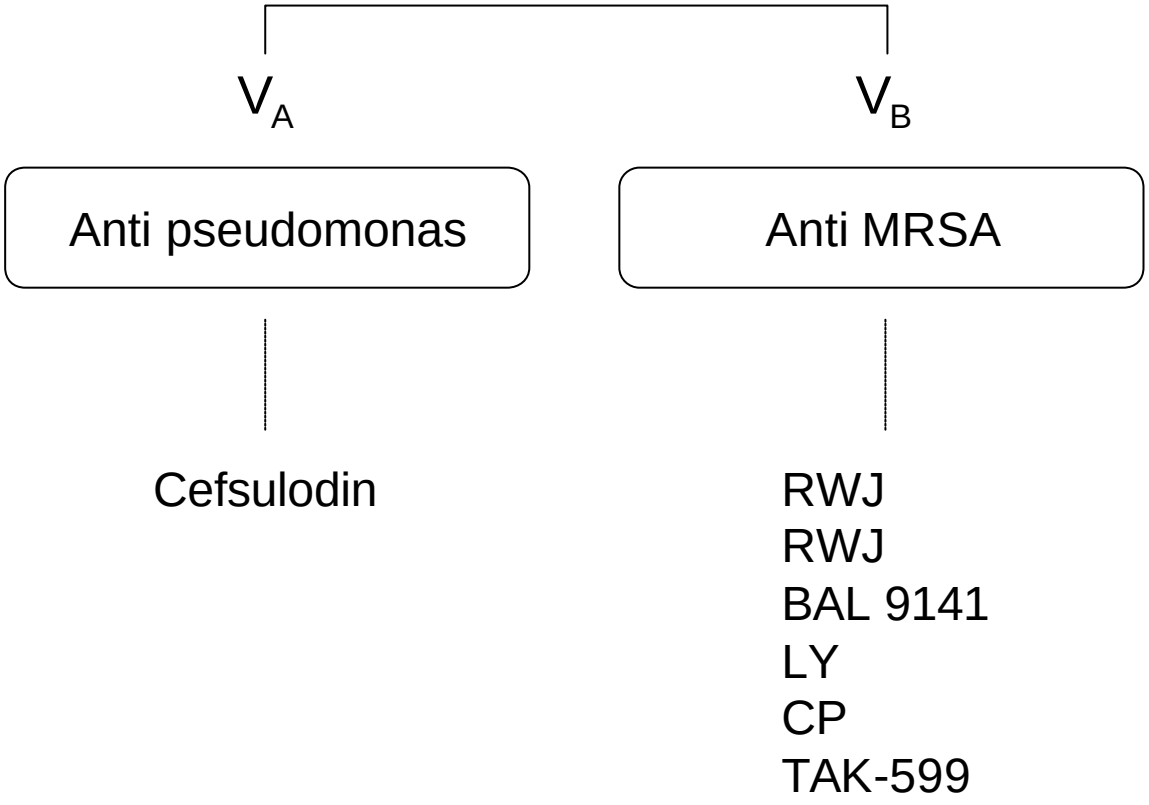
. *In vitro* .... MIC

. Bactericidal activity

. Affinity for PBP_{2a}

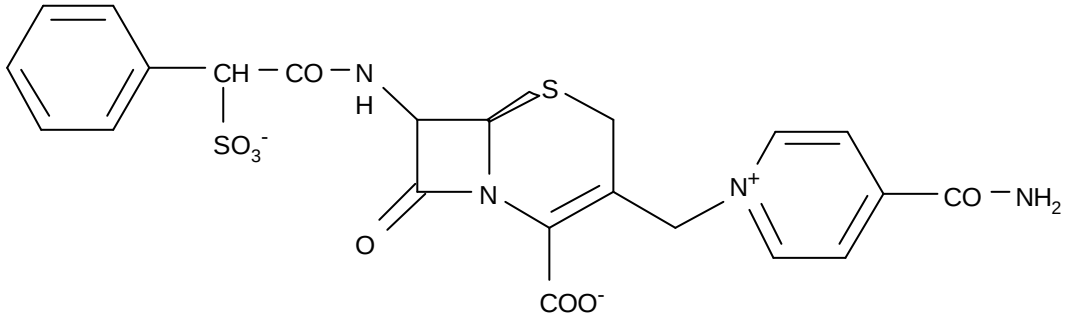
. *In vivo*

Cephems - Group VI



Cephems - Group VI

- Cefsulodin
  - . Dianionic compound : derived from sulbenicillin
- Anti pseudomonal activity



Cephems group VI

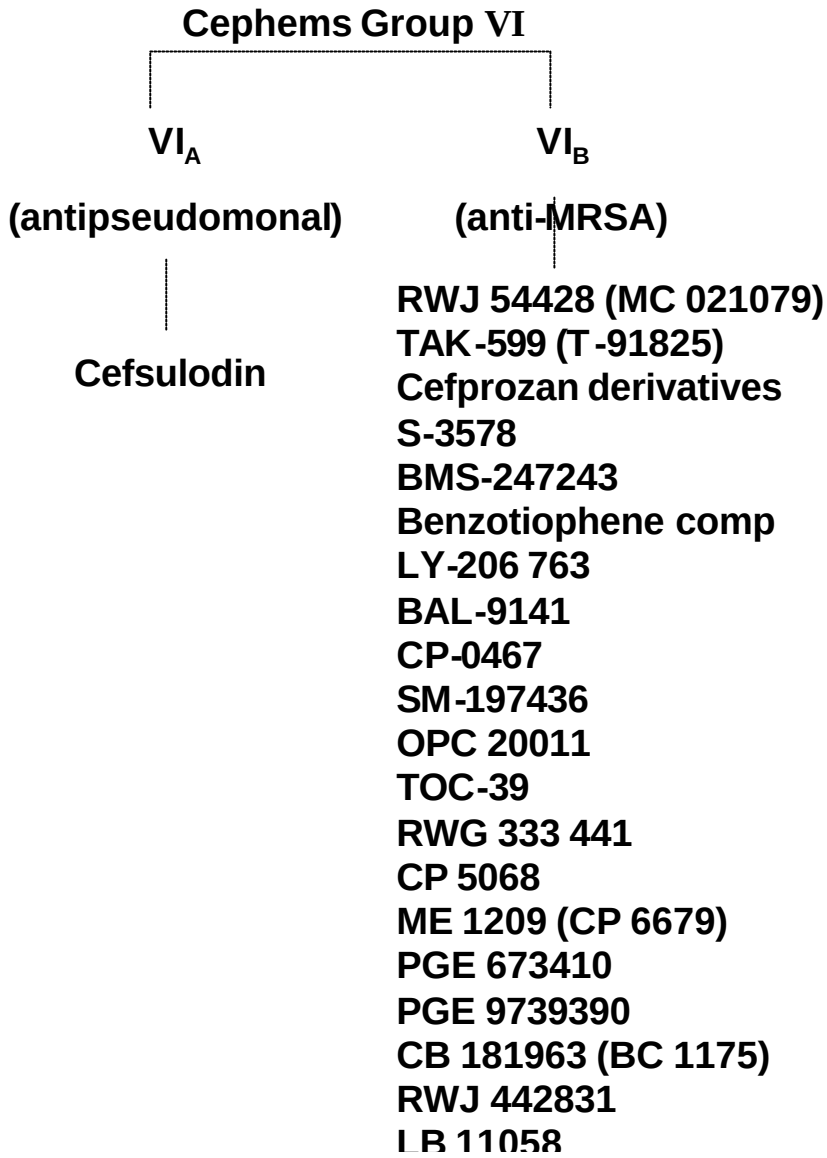
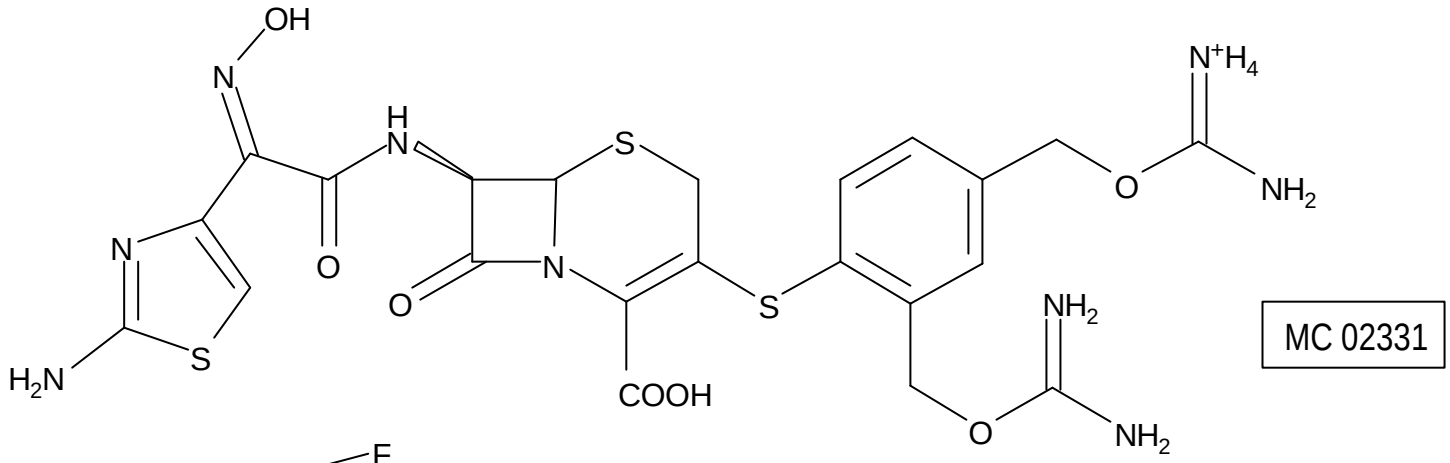


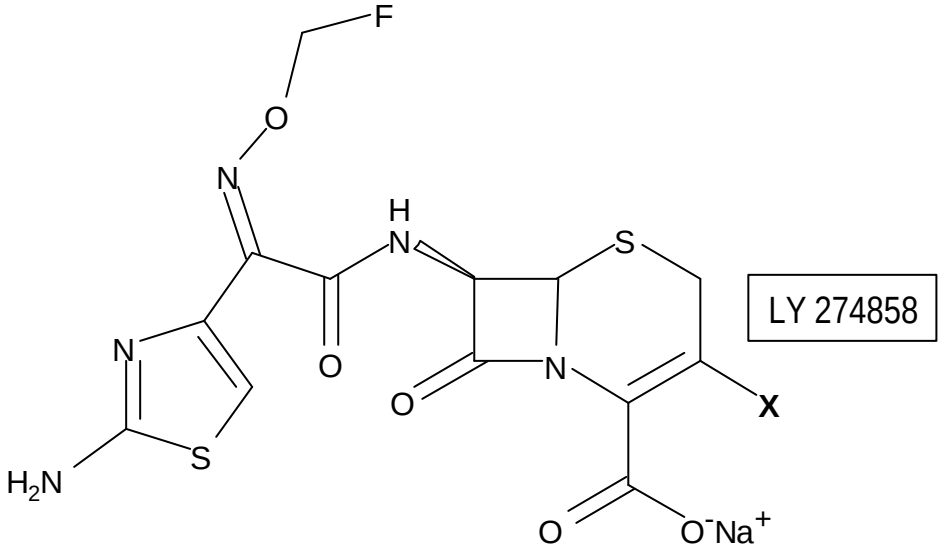
Figure 57

Cephems - Group VI

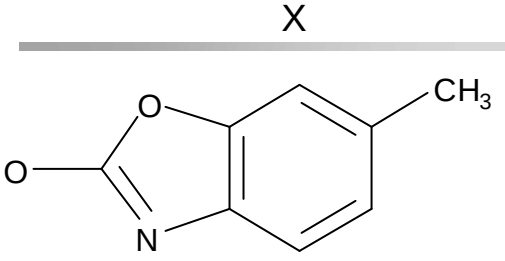
Cephems designed for an anti MRSA activity



MC 02331



LY 274858



Cephems - Group VI

Antibacterial activity

	MIC (mg/l)		IC_{50} (mg/l)
	MRSA heterogenous	MRSA homogenous	PBP 2’
LY 274858	1.0	1.9	3.5
Methicillin	256.0	> 52	456.0
Nafcillin	32.0	128	200.0

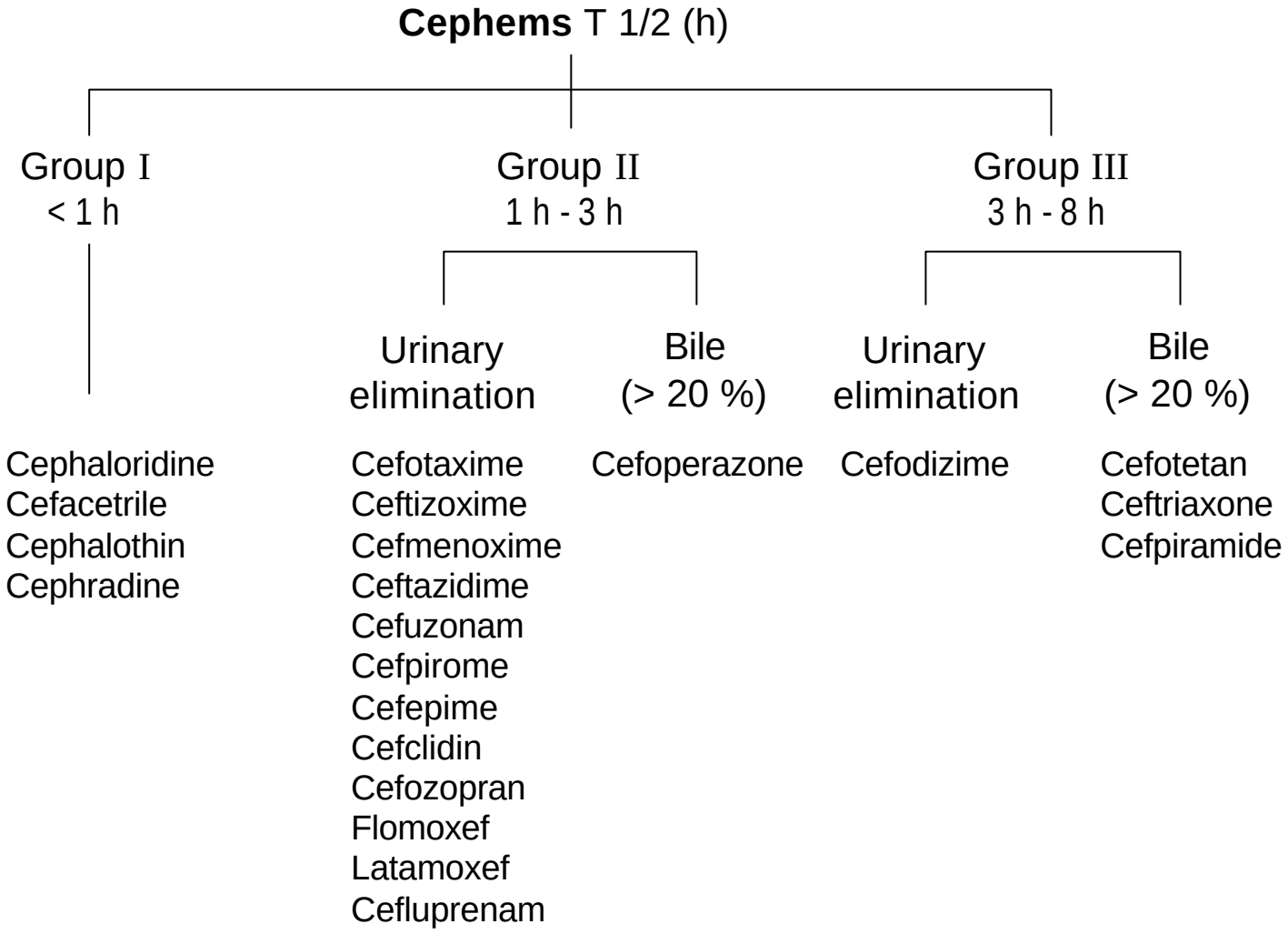
Cephems

Pharmacokinetics classification *(1)*

- Apparent elimination half- life (three groups)
- Subdivision : elimination route

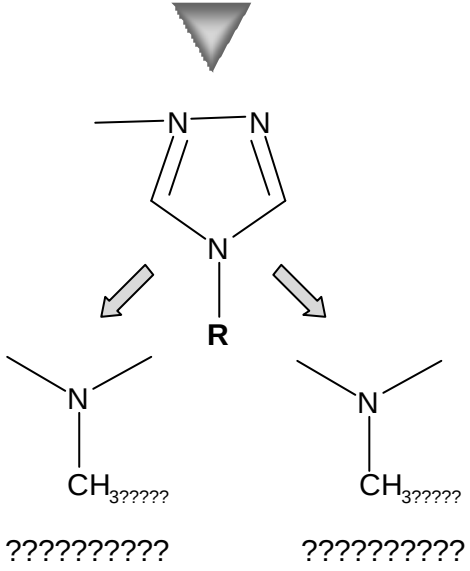
Cephems

Pharmacokinetics classification



Cephems - Group II

- Improvement of antibacterial activity
- Fixed on numerous cephems within group II and III
- Side effect doe to the methyl group :
  disulfiram-like and hypothrombinemia
- removal of CH_3 group



Cephems - Group VI

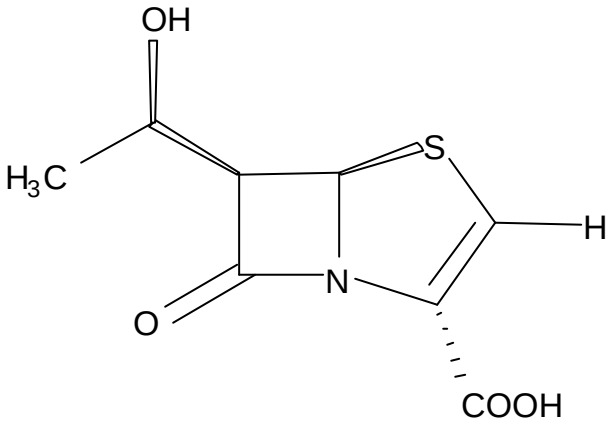
Conclusion

Powerfull successful research to meet medical needs

- *S. aureus* peni-R
- Gram-negative bacilli
- MRSA

Penems

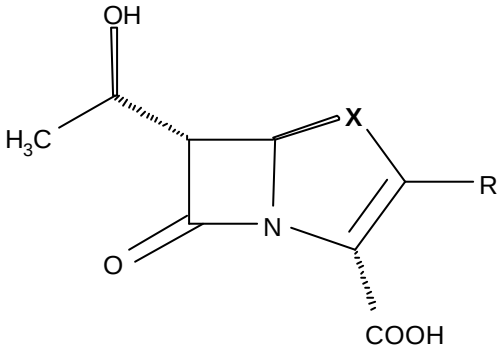
Penem

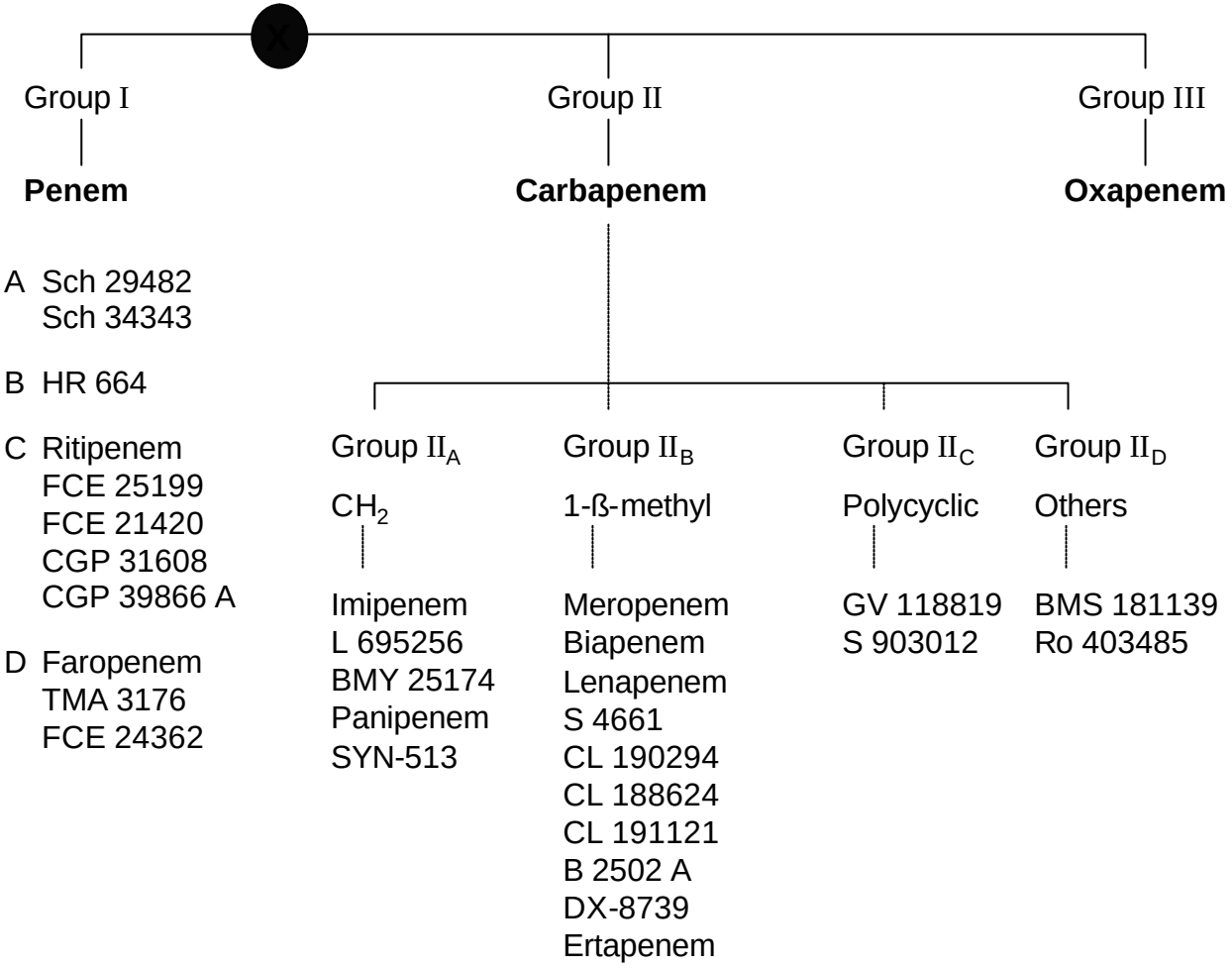


- Synthetic compounds
- Antibacterial agents
- ß lactams.

Penem

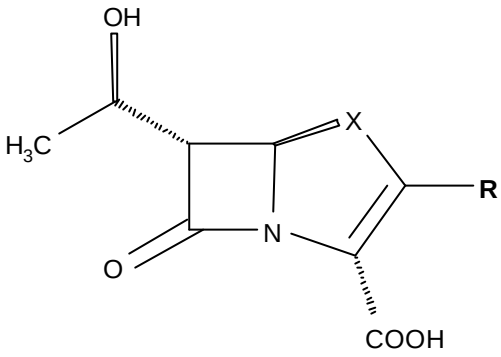
Classification

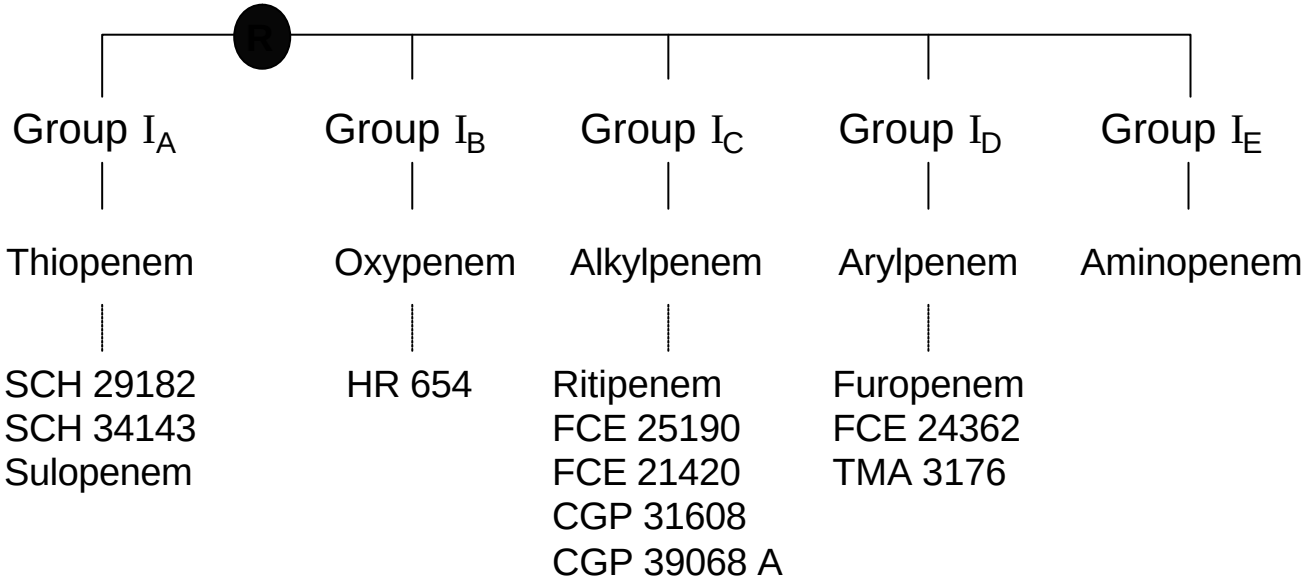




Penem

Classification



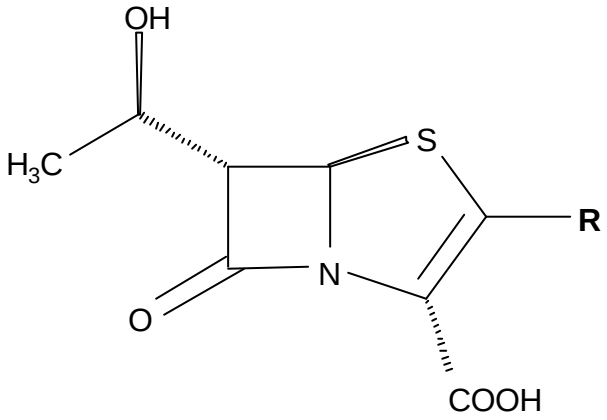


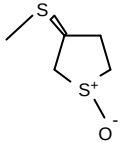
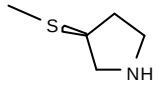
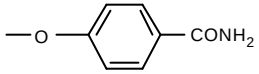
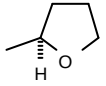
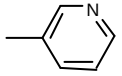
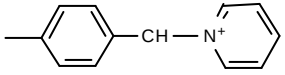
Penem

Synthetic compounds

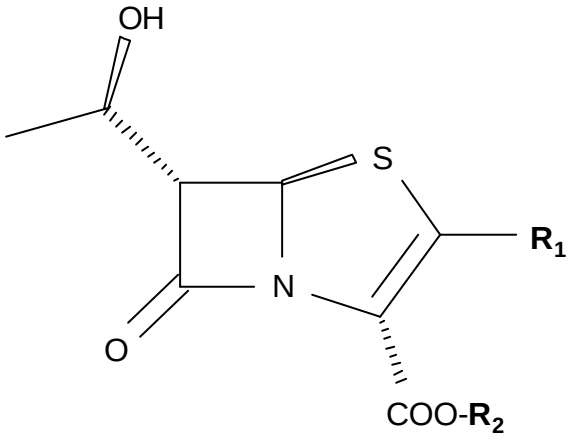
New compounds

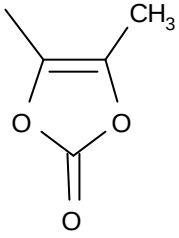
Penem



	R
SCH 29482	-S-C_2H_5
SCH 34343	-S-$C_2H_4OCONH_2$
Sulopenem	
Zeneca derivatives	
HR 664	
CGP 31608	-CH_2-NH_2
Ritipenem	-CH_2OCONH_2
FCE 21420	-CH_2OCOCH_3
FCE 24964	-CH_2OCH_3
Faropenem	
TMA 3176	
FCE 24362	

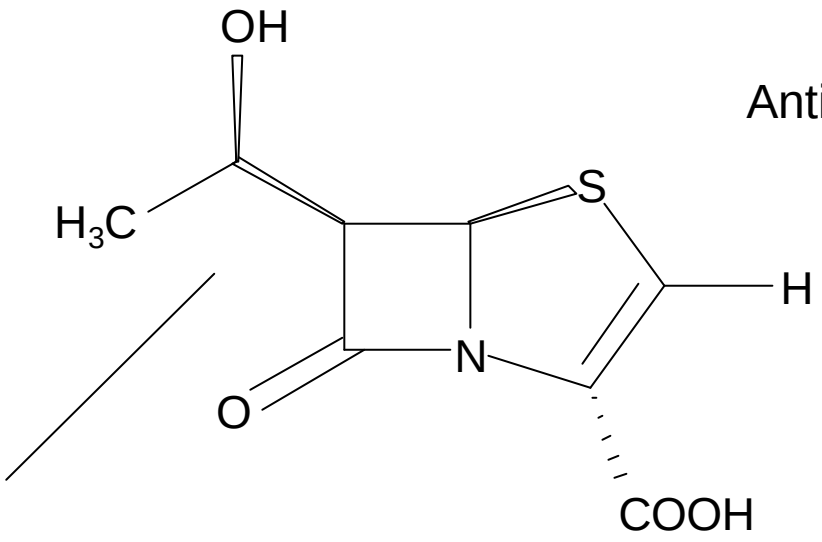
Penem



	Ester (R_2)	Parent compound
FCE 25199		FCE 24964
SUN A 0026		Fropenem
CP 65207	$-CO-C(CH_3)_3$	Sulopenem
Ritipenem-acoxil	$-CH_2OCOCH_3$	Ritipenem
TMA-230	$-CH_2OCOCH_3$	TMA-3176

Penem

Structure-activity



Antibacterial activity

ß-lactamase stability

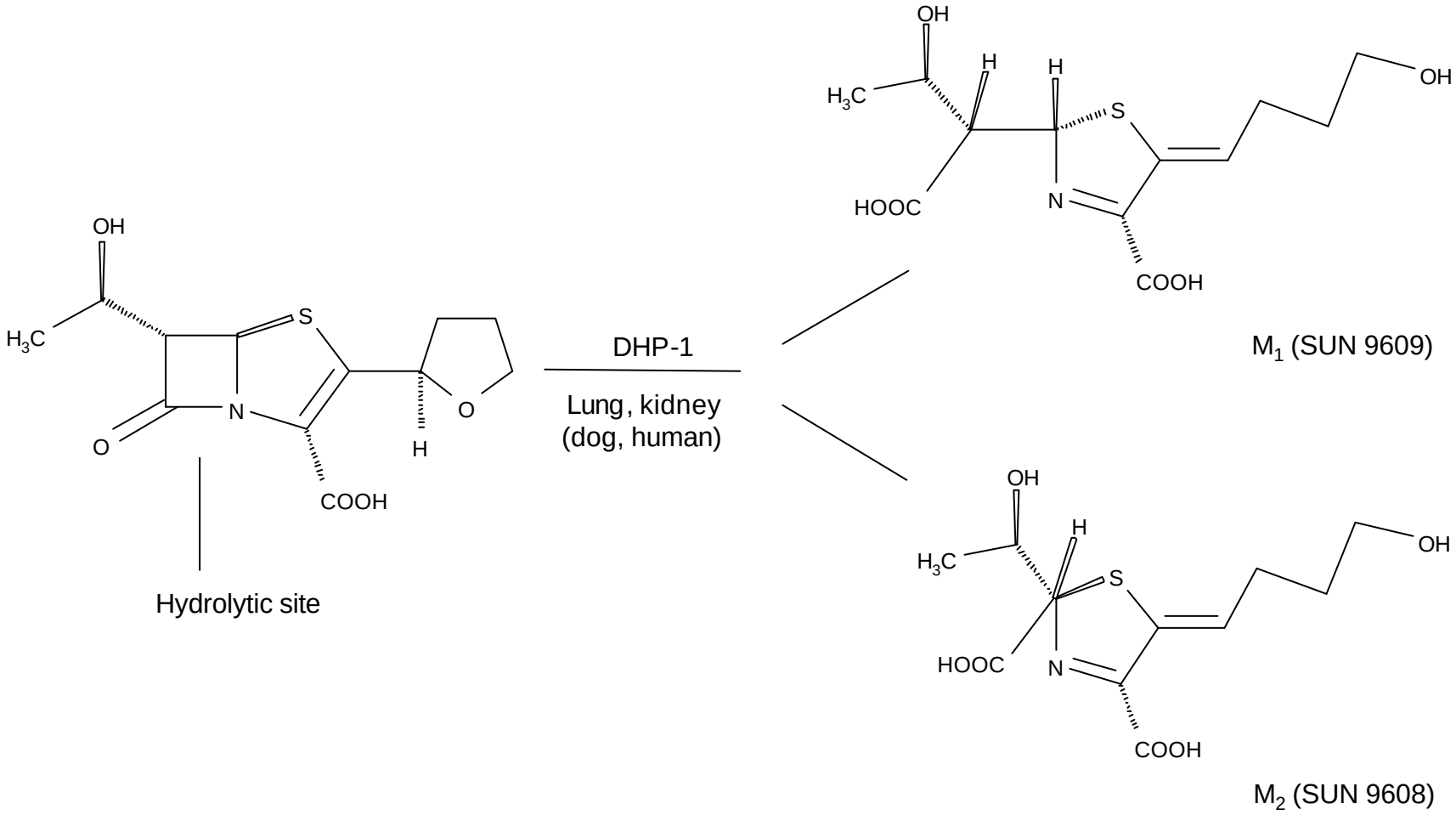
Penem

Structure-activity

- Penem nucleus displays an antibacterial activity equal to that of ampicillin against ampi-S strains
- The stereochemistry of the 6-side-chain is compulsary
- C-2 side-chain retains the antibacterial activity.

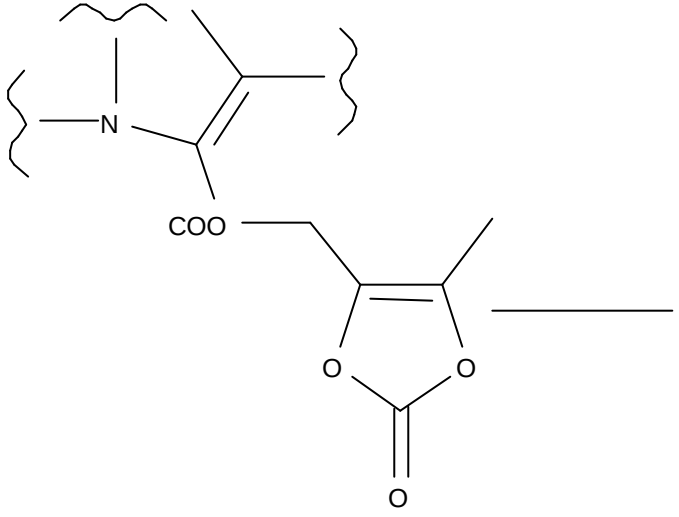
Penem

Hydrolysis by DHP-1



Penem

Dioxolenone ester : metabolism



CH_3-CO-CO-CH_3

diacetyl

Gastro intestinal tract/liver

CH_3COH-CO-CH_3

acetoine

Liver

CH_3COH-COH-CH_3

2, 3 butenadiol

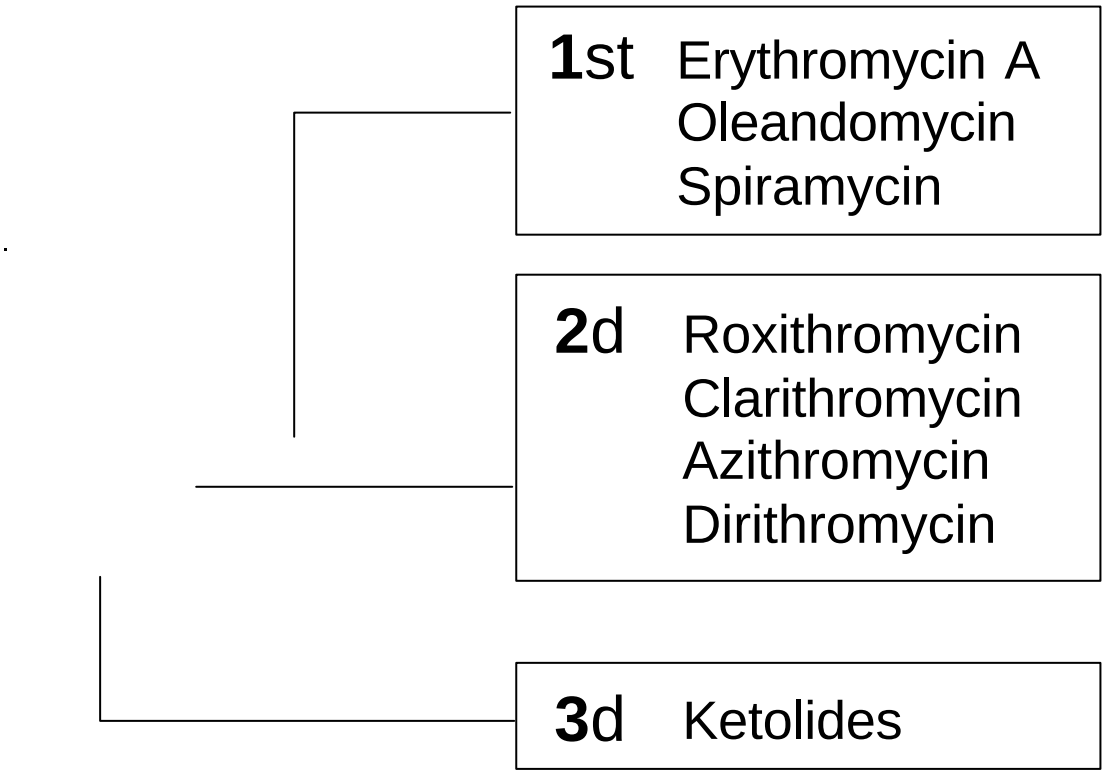
Penem

- André, je suis vraiment désolé.....
- mais je ne vois rien du tout pour la figure
- page « Fro-penem 3 »

- Macrolides

Macrolides

Three waves of macrolides



Objectives

- *S. aureus* peni-R

- Atypical microorganisms
- Improvement of pharmacokinetics

- Overcome resistance to erythromycin A
- Enhance activity against Gram-positive bacteria

Macrolides

Second wave of molecules

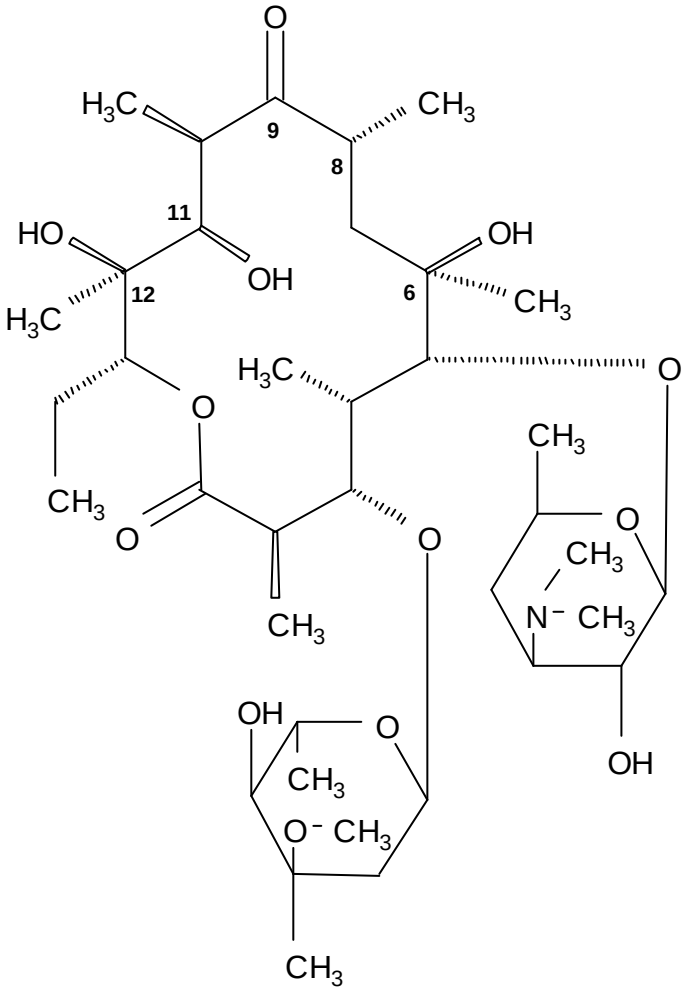
- Increase absorption
- Good stability in acid conditions
- No enhancement of *in vitro* activity against common pathogens
- Increase *in vitro* activity against atypical pathogens.

Macrolide

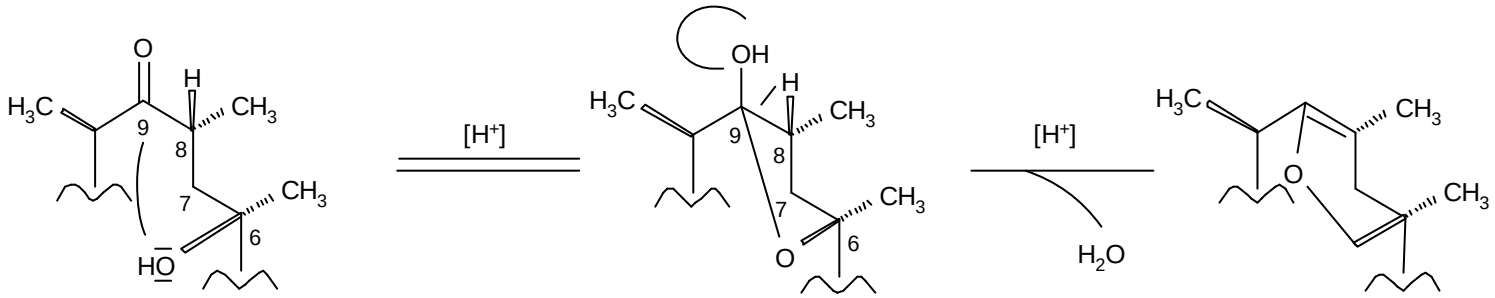
Erythromycin A	*Saccaropolyspora erythrea*	1952
Oleandomycin	*Streptomyces antibioticus*	1954
Spiramycin(s)	*Streptomyces ambofaciens*	1954
Josamycin	*Streptomyces narbonensis var. josamyceticus vova sp*	1957
Midecamycin	*Streptomyces mycarofaciens*	1971
Tylosin	*Streptomyces fradiae*	1961

Macrolide

Erythromycin A



Macrolide



Erythromycin A

Hemicetal

Didehydro-erythromycin A

Macrolide

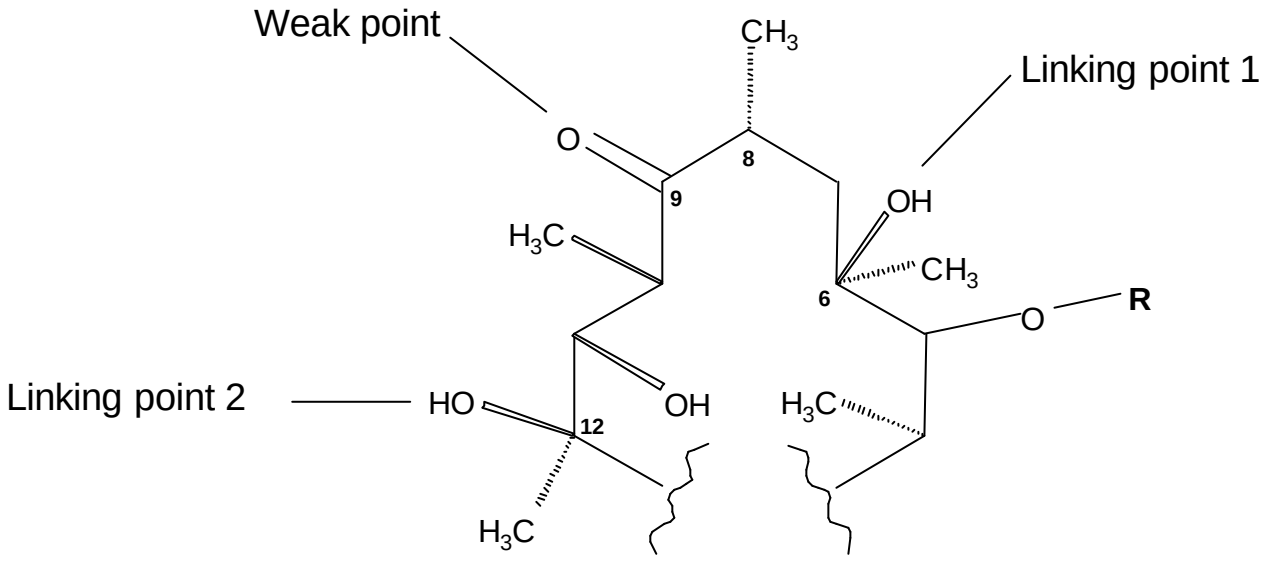
	Concentrations (plasma)	
2'-ester erythromycin A	100 mg/L	5 mg/L
2'-propionyl	69	247
2'-ethylsuccinyl	39	60
2'-acethyl	39	170
2'-butyryl	188	353
2'-valeryl	492	478

Macrolide

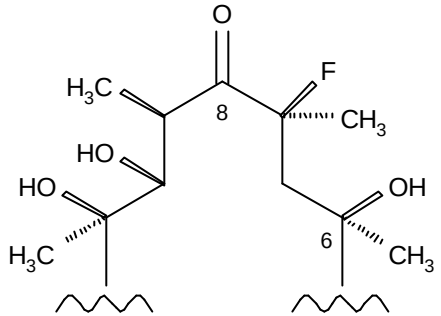
	Erythromycin A	Anhydroerythromycin A
Cmax (mg/L)	0.9 ± 0.2	0.3 ± 0.1
Tmax (h)	2.6 ± 0.4	2 ± 0
T ½ (h)	1.5 ± 0.1	3.3 ± 2.4
AUC_{0-8} (mg.h/L)	4.5 ± 1	4.1 ± 2.4

Macrolide

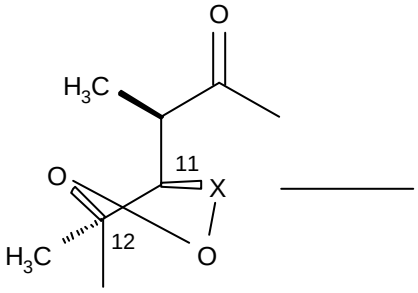
Weak and link points of erythronolide A



Macrolide

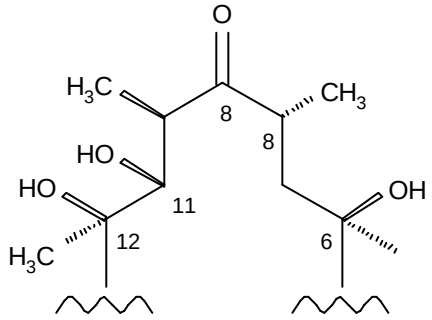


Flurithromycin

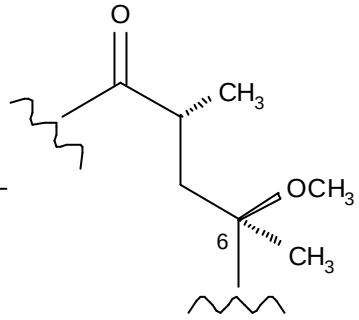


X = O : Davercin
X = N : Carbamate

R
other



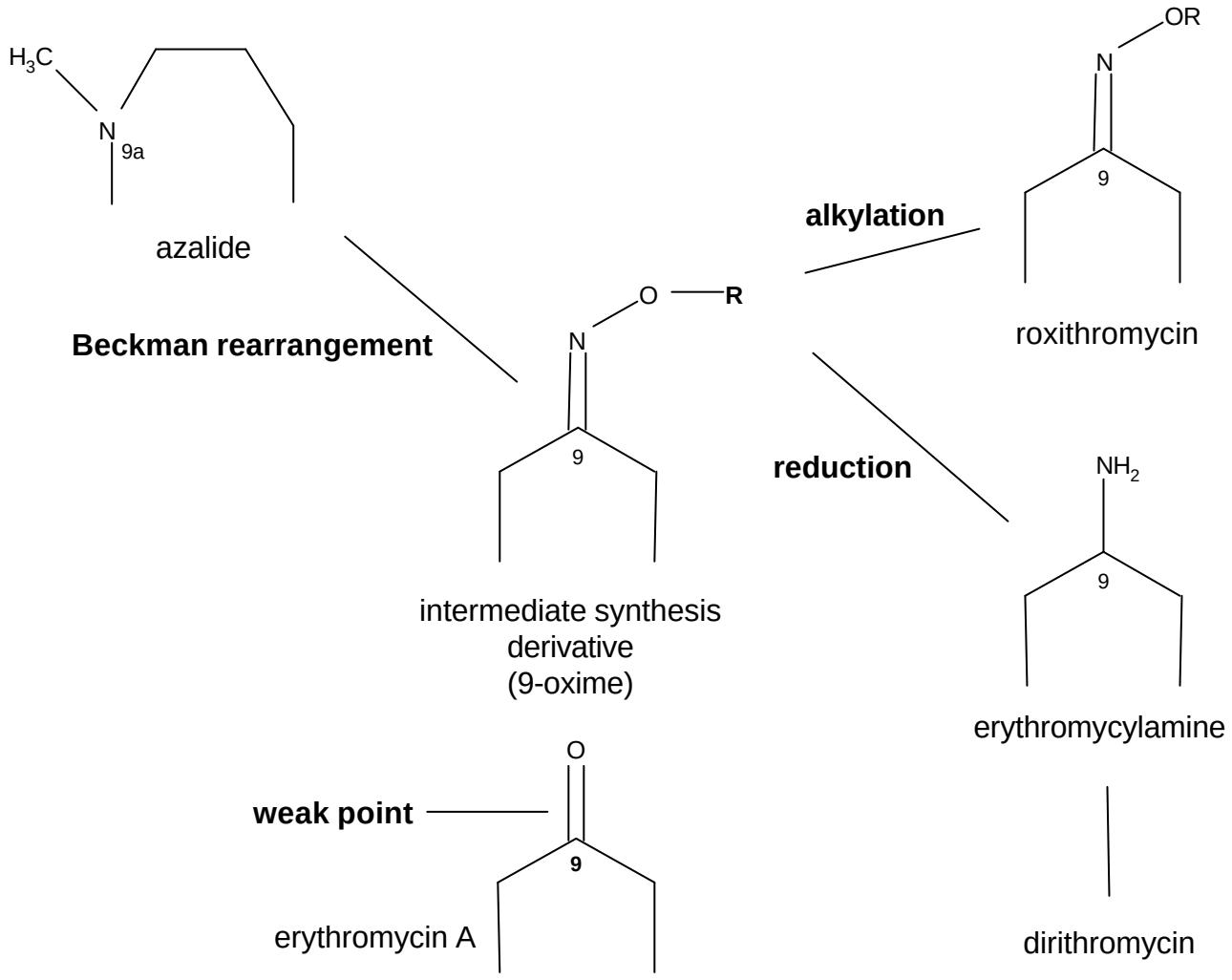
Erythromycin A



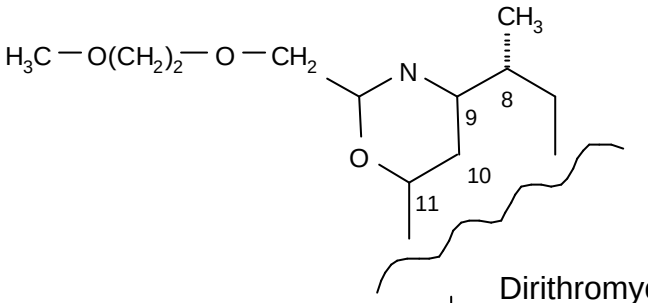
Clarithromycin

Macrolide

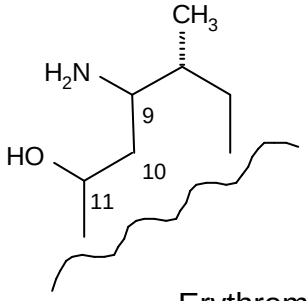
Semisynthetic derivatives of 9-erythromycin A



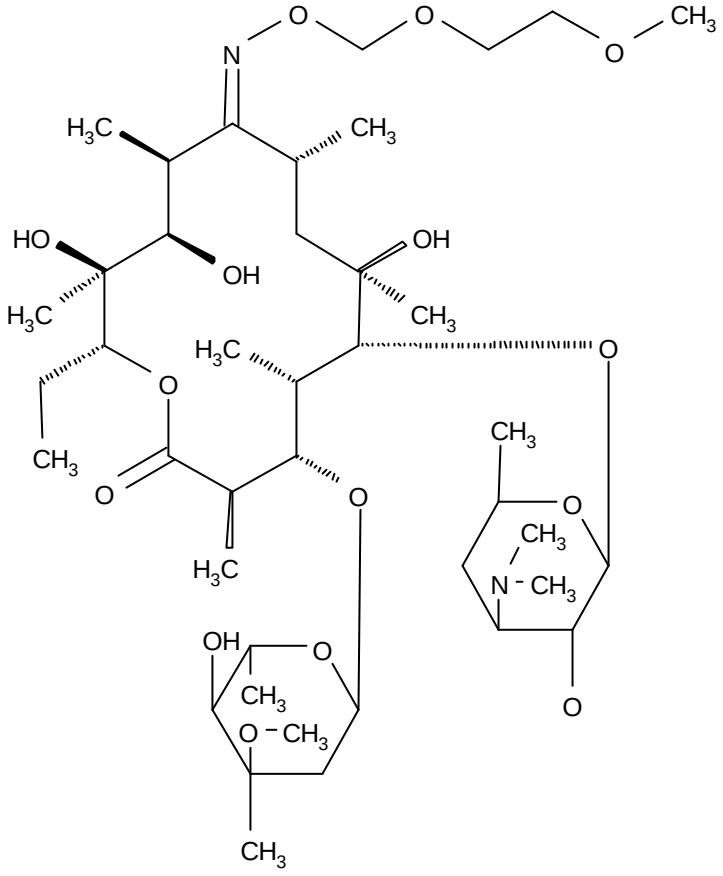
Macrolide



Dirithromycin

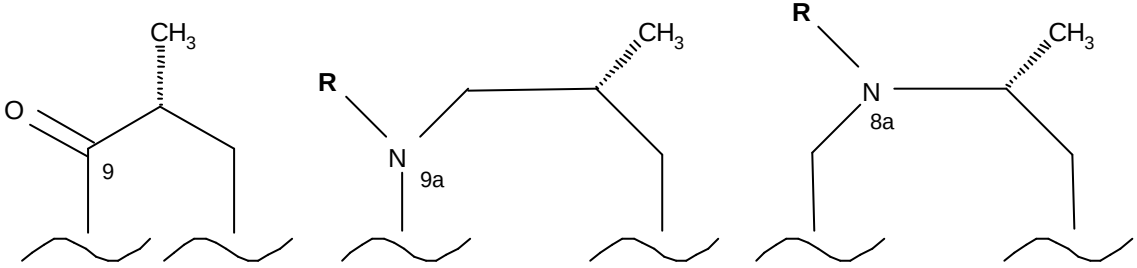


Erythromycylamin

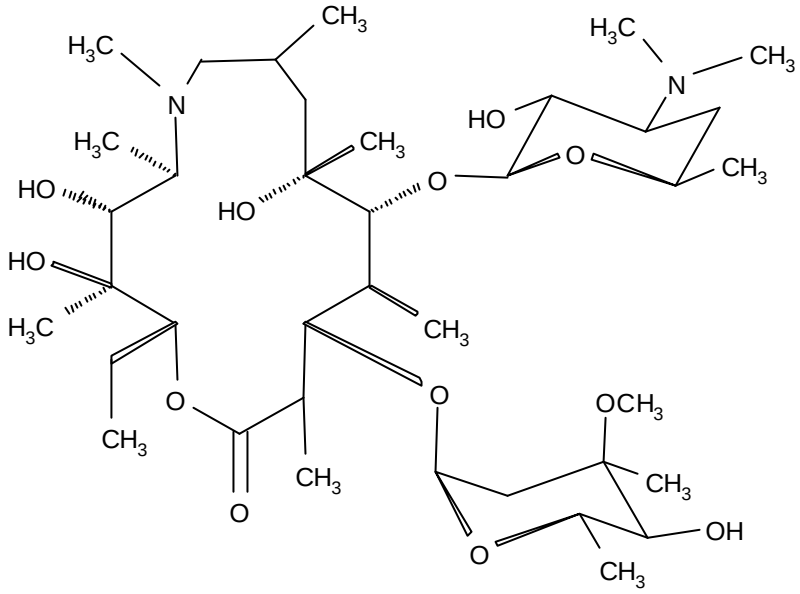


Roxithromycin

Macrolide

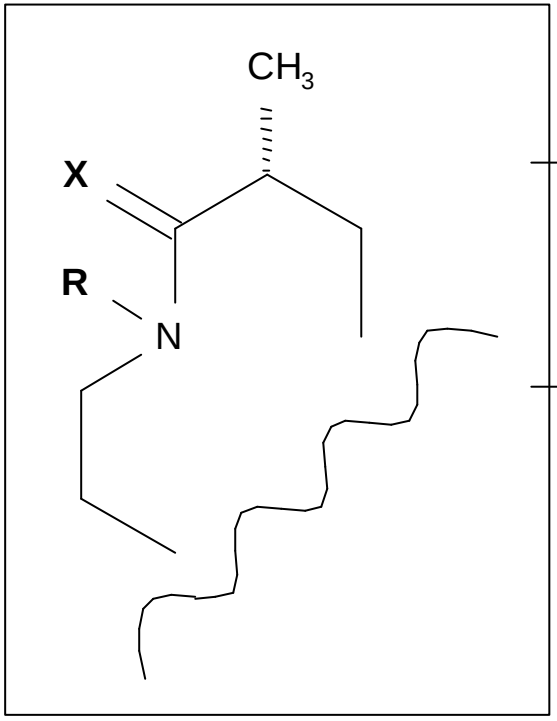


Erythromycin A 9a-azalide 8a-azalide



Azithromycin

Macrolide



N-10-methyl azalide

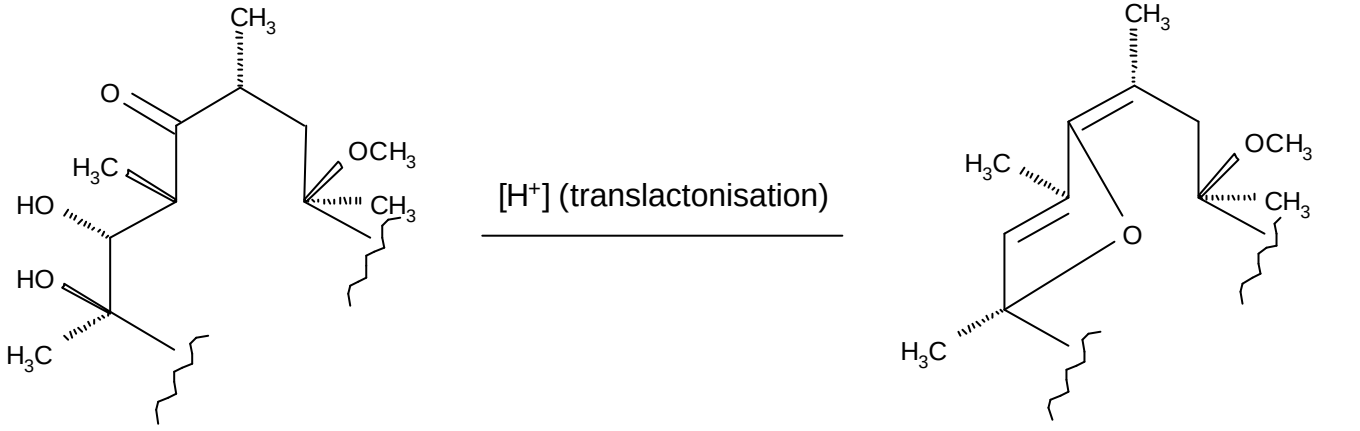
X = H
R = CH_3

Lactam

X = O
R = H

14-membered ring azalides

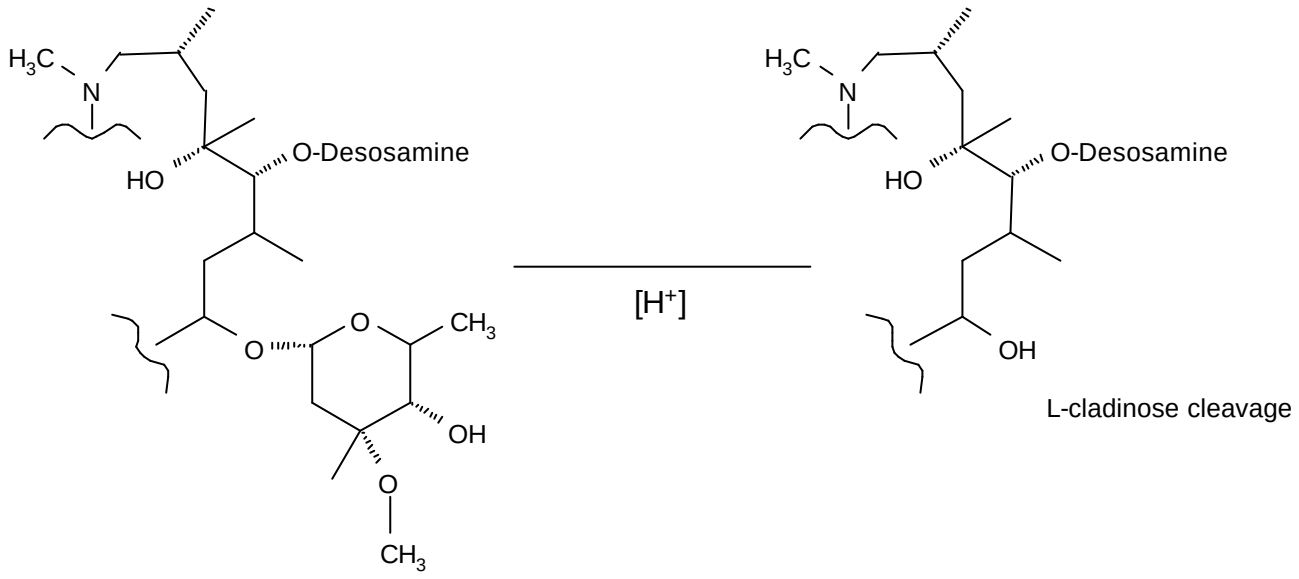
Macrolide



Clarithromycin

Pseudoclarithromycin

Macrolide

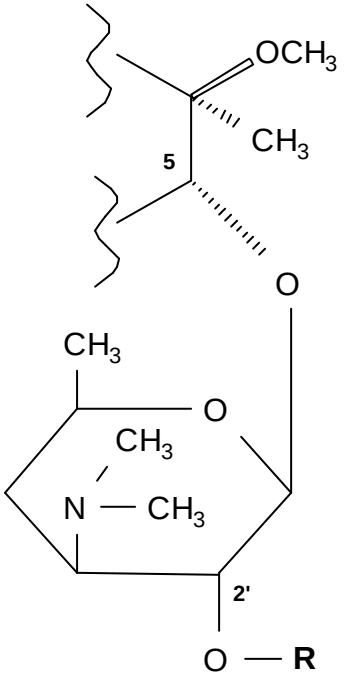


Azithromycin

CP 66458

Macrolide

2'-esters erythromycin A



D-desosamine

2'-ester	R	Sale
Acistrate	$COCH_3$	$CH_3(CH_2)_{16}COOH$
Ethylsuccinate		-
Estolate	$OCCH_2CH_3$	$C_{12}H_{25}OSO_3H$
Propionate	$OCCH_2CH_3$	-
RV-11	$OCCH_2CH_3$	HS—CH—COOH H2C—COOH

- Ketolides

Ketolides

Definition

- Semisynthetic derivatives of erythromycin A
- Lack of α L cladinose
- Highly stable in acidic environment
- Overcome erythromycin A resistance (MLS_B inducible, efflux)
- Unable to induce MLS_B resistance.

ketolides

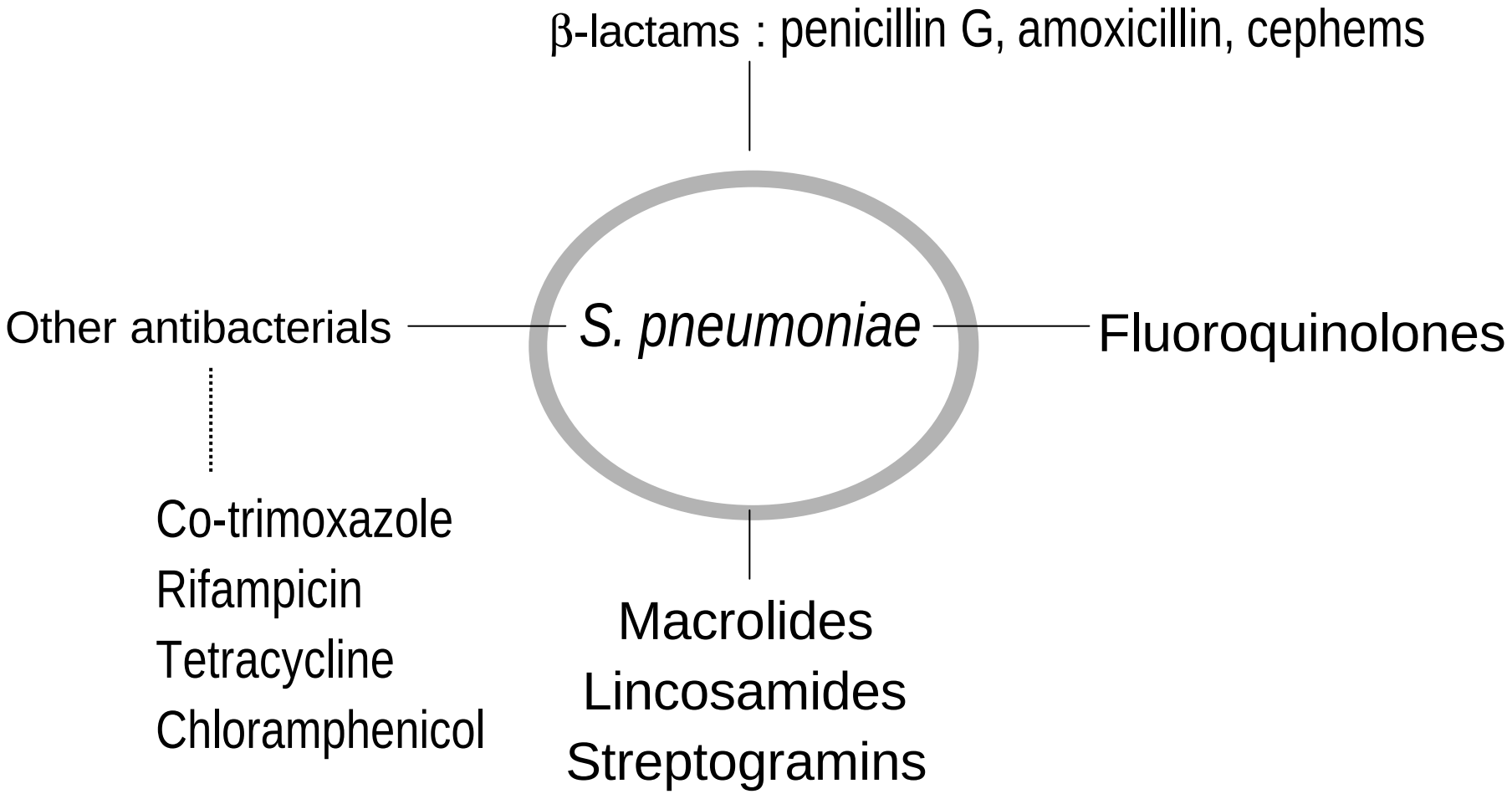
- Same pharmacokinetic profile
- Activity against erythromycin A resistant isolates.

ketolides

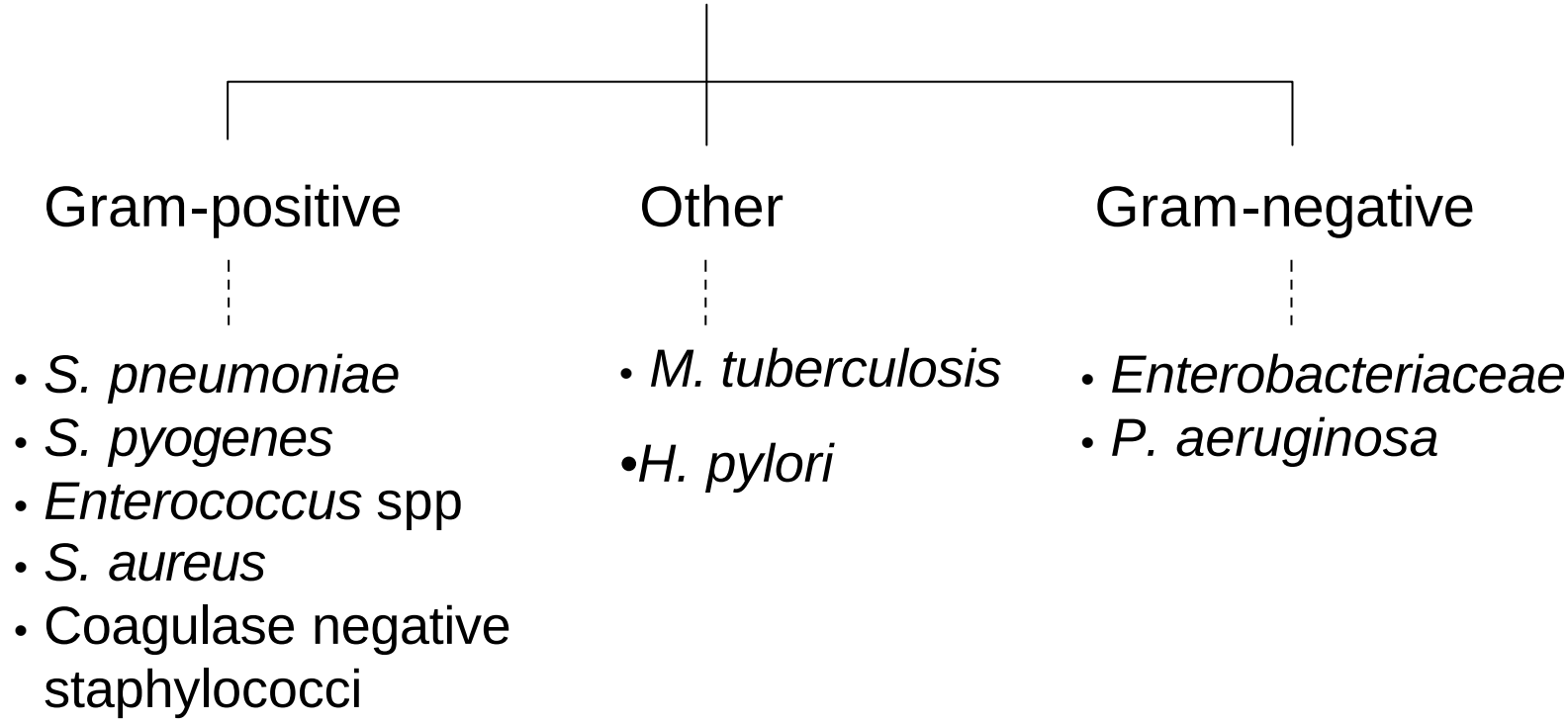
Target

- Same pharmacokinetic profile of macrolides
- Activity against erythromycin A resistant isolates

Bacterial resistance to antibacterials

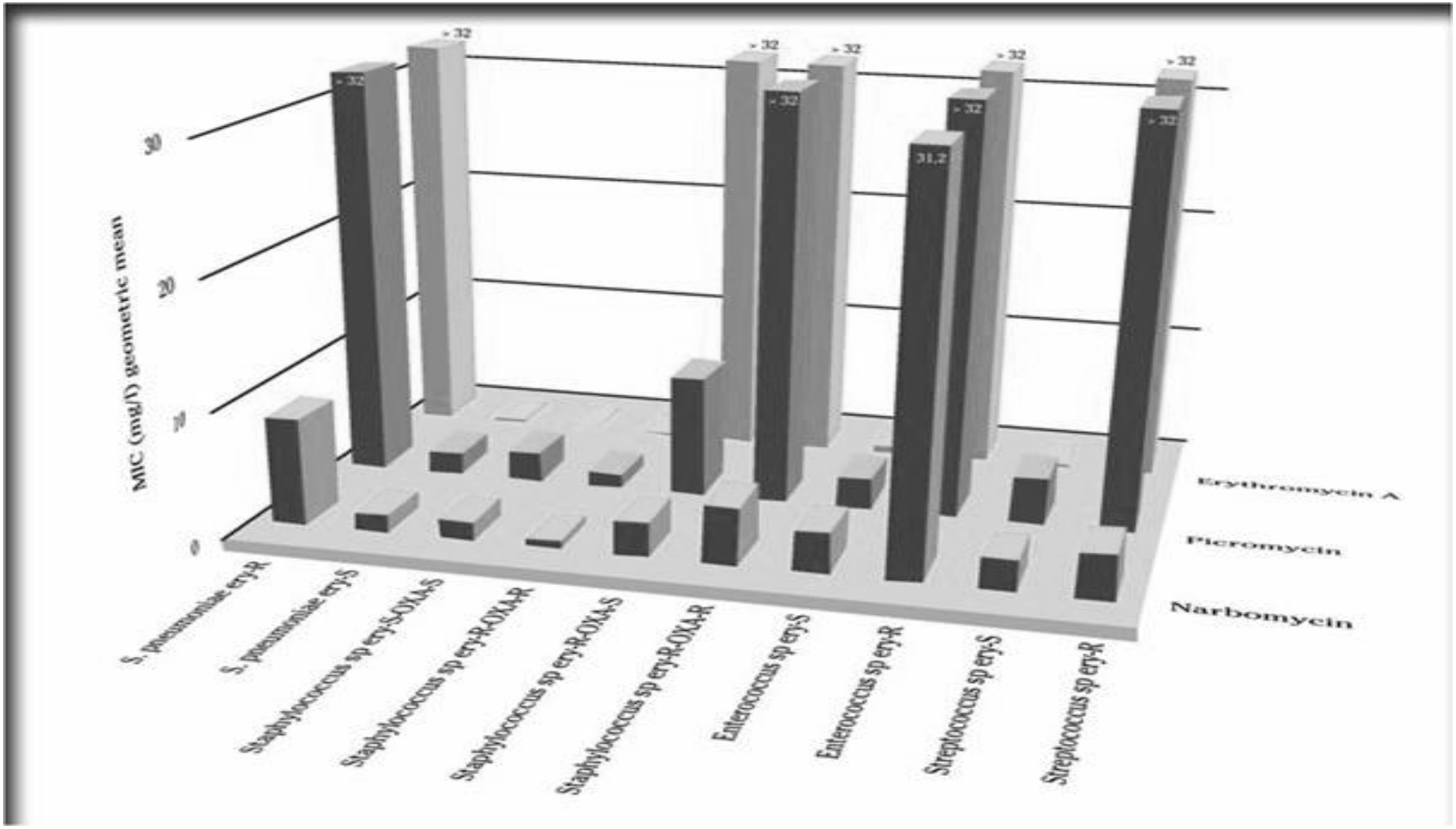


Spread of bacterial resistance



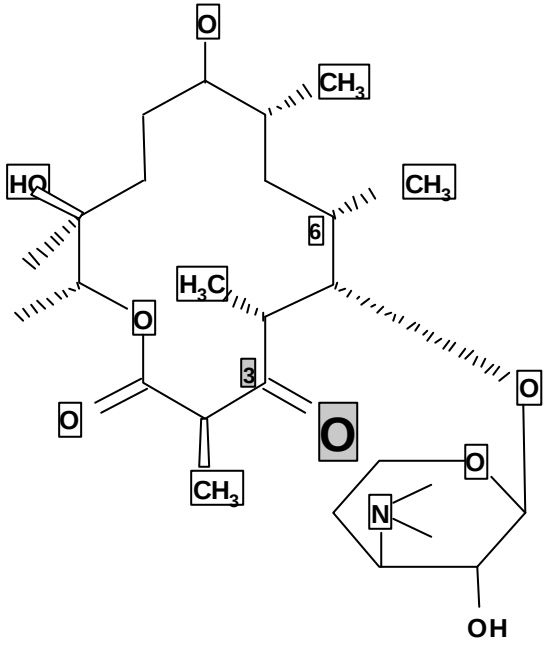
Ketolides

In vitro activity of narbomycin

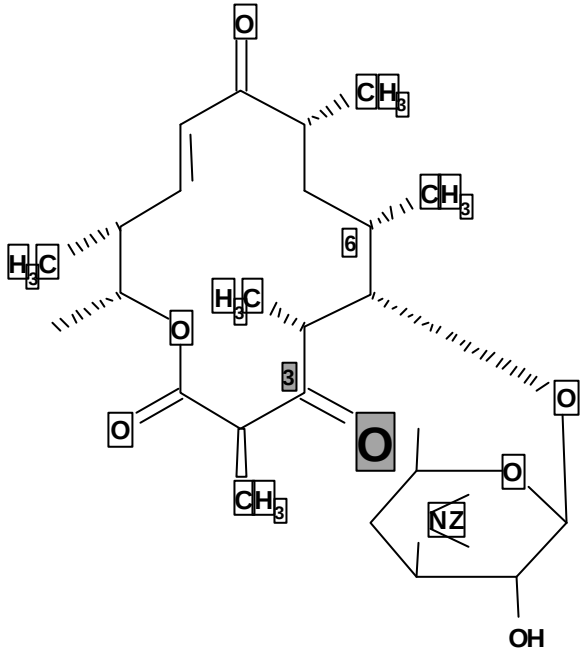


Ketolides

Natural ketolides

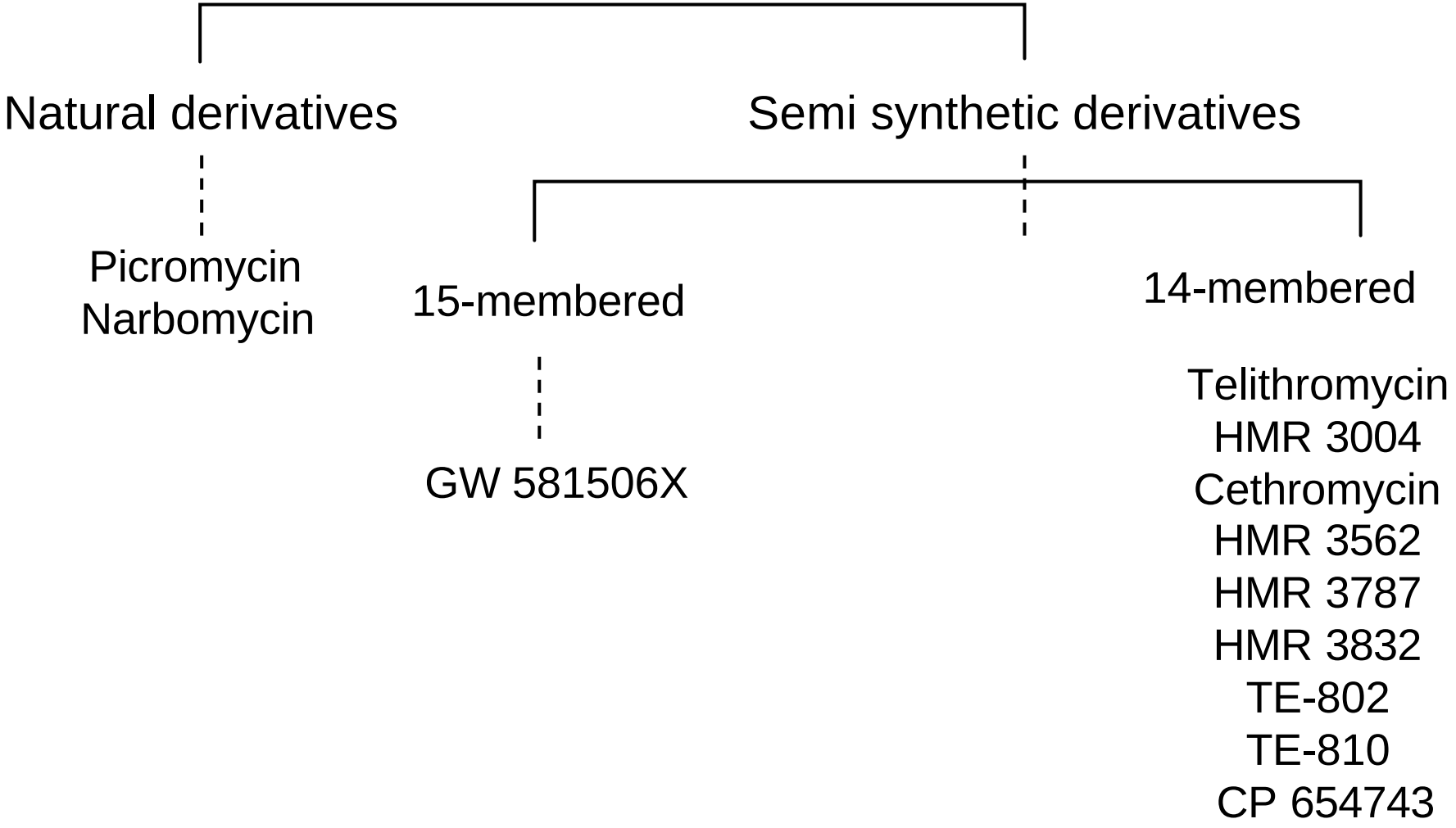


Picromycin

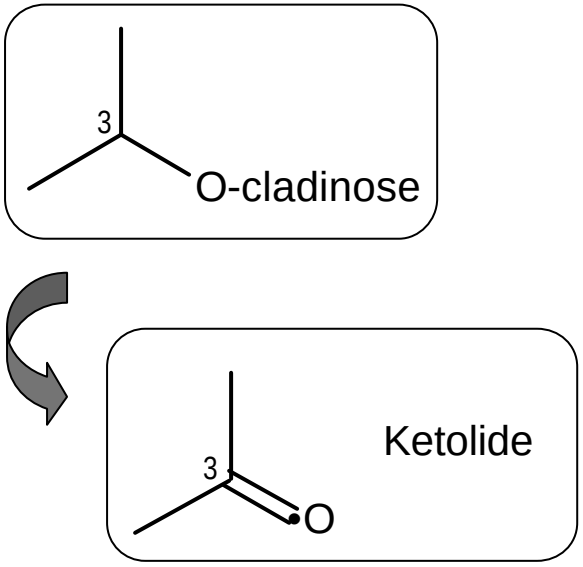


Narbomycin

Ketolides


Natural derivatives
Semi synthetic derivatives
Picromycin
Narbomycin
15-membered
14-membered
GW 581506X
Telithromycin
HMR 3004
Cethromycin
HMR 3562
HMR 3787
HMR 3832
TE-802
TE-810
CP 654743

Ketolides



Telithromycin	
HMR 3004	
HMR 3787	Aventis Pharma
HMR 3832	
HMR 3562	
Cethromycin	Abbott
TE 802, TE 810	Taisho
RWJ 415 663	RW Johnson and pharmaceutical
RWJ 415 667	
CP 654 743	Pfizer

Ketolides

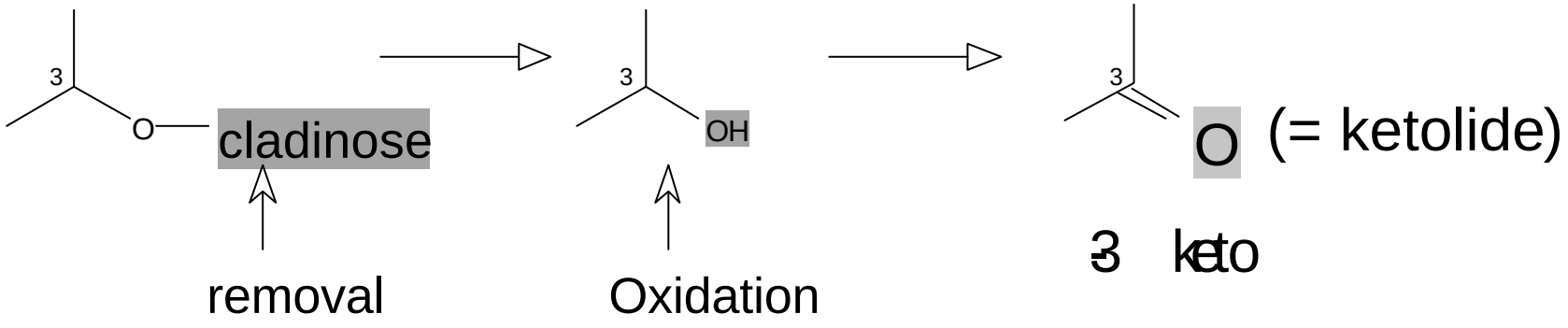
They are composed of three specific chemical structures

- 3-keto function (lack of L-cladinose)
- Side-chain C_{11}-C_{12}
- Side chain C_6

3 KETO FUNCTION

Ketolides

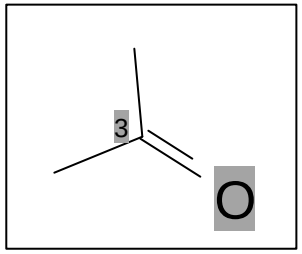
- New medicinal chemical entities
- Semisynthetic compounds obtained from erythromycin A
- Removal of the L-cladinose (neutral sugar), and oxidation of the 3-hydroxyl (OH) yields to a 3-keto group (ketolide).



Ketolides

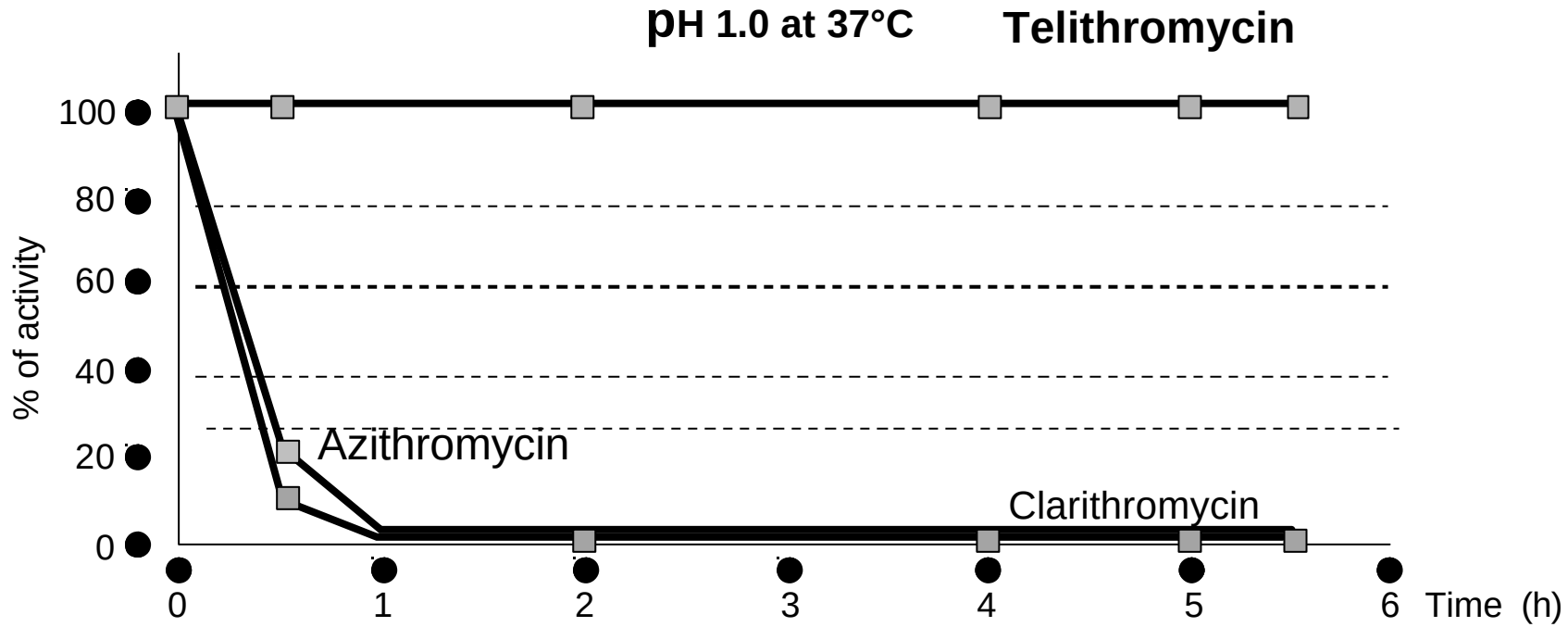
3-keto function

3-keto function imparts the following biological properties



- Antibacterial activities against *erm*-containing-Gram-positive cocci
- Absence of ability to induce MLSB resistance
- High stability in acidic environment.

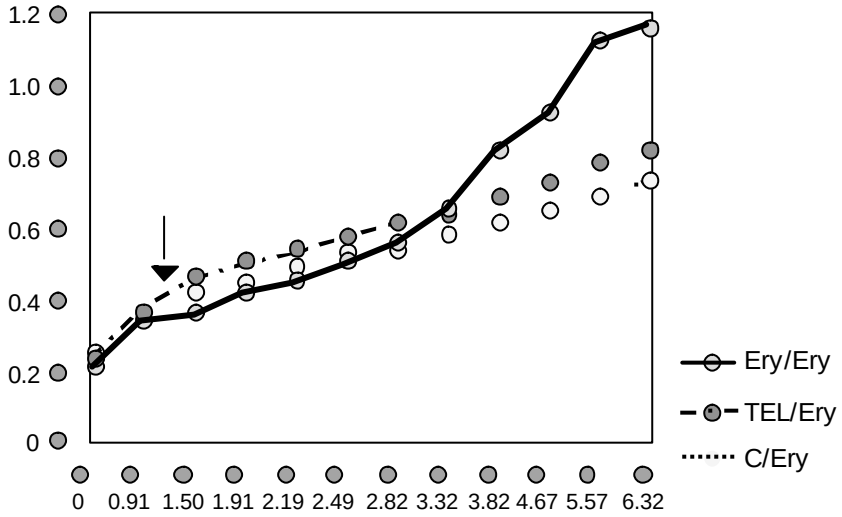
Stability in acidic environment

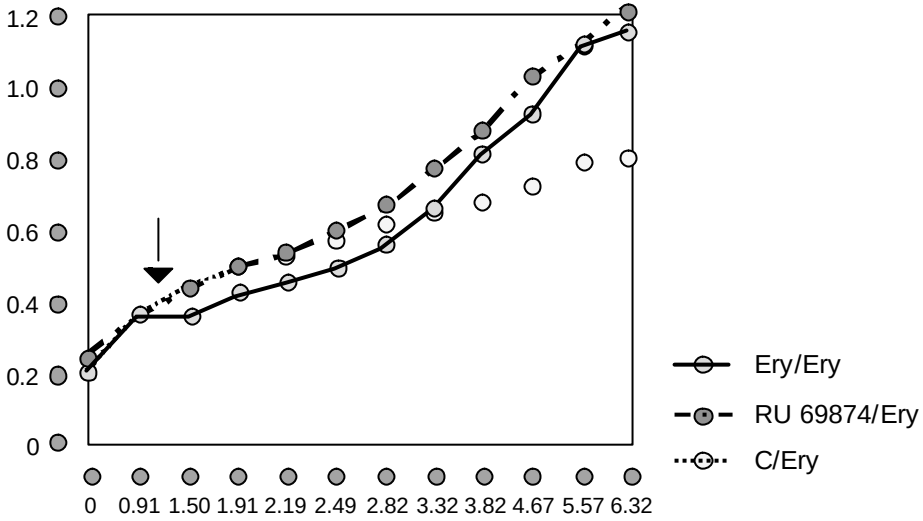


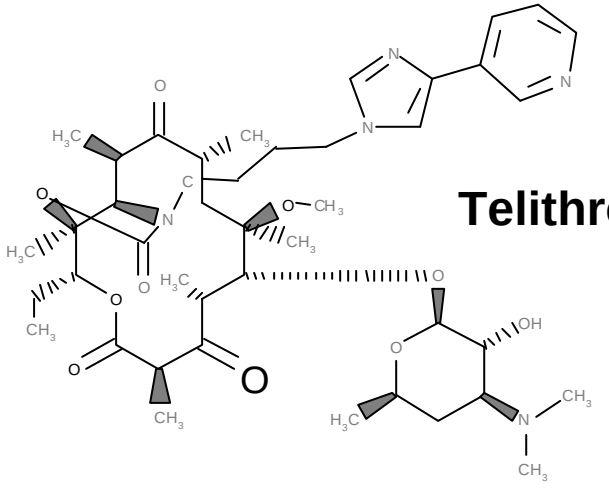
Interaction with human motilin receptor

	IC_{50} (μM) (inhibition of motilin binding)
Telithromycin	> 100μM (23 % inhibition at 100 μM)
Clarithromycin	≥ 100 μM (54 % inhibition at 100 μM)
Erythromycin A	< 3 μM (60 % inhibition at 3 μM)

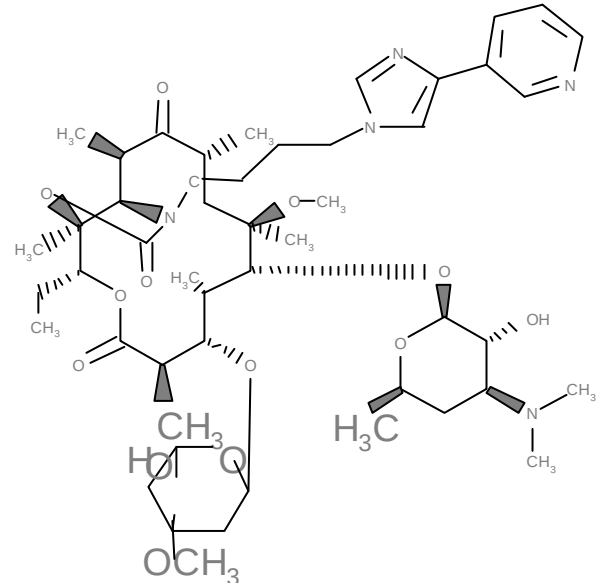
No inducer of MLS_B resistance







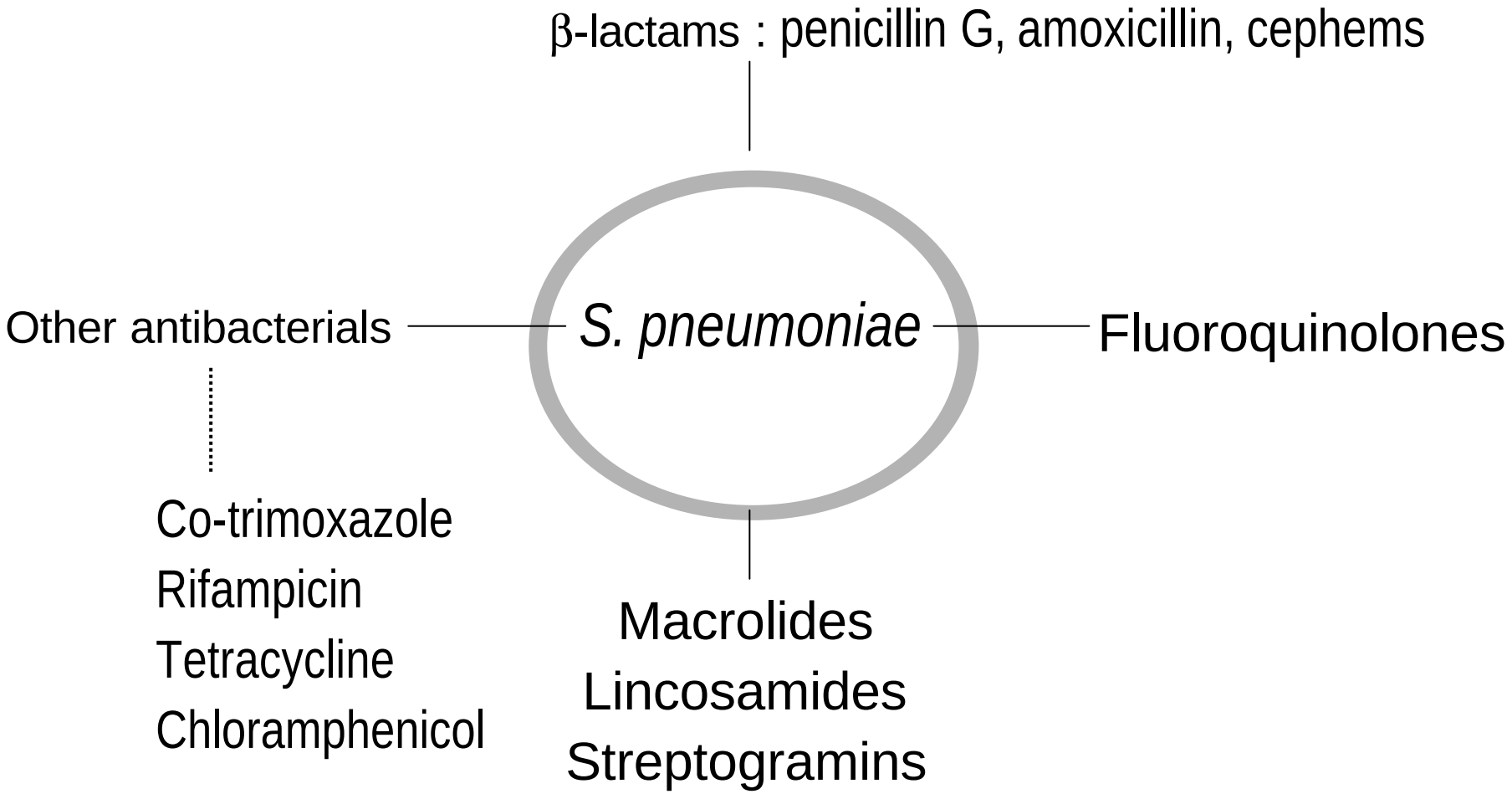
Telithromycin



RU 69874

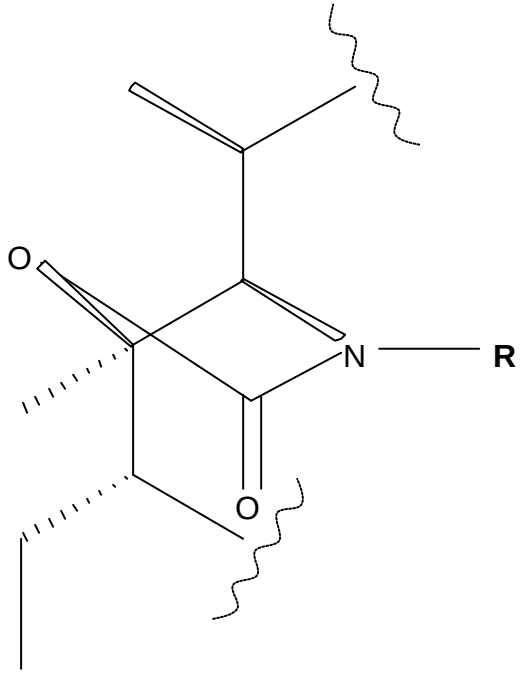
C11–C12 carbamate residue

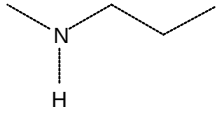
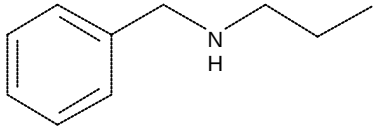
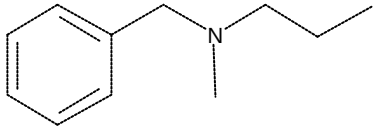
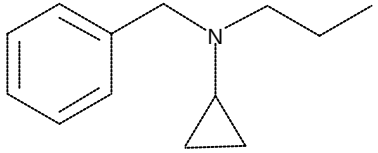
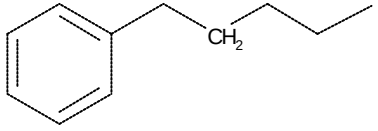
Bacterial resistance to antibacterials



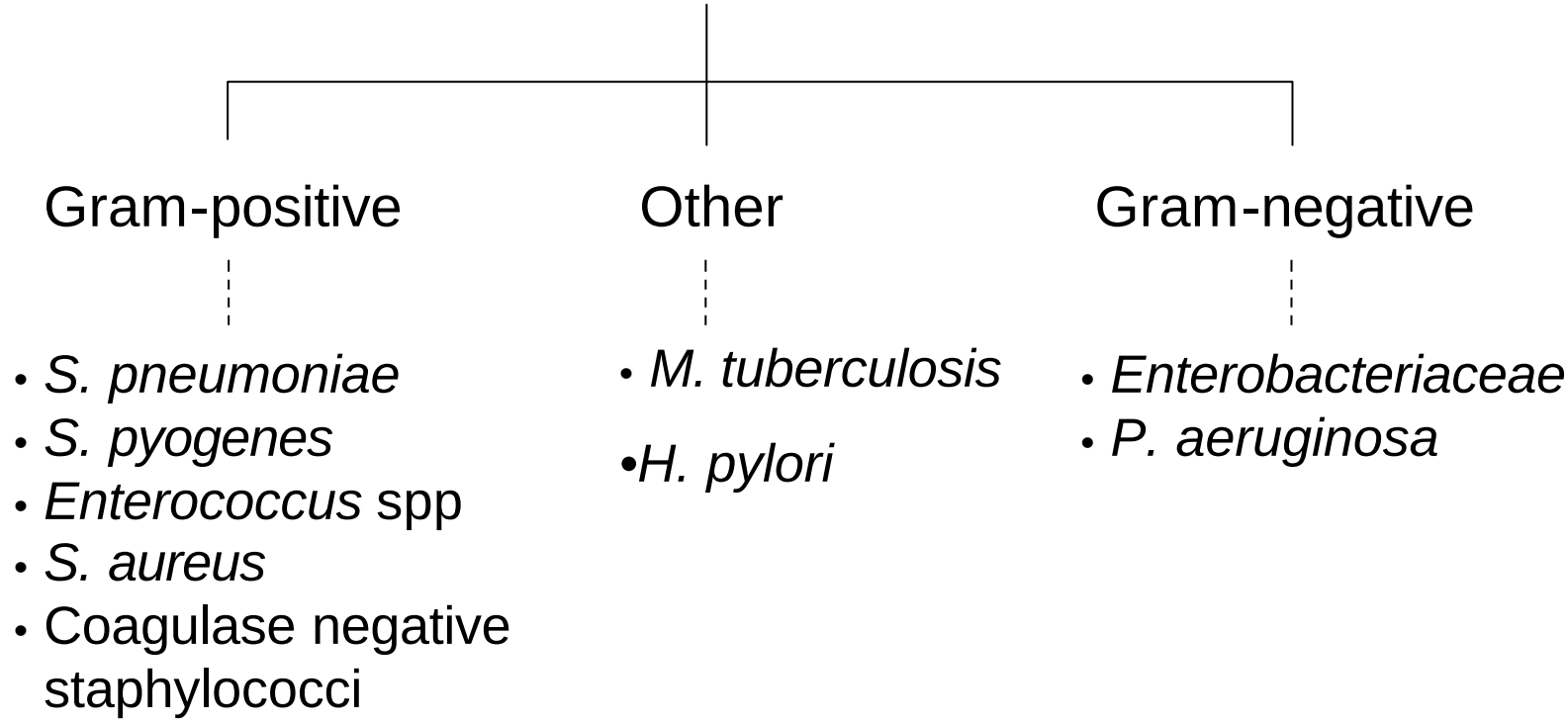
Clarithromycin...

...carbamates analogues



	R
A-61795	H
A-62514	
A-66173	
A-66005	
A-64239	
A-66321	

Spread of bacterial resistance

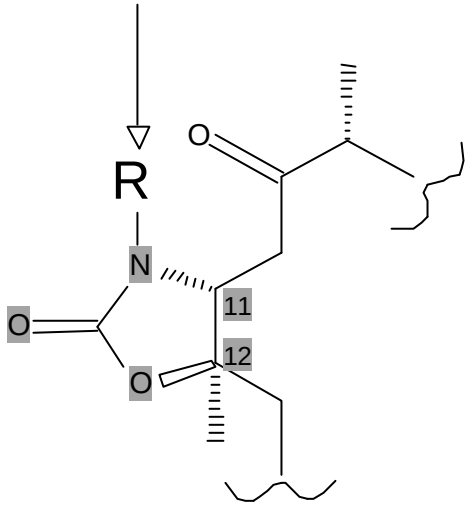


Ketolides

C_{11}-C_{12} carbamate substituted side chain

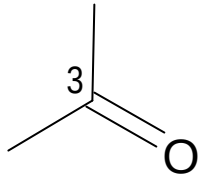
- *In vitro* activity
- Pharmacokinetics
- Pharmacodynamics
- Intracellular kinetics
- Efflux
- Mechanism of action
- Tolerance- toxicity.

Carbamate residue



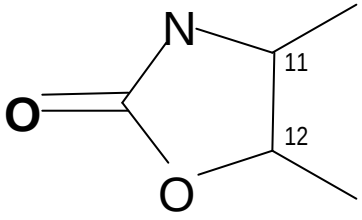
Research in anti-infectives

Ketolides



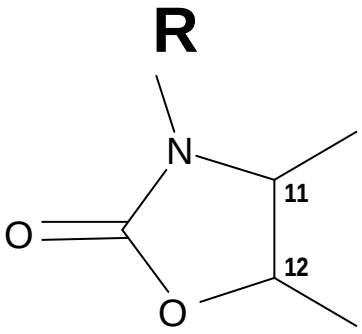
New chemical entities

$C_{11} – C_{12}$
carbamate ketolide

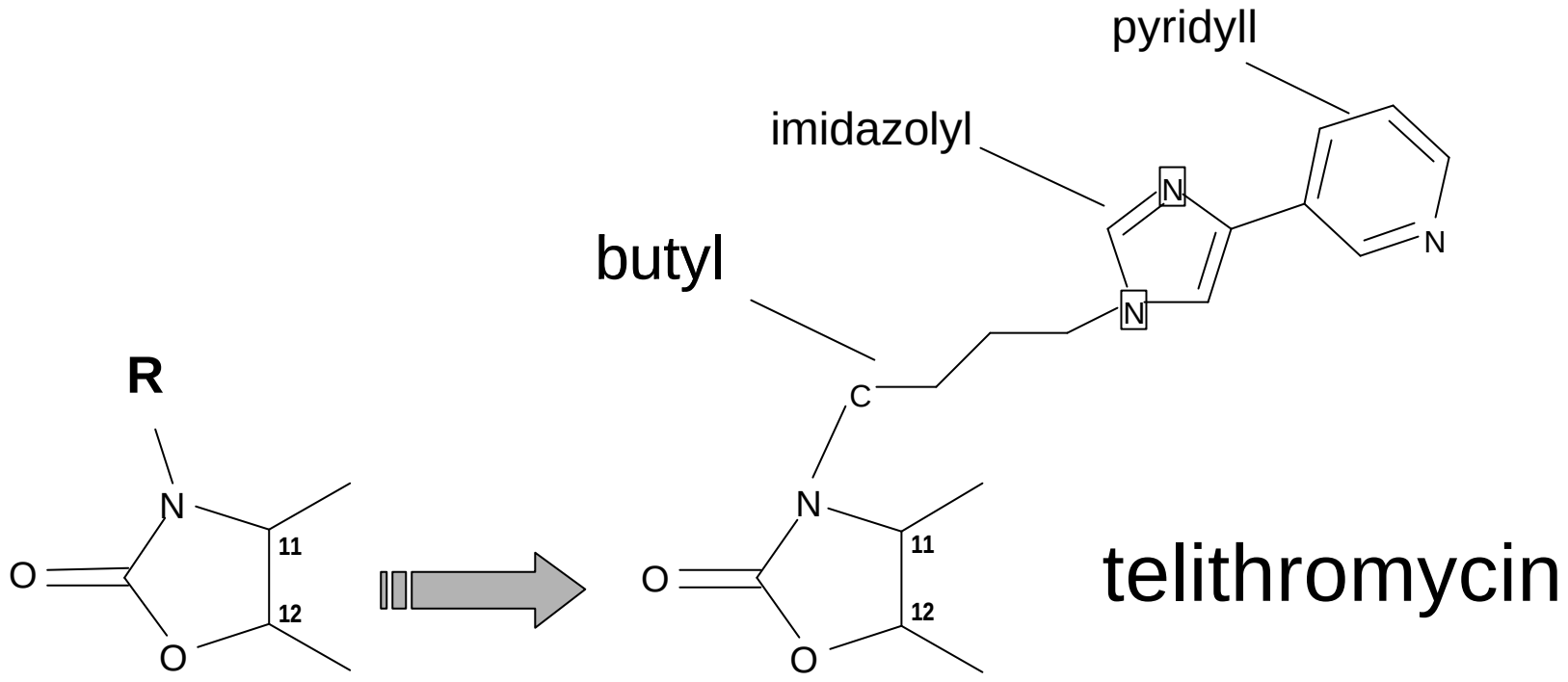


Optimisation

Substituted
carbamate ketolide



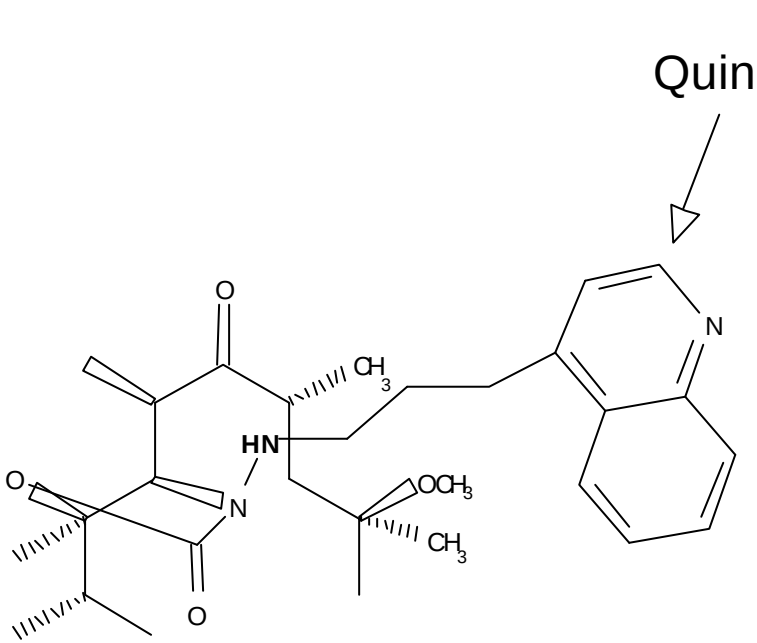
Innovation



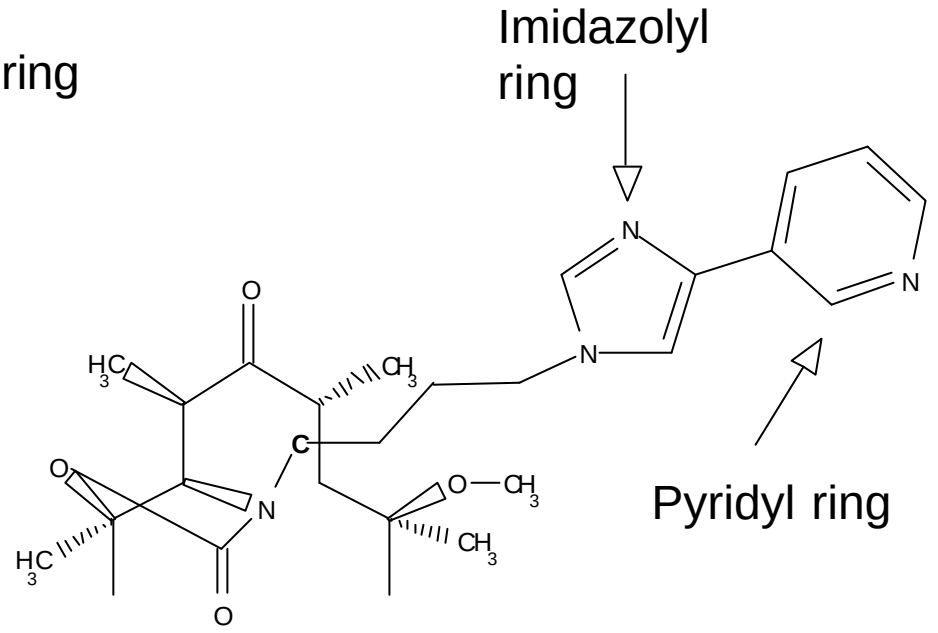
Carbamate ketolide

Ketolides

C_{11} – C_{12} carbamate substituted side chain



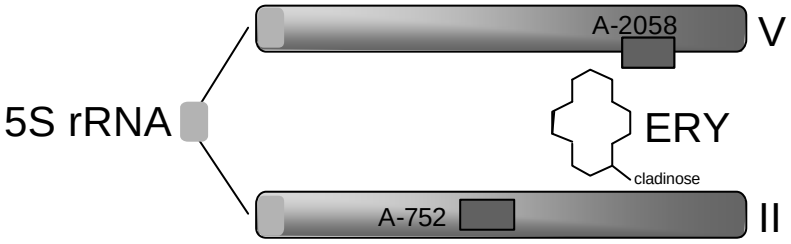
HMR 3004



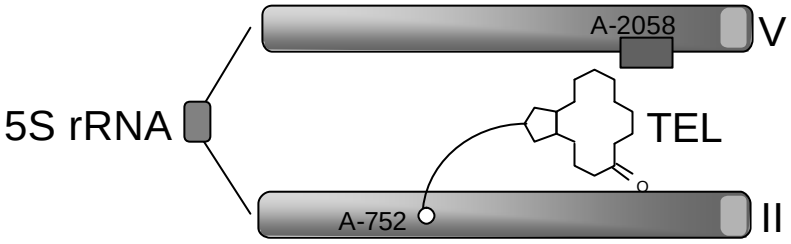
Telithromycin

Mode of action

Significant of different mode of action



No link with domain V



Link with domain II

Resistance to erythromycin A, azithromycin, roxithromycin, clarithromycin,

Telithromycin retains activity against erythromycin A-resistant organisms

Ketolides

C_{11} – C_{12} carbamate substituted side chain (1)

Pharmacokinetics in mice

	Cmax (mg/l)	Tmax (h)	Concentrations (mg/l) at 0.25*	0.50	1.0	2.0	3.0	4.0
Telithromycin	4.67	0.50	3.97	4.67	3.52	1.60	1.70	0.90
HMR 3004	1.70	0.25	1.70	0.90	1.00	0.20	0.10	0.06

* sampling time (hours)

Craig, 1996

Ketolides

C_{11} - C_{12} carbamate substituted side chain (2)

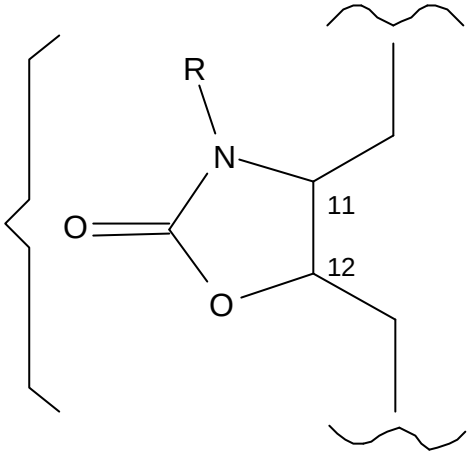
Pharmacokinetics in mice

	Cmax (mg/l)	Tmax (h)	$AUC_{0\text{-}24h}$ (mg.h/l)
Telithromycin	4.67	0.50	17.92
HMR 3004	1.67	0.25	2.47

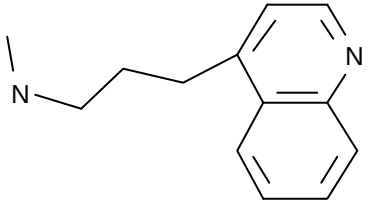
Craig, 1996

Ketolides

C_{11}-C_{12} carbamate residue

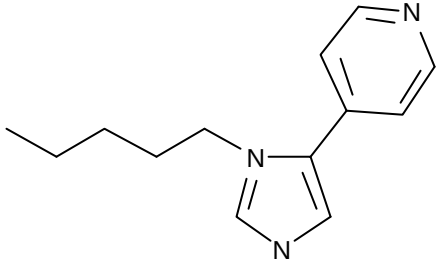


Comparative human pharmacokinetics after a single oral dose 600 mg



HMR 3004

Cmax (mg/l)	0.16 ± 0.30
Tmax (h)	1.75
AUC $_{0\text{-}8}$ (mg.h/l)	0.59 ± 0.11
C_{24h} (mg/l)	ND
t1/2 (h)	2.25 ± 0.16

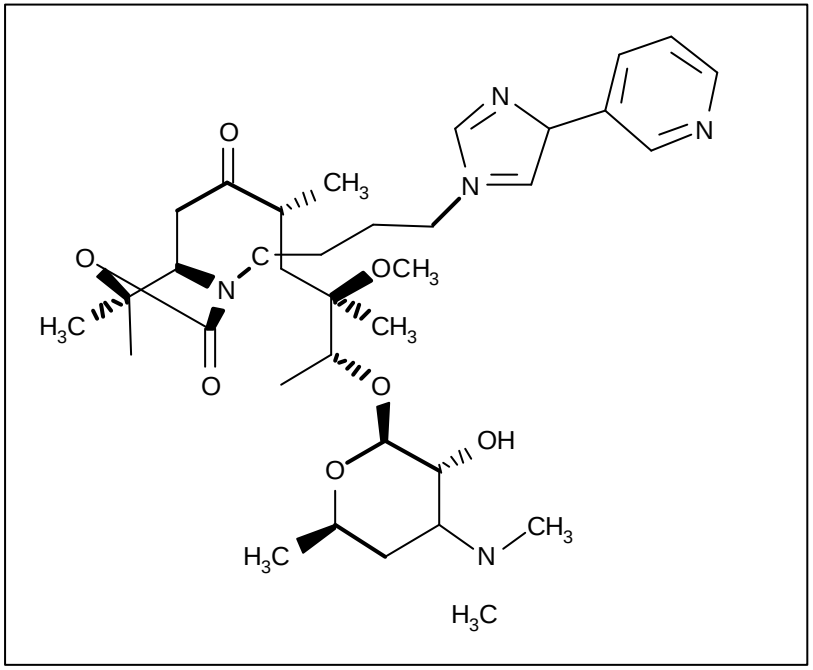


HMR 3647

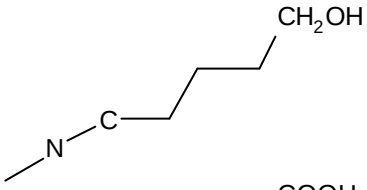
Cmax (mg/l)	0.90 ± 0.13
Tmax (h)	1.5
AUC $_{0\text{-}8}$ (mg.h/l)	4.09 ± 0.55
C_{24h} (mg/l)	0.01 ± 0.01
t1/2 (h)	11.0 ± 1.90

Telithromycin

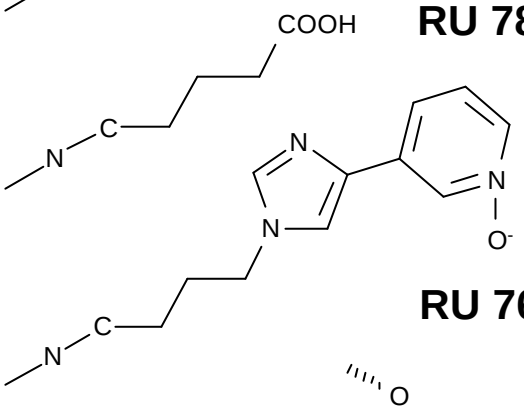
Metabolites (1)



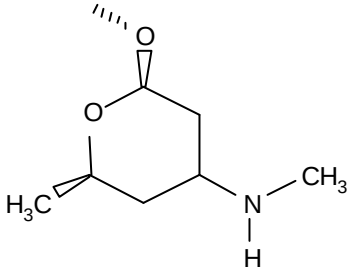
Telithromycin



RU 78849 (N-propyl carboxylic)



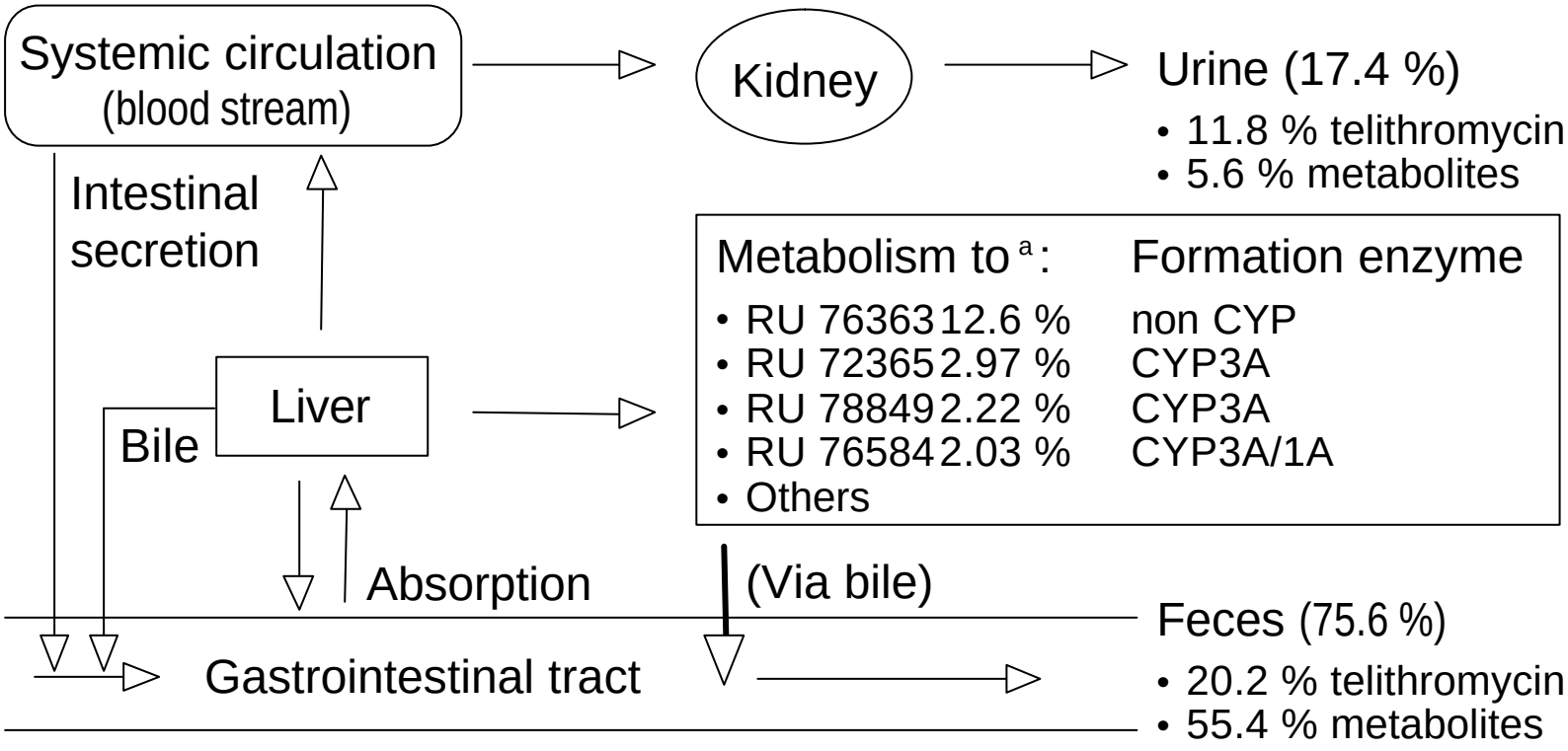
RU 76584 (N-pyridine oxide)



RU 72365 (N-monodemethyl desosamine)

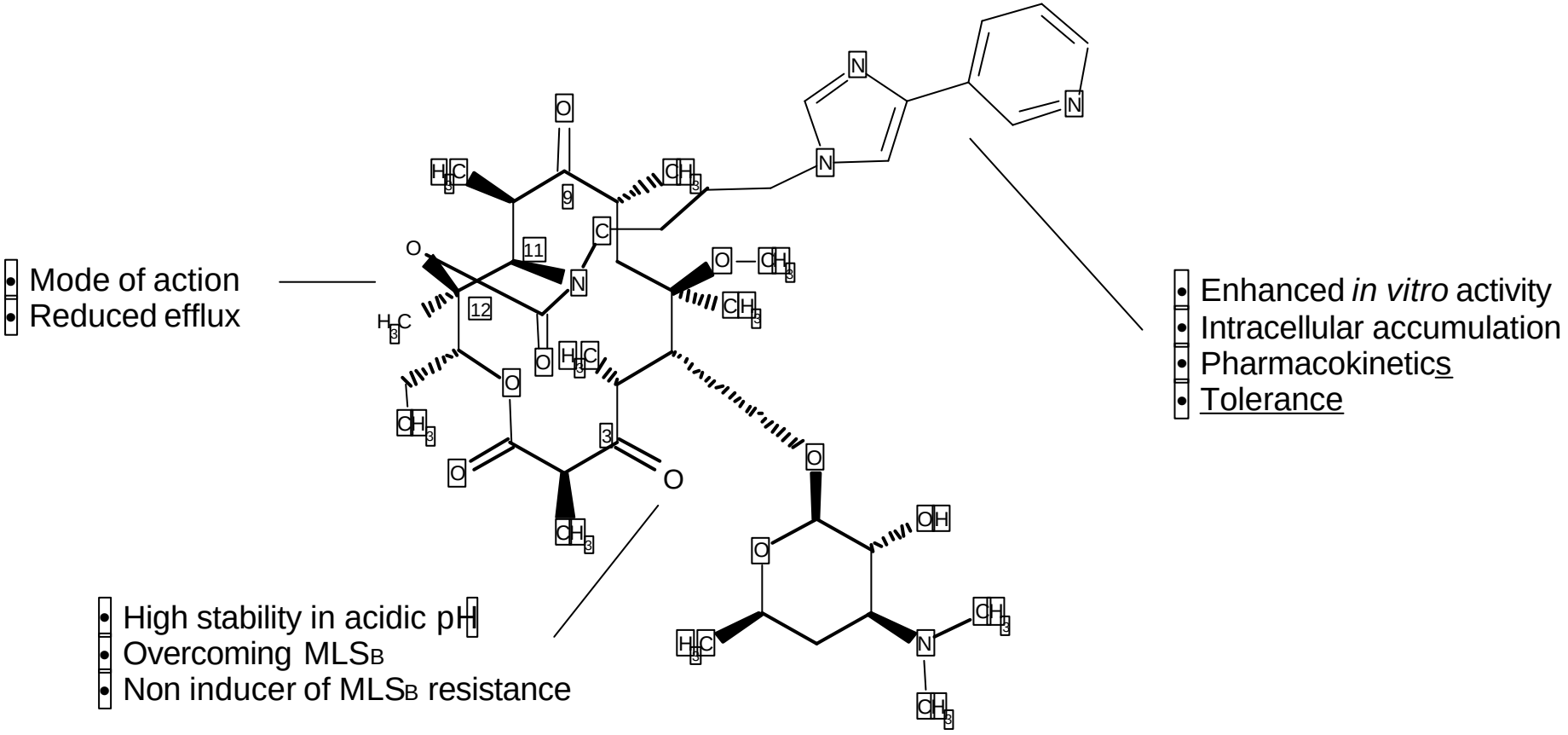
Telithromycin

Disposition



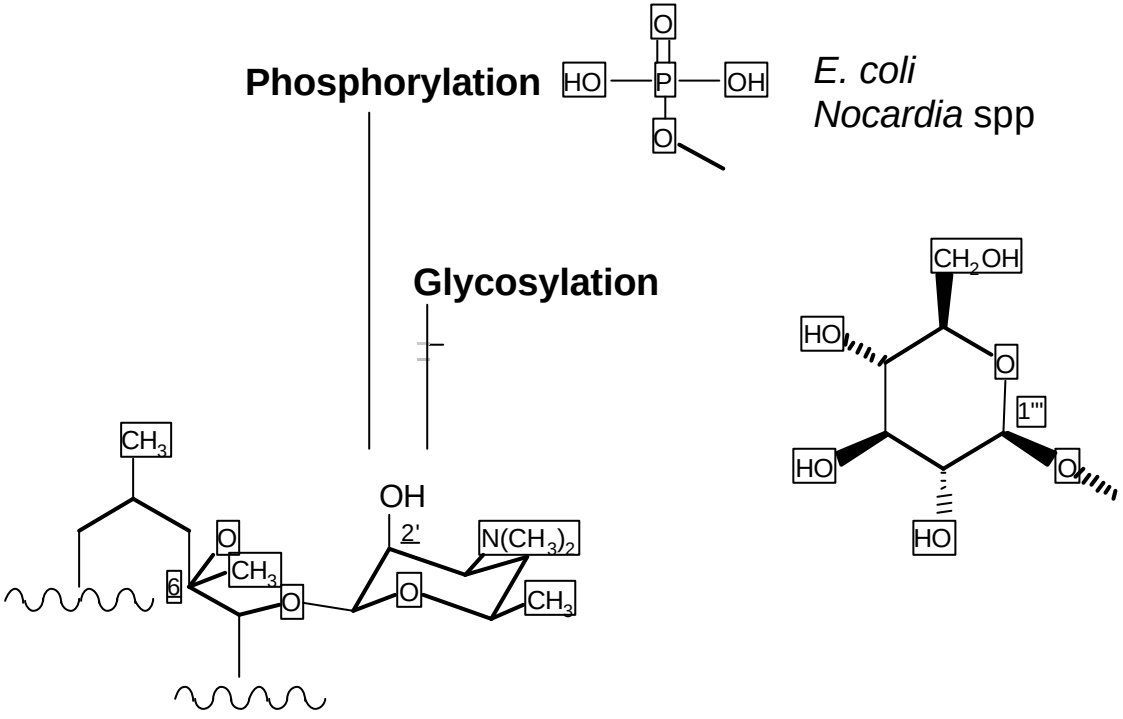
Metabolites expressed as relative radioactivity in circulation (metabolite AUC / telithromycin AUC)

Structure activity relationships



Macrolide resistance

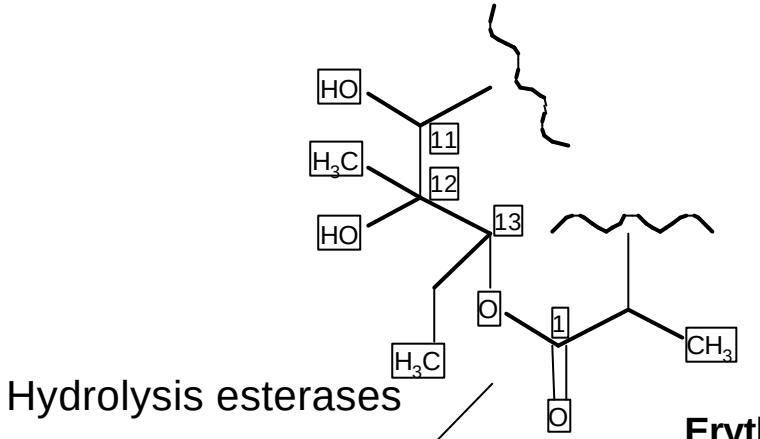
14-membered ring macrolide



Macrolide resistance

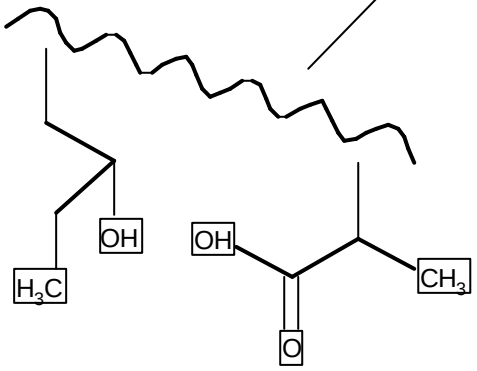
Ring hydrolysis

by esterases



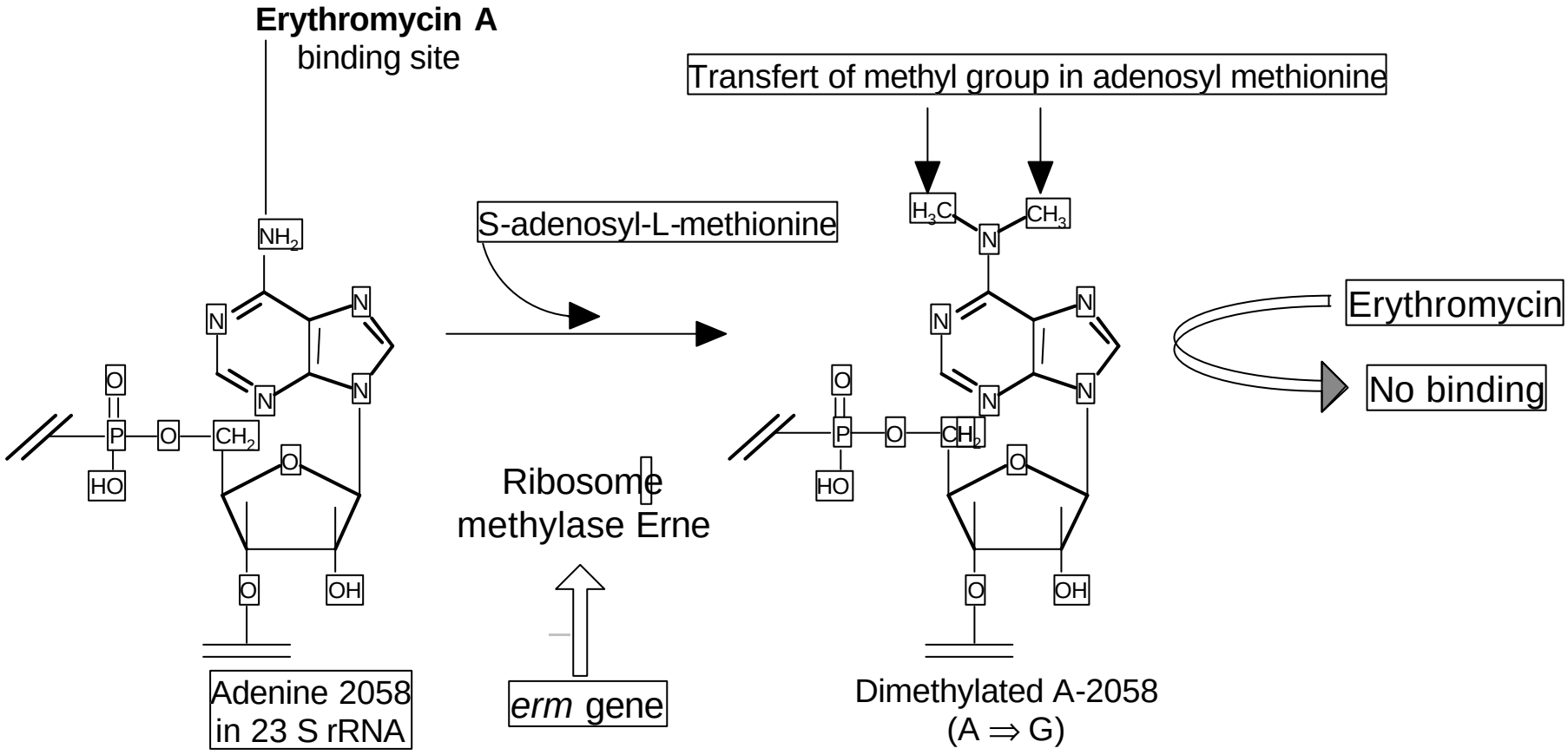
Hydrolysis esterases

Erythromycin A



Described in *S. aureus - E. coli*

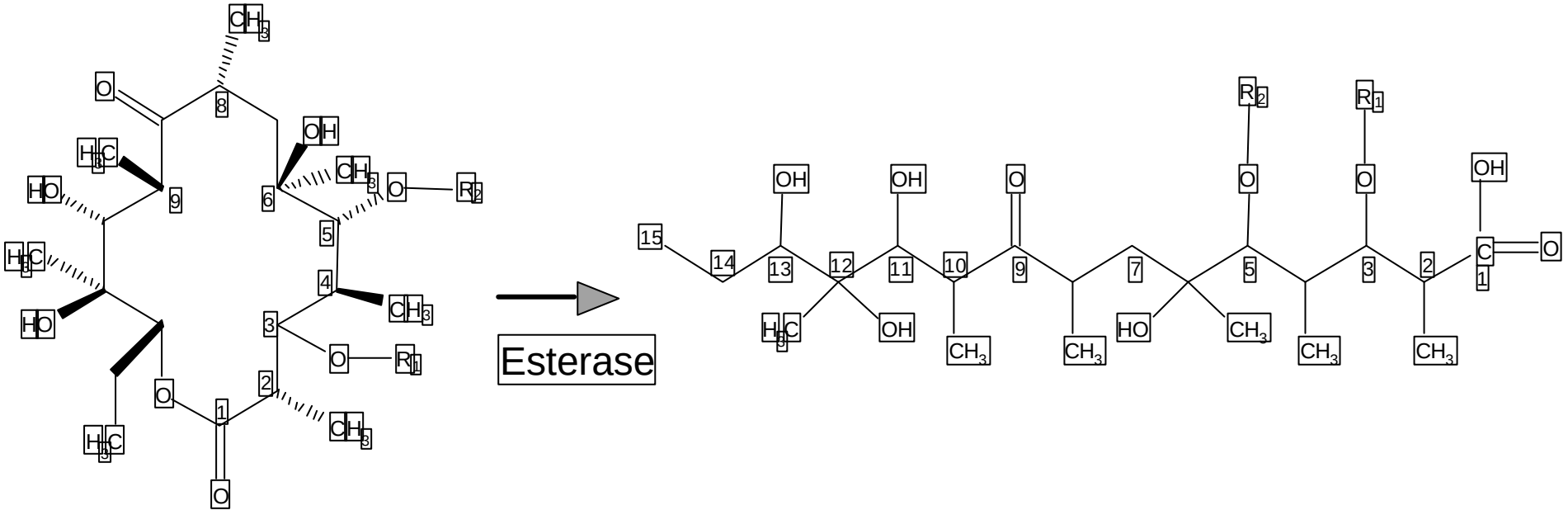
Macrolide resistance



Adapted from K. O'hara, 2000

Macrolides

Mechanism of resistance



R_1 : α-L cladinose

R_2 : D-desosamine