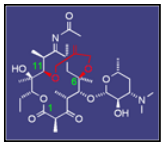
Chemistry 259

Medicinal Chemistry of Modern Antibiotics

Spring 2008



Lecture 10: *Antibiotics Classes & Targets*

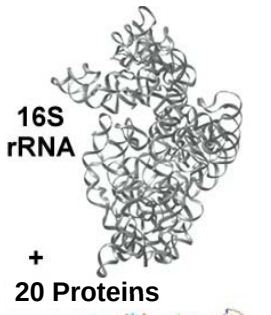
Part IV: Drugs Targeting Protein Biosynthesis

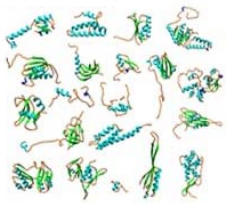
50S Subunit and Other Targets

Thomas Hermann

Department of Chemistry & Biochemistry
University of California, San Diego

Bacterial Protein Synthesis: Ribosome Composition





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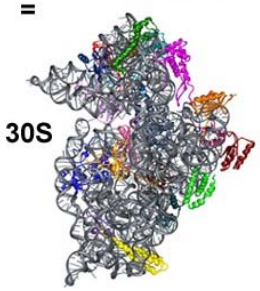


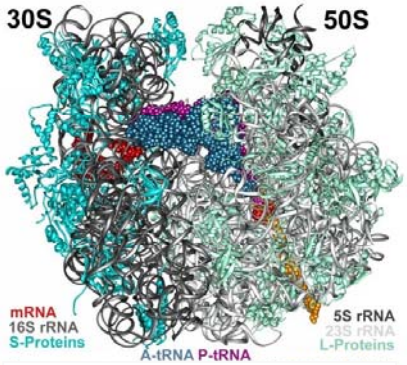
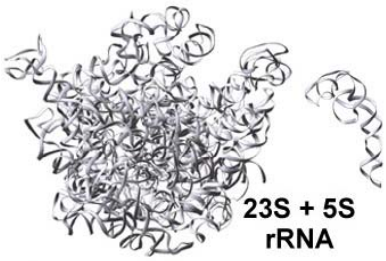
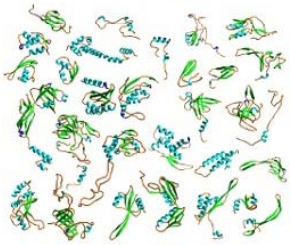


Table 1 | **Ribosome components from the three domains of life**

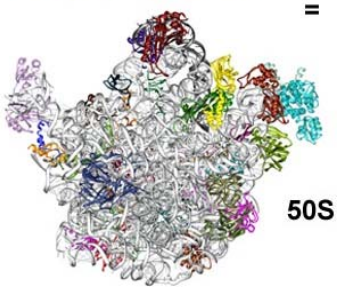
Characteristics	Bacteria	Archaea	Mitochondria	Eukarya
Ribosome size	70S	70S	55S	80S
Small subunit				
Size	30S	30S	28S	40S
Mass (MDa)	0.8	0.8	1.2	1.4
rRNAs	16S	16S	12S	18S
Number of r-proteins	20	26	33	32
Large subunit				
Size	50S	50S	39S	60S
Mass (MDa)	1.6	1.6	2.4	2.6
rRNAs	23S, 5S	23S, 5S	16S	28S, 5.8S, 5S
Number of r-proteins	34	40	52	46



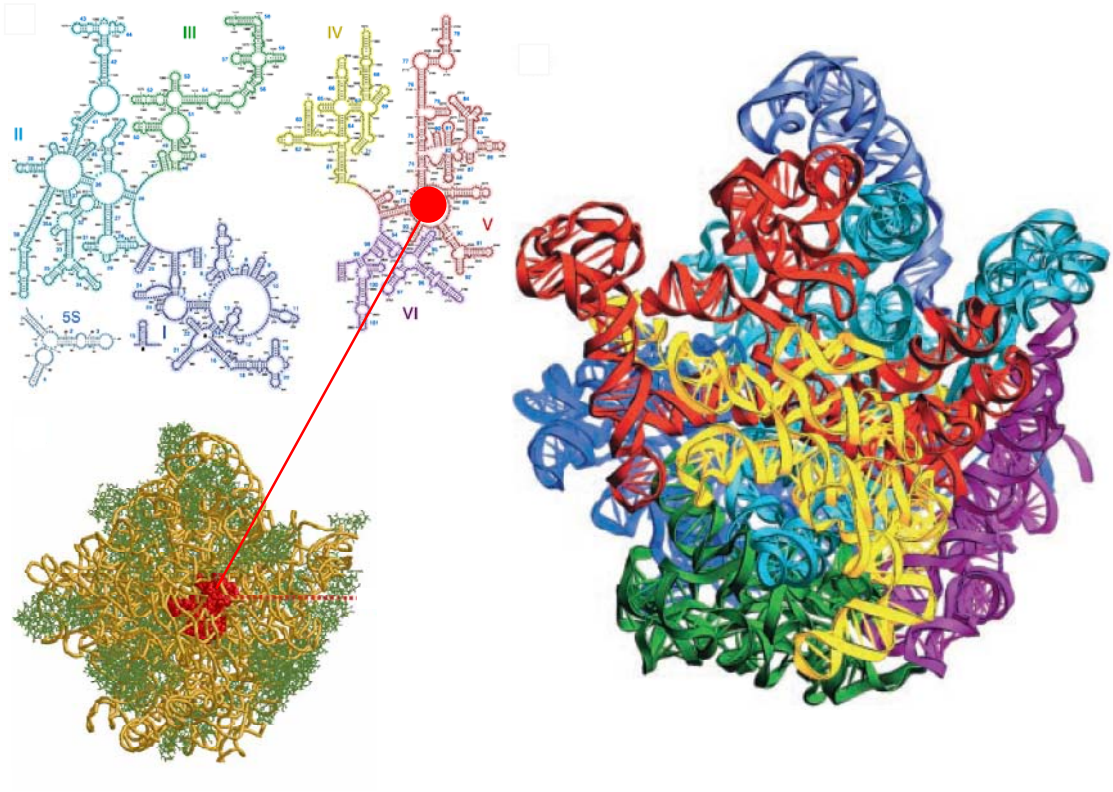


+
34
Proteins

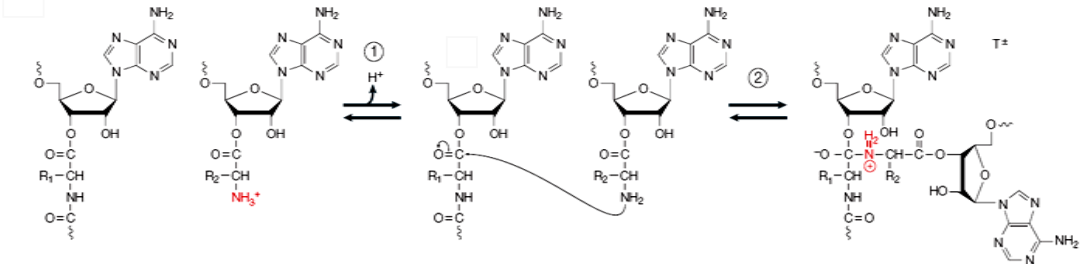
=

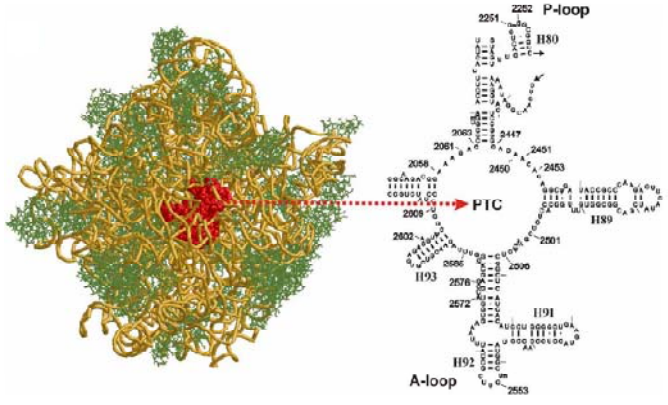


Bacterial Protein Synthesis: 23S & 5S Ribosomal RNA

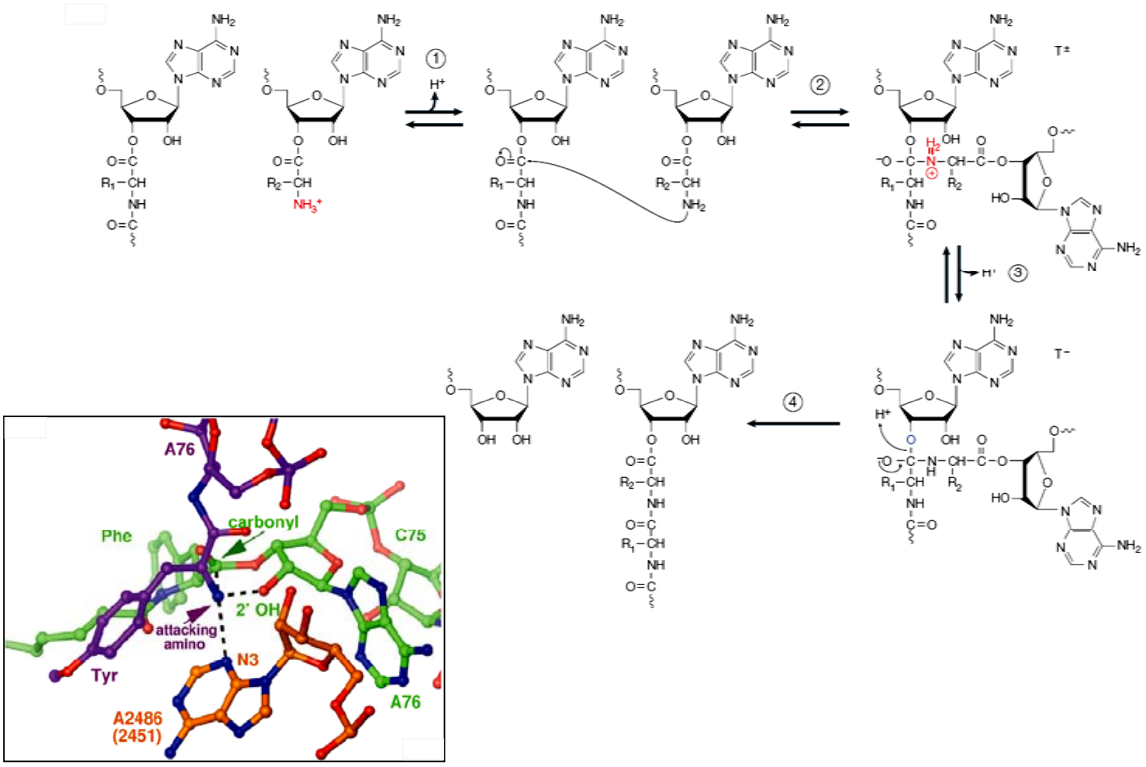


Bacterial Protein Synthesis: The Ribosome is an RNA Enzyme (Ribozyme)

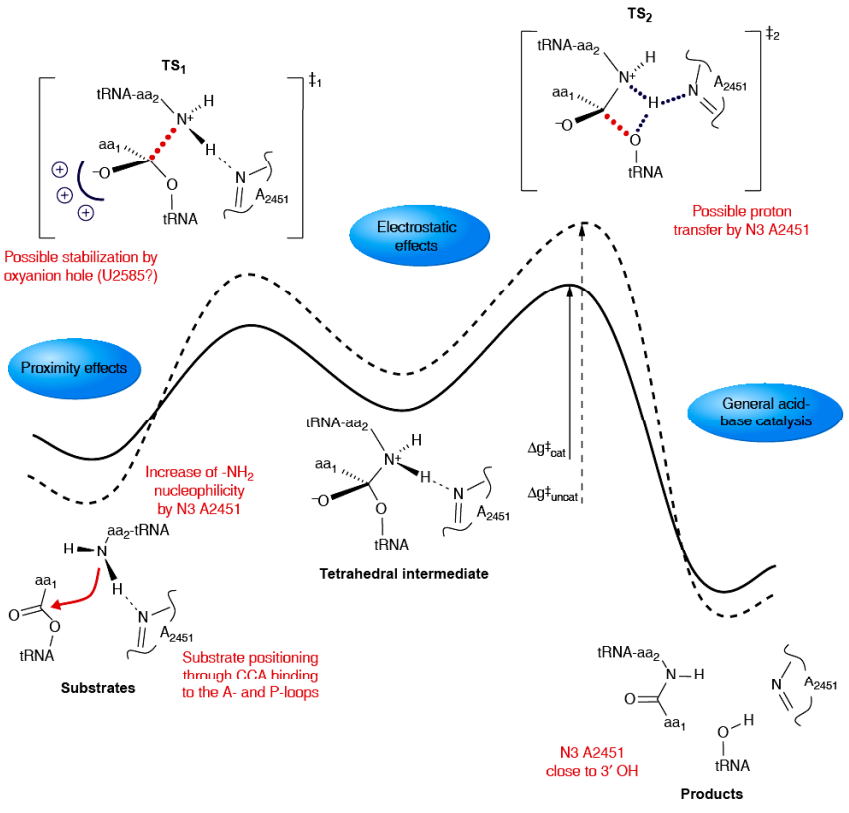




Bacterial Protein Synthesis: The Ribosome is an RNA Enzyme (Ribozyme)



Bacterial Protein Synthesis: The Ribosome is an RNA Enzyme (Ribozyme)



Bacterial Protein Synthesis: 50S Subunit as a Target

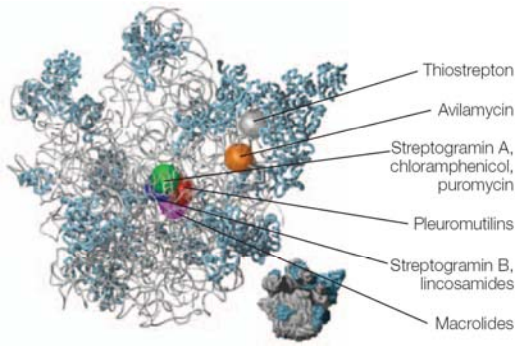
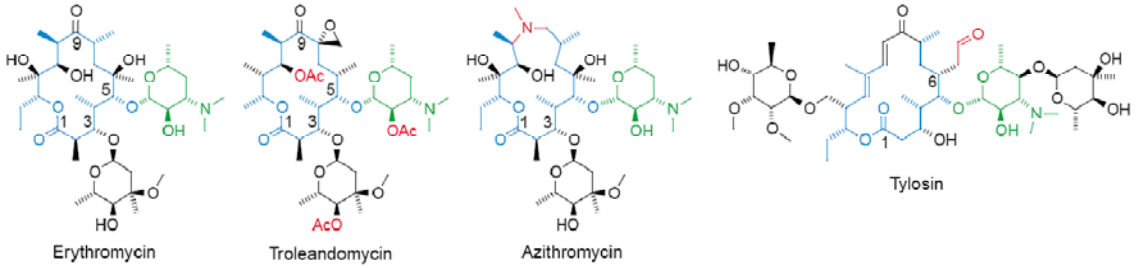


Table 3 | **Antibiotics that target the 50S ribosomal subunit**

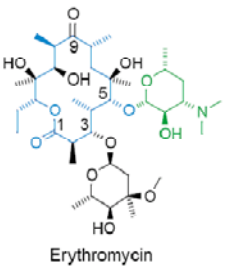
Drug	PDB	Resolution (Å)	System
Macrolides and ketolides*			
Carbomycin	1K8A	3.0	*Haloarcula*
Spiramycin	1KD1	3.0	*Haloarcula*
Tylosin	1K9M	3.0	*Haloarcula*
Azithromycin	1M1K	3.2	*Haloarcula*
Azithromycin	1NWY	3.3	*Deinococcus*
Azithromycin	1YHQ	2.4	*Haloarc-2058A*
Erythromycin	1JZY	3.5	*Deinococcus*
Erythromycin	1YI2	2.7	*Haloarc-2058A*
Clarithromycin	1J5A	3.5	*Deinococcus*
Roxithromycin	1JZZ	3.8	*Deinococcus*
Troleandomycin	1OND	3.4	*Deinococcus*
ABT 773	1NWX	3.5	*Deinococcus*
Telithromycin	1P9X	3.4	*Deinococcus*
Telithromycin	1YIJ	2.6	*Haloarc-2058A*
Streptogramin A‡			
Virginiamycin M	1N8R	3.0	*Haloarcula*
Virginiamycin M	1YIT	2.8	*Haloarc-2058A*
Dalfopristin	1SM1	3.4	*Deinococcus*
Streptogramin B§			
Quinupristin	1SM1	3.4	*Deinococcus*
Quinupristin	1YJW	2.9	*Haloarc-2058A*
Lincosamides‖			
Clindamycin	1JZX	3.1	*Deinococcus*
Clindamycin	1YJN	3.0	*Haloarc-2058A*
Nucleoside based¶			
Chloramphenicol	1K01	3.5	*Deinococcus*
Chloramphenicol	1NJI	3.0	*Haloarcula*
Anisomycin	1K73	3.0	*Haloarcula*
Sparsomycin	1M90	2.8	*Haloarcula*
Blasticidin S	1KC8	3.0	*Haloarcula*
Puromycin derivative	1FFZ	3.2	*Haloarcula*
Pleuromutilins‡			
Tiamulin	1XBP	3.5	*Deinococcus*
Valnemulin	–	–	nd
Oxazolidinones†			
Linezolid	–	–	np
XA043	–	–	nd

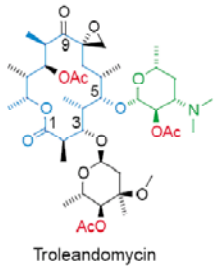
Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides



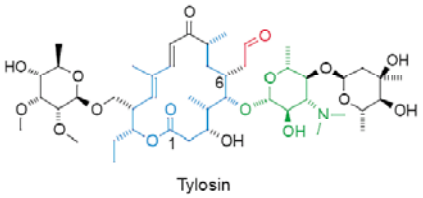
- Isolated first in 1949 from *Streptomyces erythreus* (erythromycin)
- Obtained by fermentation & semi-synthesis starting from natural products
- Naturally occuring macrolides (erythromycin) are acid-labile, have short $t_{1/2}$ (1.5h) and narrow spectrum (Gram-positives, *Staphylococci*, *Streptococci*, *Bacilli*)
- Semi-synthetic derivatives (clarithromycin, $t_{1/2}$=3-7h), azalides (azithromycin, $t_{1/2}$>35h!), have improved stability, PK properties and spectrum (Gram-negatives, *Haemophilus influenzae*, atypical bacteria: *Legionella*, *Chlamydia*, *Mycoplasma*)
- Bind to the peptidyl transferase center at the peptide exit tunnel and block egress of the nascent peptide
- Bacteriostatic (may be bactericidal at high concentrations)
- Used orally
- Reasonable bioavailability: erythromycin: 15-45% (acid lability!), clarithromycin: 55%, azithromycin: 38%
- Extensive tissue and cellular distribution
- Resistance by active efflux (~80% of resistant isolates in US), altered target sites (primary mechanism in Europe)
- Cross-resistance by target modification (ermA/ermC) occurs between all macrolides, and against clindamycin (lincosamides) and synercid (streptogramins)

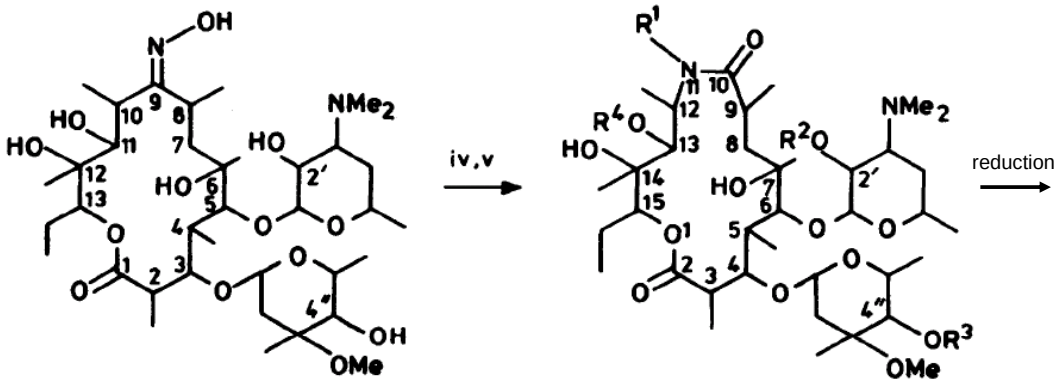
Bacterial Protein Synthesis: 50S Subunit as a Target: Azalides


Erythromycin


Troleandomycin

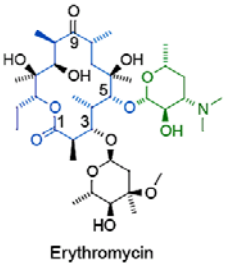

Azithromycin

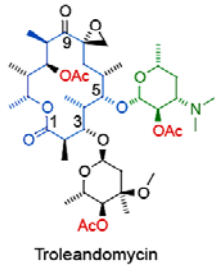

Tylosin

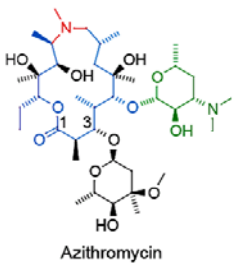


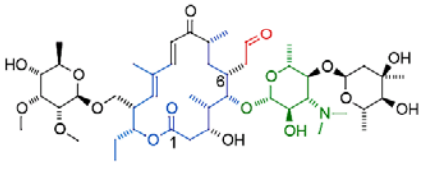
Azalides: synthesis from oximes by Beckmann rearrangement

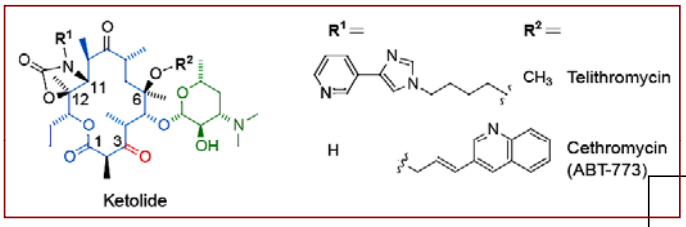
Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides

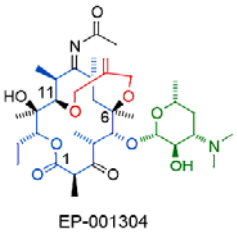

Erythromycin

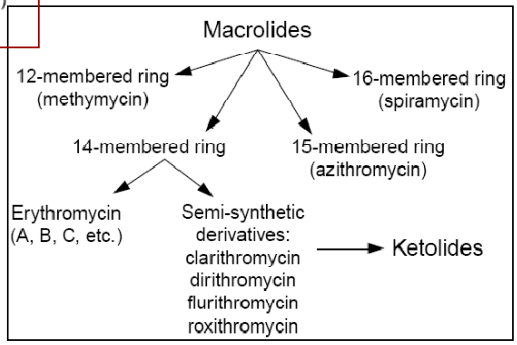

Troleandomycin


Azithromycin


Tylosin




EP-001304



Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides - Ketolides

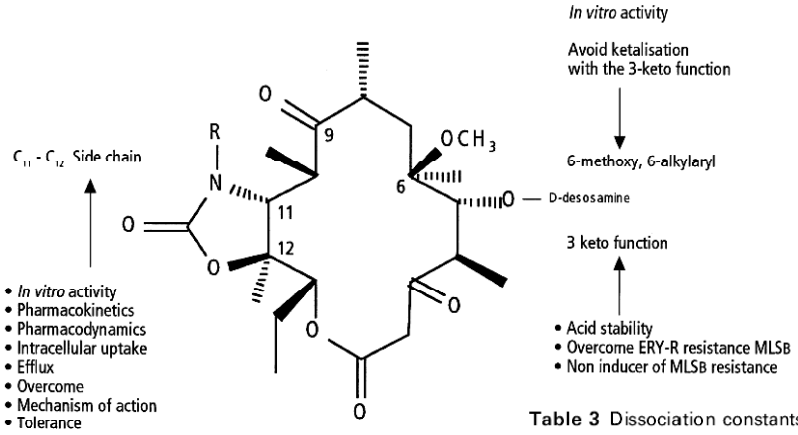
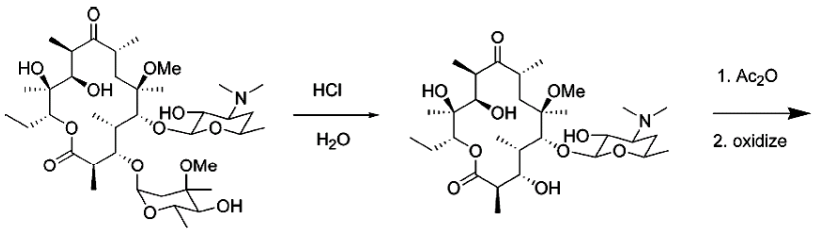
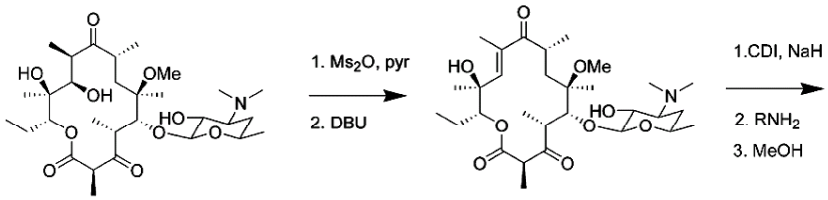


Table 3 Dissociation constants (K_{diss}) of ketolide and macrolide antibiotics for wild-type and A2058G mutant (MLS_B-resistant) ribosomes

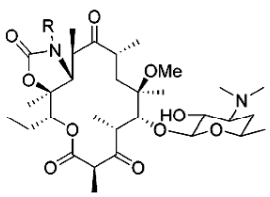
Antibiotic	Mean (± SD) K_{diss} (M)[a]		K_{diss} ratio (mutant/ wild-type)
	Wild-type	Mutant A2058G	
Erythromycin	$1.4 \pm 0.2 \times 10^{-8}$	$1.9 \pm 0.3 \times 10^{-4}$	14 000
Clarithromycin	$9.5 \pm 1.4 \times 10^{-9}$	$1.7 \pm 0.4 \times 10^{-4}$	18 000
HMR 3004	$1.6 \pm 0.3 \times 10^{-9}$	$6.9 \pm 1.4 \times 10^{-6}$	4300
Telithromycin	$1.3 \pm 0.2 \times 10^{-9}$	$7.9 \pm 1.9 \times 10^{-6}$	6100
RU 66252	$2.9 \pm 0.2 \times 10^{-9}$	$2.7 \pm 1.2 \times 10^{-6}$	9300
RU 56006	$9.8 \pm 1.1 \times 10^{-7}$	$> 2.5 \times 10^{-2}$	> 25 000

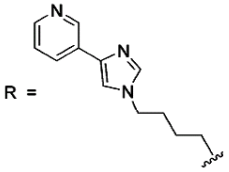
Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides - Ketolides





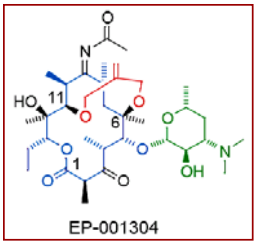
CDI=N,N'-Carbonyldiimidazole



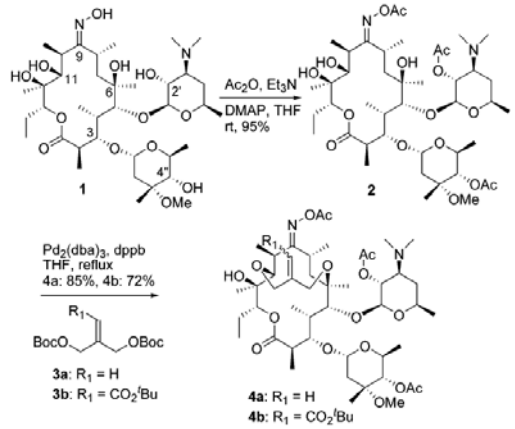


Telithromycin

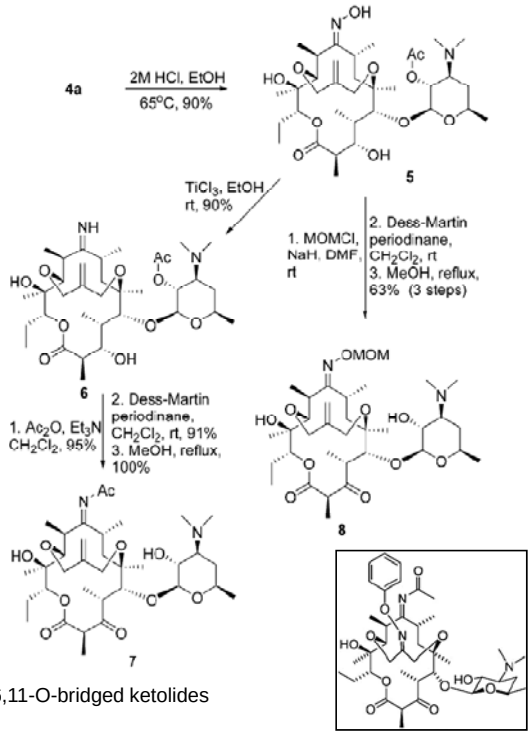
Bacterial Protein Synthesis: 50S Subunit as a Target: Bridged Ketolides



- substituents at 6- and 11-position force macrolide ring into a favorable conformation for rRNA binding
- O-bridge is "atom efficient" (compare to 6,11-disubstituted ketolides Telithromycin and Cethromycin)



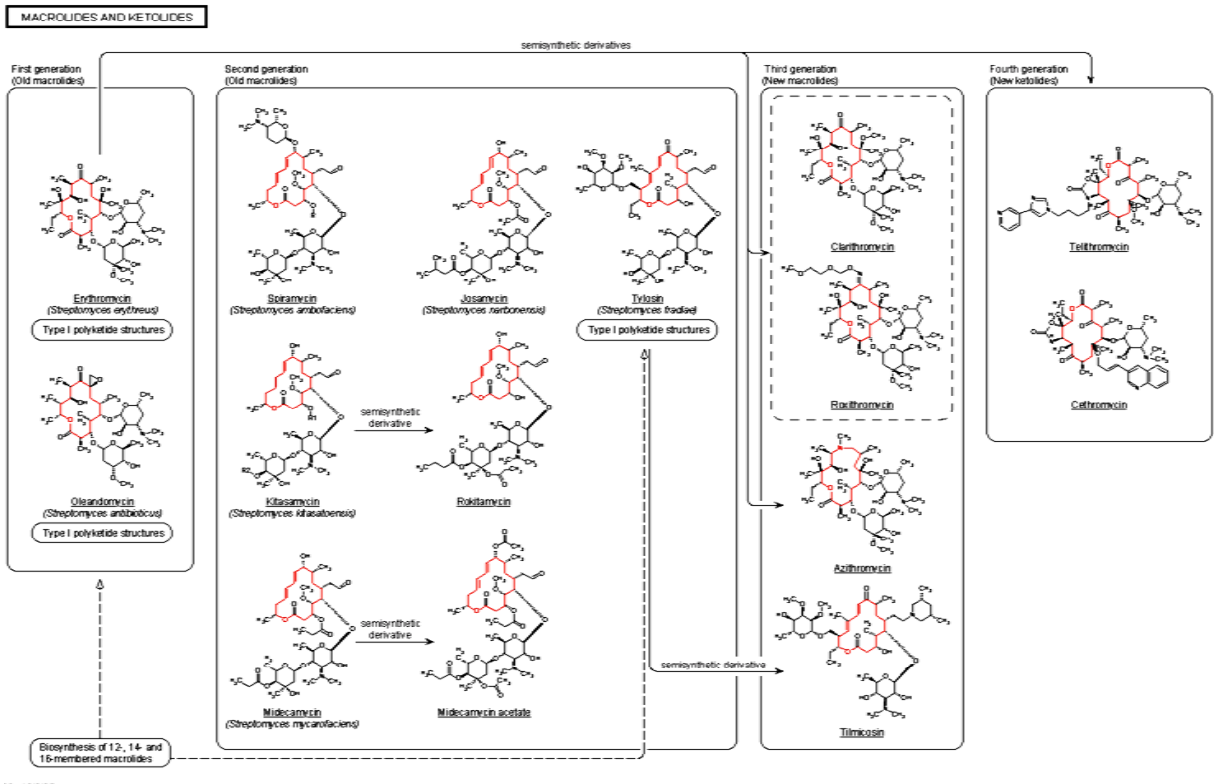
6,11-O-bridged macrolides



6,11-O-bridged ketolides

(Wang et al., *Org. Lett.* 2004, 6, 4455)

Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides



Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides

Organism (number of strains)	Telithromycin	Erythromycin A	Azithromycin	Clarithromycin
S. pneumoniae				
pen^r (110)	0.12/1	8/>64	4/>16	
ery^{r}clin^r (57)	0.06/1	>64/>64	>16/>16	
ery^{s}clin^s (537)	≤0.06/≤0.06	≤0.06/≤0.06		
ery^{r}clin^s (50)	0.25/0.5	4/8		
ery^{s}clin^r (24)	≤0.06/≤0.06	>32/>32		
14.8 (1)	MIC 0.0002 MBC 0.001	>64/>64	MIC 0.0006 MBC 0.02	
erm(B)$^+$*mef*(A)$^-$ (73)	0.03/0.12	MIC 64		
erm(B)$^+$*mef*(A)$^+$ (1)	MIC 0.06			
S. pyogenes (80)	range	range	range	range
ery^s	0.003–0.06 HMR 3004	0.003–0.25	0.015–0.25	0.007–0.0125
ery^r cMLS phenotype (64)	1/4	>128/>128	>128/>128	>128/>128
ery^r iMLS phenotype (120)	0.5/4	>128/>128	>128/>128	>128/>128
M phenotype (203)	0.06/0.12	8/16	4/8	8/8
S. aureus				
MSSA ery^{s}clin^s (278)	0.06/0.12	0.5/0.5	2/2	
MRSA ery^{r}clin^r (140)	>64/>64	>64/>64	>16/>16	
coagulase-negative staphylococci				
oxa^{s}ery^{s}clin^s (125)	0.06/0.12	0.25/0.5	0.5/1	
oxa^{r}ery^{r}clin^r (148)	>64/>64	>64/>64	>16/>16	
Enterococcus faecalis				
van^s (377)	0.06/4	4/>64	16/>16	
ery^{r}clin^r (107)	2/8	>32/32	>32/>32	>32/>32
van^r (13)	2/8	>32/>32	>32/>32	>32/>32
Enterococcus faecium				
van^r (90)	8/16	>64/>64	>16/>16	
Bordetella pertussis	0.015/0.03 (n = 51)	0.03/0.06 (n = 34)	0.03/0.06 (n = 40)	0.06/0.06 (n = 37)
Corynebacterium diphtheriae (410)	0.004/0.008	0.015/0.026	0.044/0.058	0.006/0.008
Listeria monocytogenes (15)	0.125/0.25	0.125/0.25	1/1	0.06/0.125
L. pneumophila (46)	0.032/0.125	0.125/0.5		0.032/0.046
C. pneumoniae	MIC/MBC			
CWL 029 (ATCC VR 1310)	0.0156/2.5–5			
G954 (1)	0.0156/0.31–2.5			
C. pneumoniae (19)	0.0625/0.25	0.125/0.25	0.125/0.25	
M. pneumoniae (25)	≤0.015/≤0.015	≤0.015/≤0.015	≤0.015/≤0.015	≤0.015/≤0.015
M. hominis (30)	16/16	>64/>64	64/>64	64/>64
Ureaplasma urealyticum (15)	0.03/0.03	0.25/0.5	0.25/0.25	0.03/0.03
Rickettsia spp. (6)	MIC range 0.5–1	MIC range 0.125–8		
Bartonella spp. (8)	MIC range 0.003–0.015	MIC range 0.006–0.25		
Coxiella burnetii (3)	MIC range 1	MIC range 8		

Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides

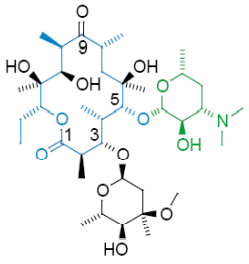
Organism (number of strains)	Telithromycin	Erythromycin A	Azithromycin	Clarithromycin	I
Neisseria meningitidis (200)	0.12/0.12	1/1	0.5/1	0.12/0.5	
Neisseria gonorrhoeae (200)	0.06/0.12	0.5/2	0.12/0.25	0.25/1	
H. influenzae					
β-Lactamase-negative (20)	1/2	4/8	1/1	4/8	
β-Lactamase-positive (24)	1/2	4/4	0.5/1	4/8	
Moraxella catarrhalis (150)	0.12/0.12 MIC pH 6.8/7.4	0.55/25	0.06/0.06	0.12/0.12 MIC pH 6.8/7.4	
M. tuberculosis (H37Rv)	>40/>40			20/5	
M. avium (ATCC 25291)	5/1.25			0.6/0.15	
Helicobacter pylori					
NCTC 11637 (1)	MIC 0.5 mg/L				
B. fragilis (62)	4/4	4/16			
Bacteroides ovatus (70)	4/4	4/>32			
Fusobacterium spp. (17)	2/4	>32/>32			
Bilophila wadsworthia (29)	2/4	8/16			
Clostridium spp. (29)	≤0.03/0.5	1/4			

Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides

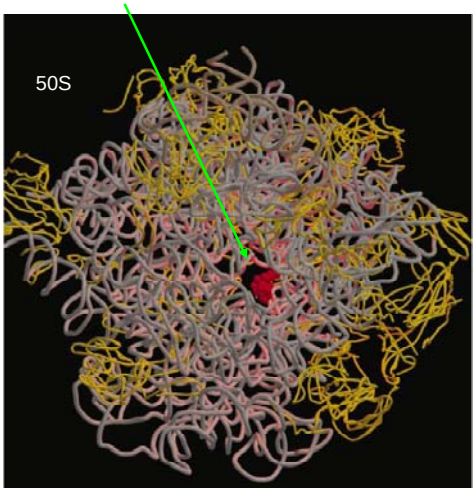
Drug	Erythromycin Base	Clarithromycin/ 14-OH metabolite		Azithromycin	Roxithromycin	Dirithromycin
Dose **	500 mg*	500 mg BID, dose 5	2 –500mg XL once daily, day 5	500mg po BID x 1; 500mg po qd x 5 days	150 mg BID x 10 days	500 mg QD, 10 days
Dosage Form	Varies	Tablet-immediate release	Tablet-extended-release	Capsule	Tablet	Tablet
Bioavailability (%)	Varies *	55%		37%	72-85%	6-14%
Cmax (mg/L)	0.3 – 3.5	2.67 +/- 0.94 0.88+/-0.20	2.59 +/-0.71 0.79 +/- 0.17	0.62	9.3 +/-1.4	0.48 +/- 0.43
Tmax (h)	4-5	2.6 +/- 1.2 2.6+/- 1.1	7.8 +/- 4.0 8.7 +/-5.2	2.3	1.4 –/-1.2	4.0 – 4.5
AUC (0-12) (mg*hr/mL)	1.04 – 8.48	19.59+/-6.49 7.37+/-1.4	**42.1 +/- 13.2 **5.1 +/- 3.2	3.18	70.8+/-14.4	**3.37
T1/2 (h)	1.5 2	4.5/6.9		35- 40	12.4 +/-4.9	42
V	0.64 (L/kg)	222 +/-206 (L) ND		23 (L/kg)	0.87 +/-0.63 (L/kg)	800 (L)
Protein binding (%)	65-90%	42-70%		7-50%	73-96%	19%

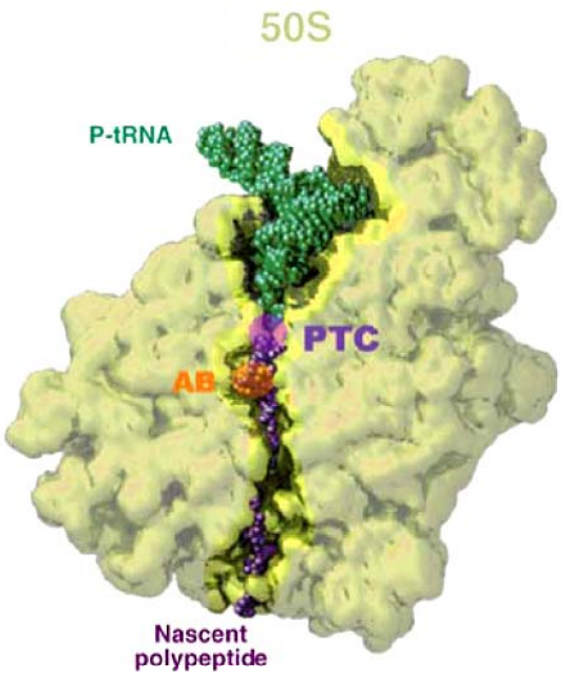
** (*qd* = *quoque die*, once daily; *bid* = *bis in diem*, twice a day)

Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides



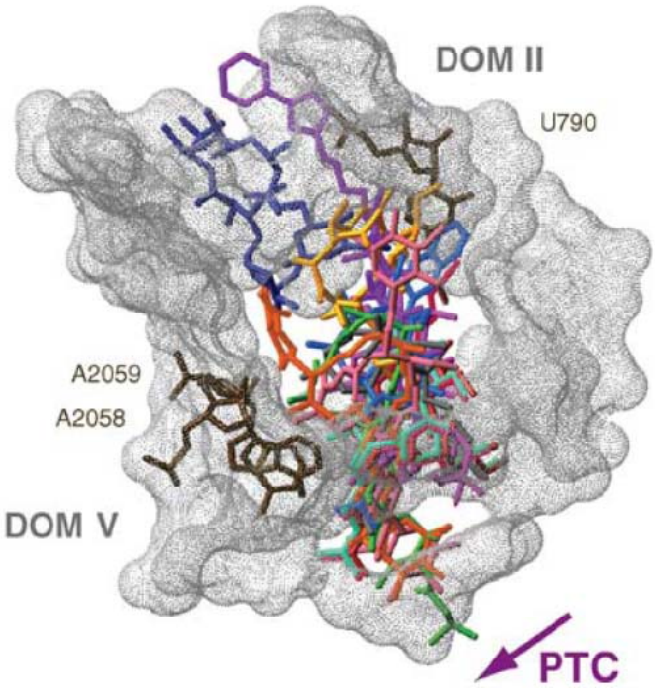
Erythromycin





(Schlunzen et al., *Nature* 2001, 413, 814)

Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides



Macrolides

14-member
- Erythromycin
- Clarithromycin
- Roxithromycin
- Troleandomycin

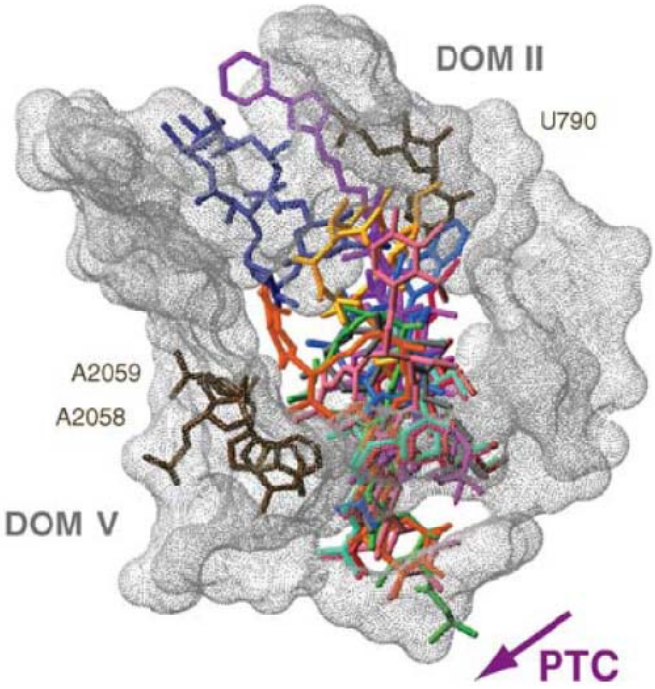
15-member
- Azithromycin (1°)
- Azithromycin (2°)
- Azithromycin (H50S)

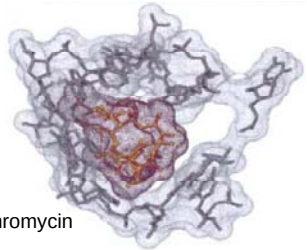
16-member
- Tylosin (H50S)
- Spiramycin (H50S)
- Carbomycin (H50S)

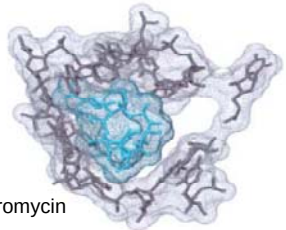
Ketolides
- ABT-773
- Telithromycin

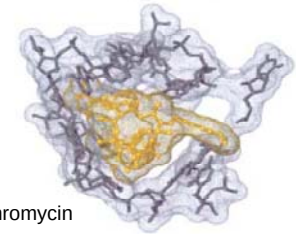
(Schlunzen et al., *Nature* 2001, 413, 814; Hansen et al., *Mol. Cell.* 2002, 10, 117; Schlunzen et al., *Structure* 2003, 11, 329; Tu et al., *Cell* 2005, 121, 257)

Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides

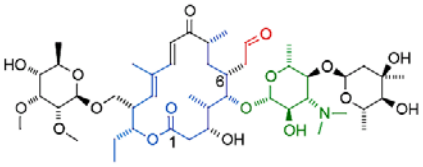



Erythromycin

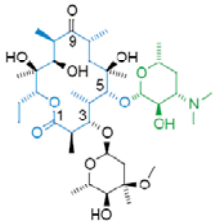

Clarithromycin


Roxithromycin

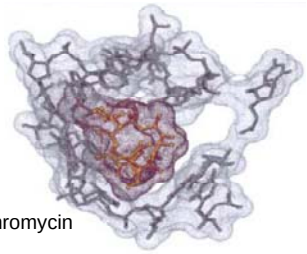
Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides



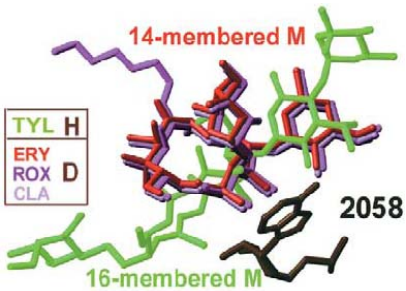
Tylosin

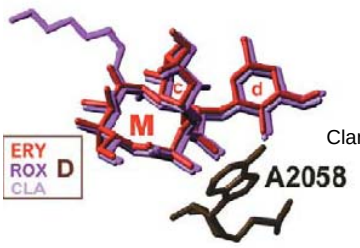


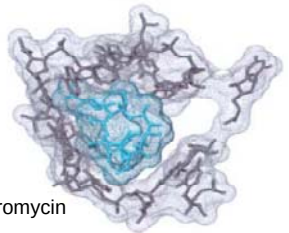
Erythromycin



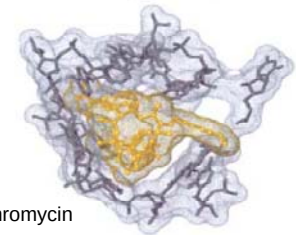
Erythromycin







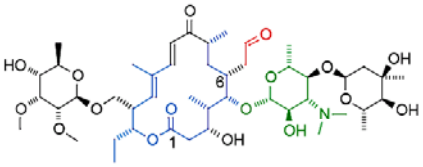
Clarithromycin



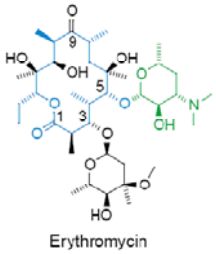
Roxithromycin

- lactone ring adapts a folded-out conformation that projects most polar groups towards one face of the molecule
- opposite side of the macrocycle is quite hydrophobic, facing toward the hydrophobic wall of the peptide exit tunnel

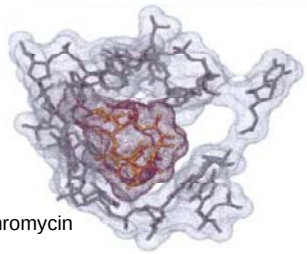
Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides



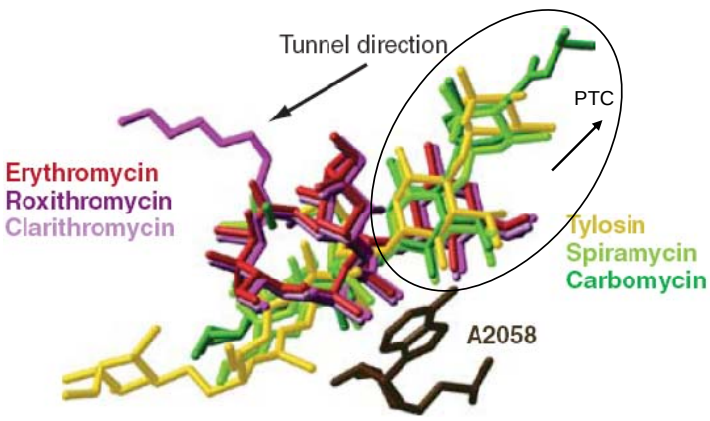
Tylosin

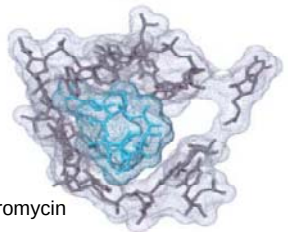


Erythromycin

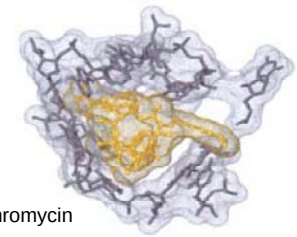


Erythromycin



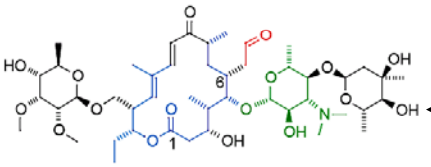


Clarithromycin



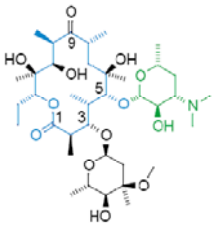
Roxithromycin

Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides

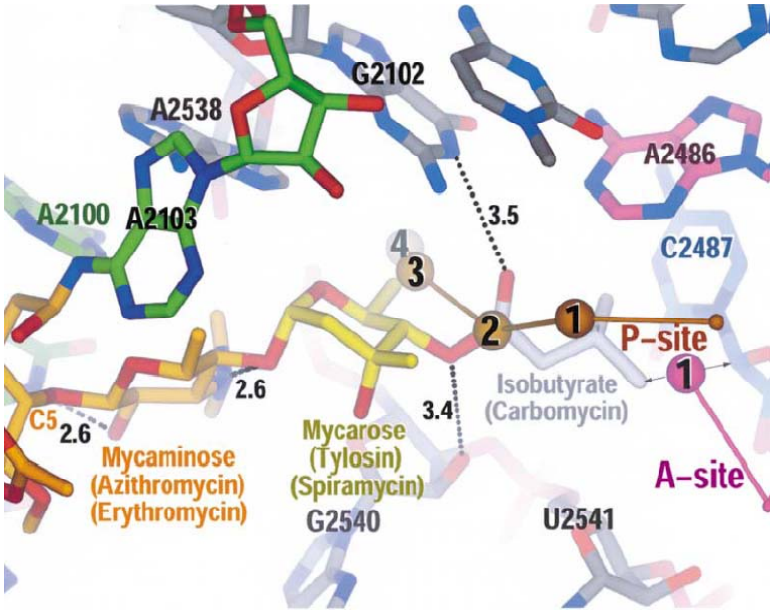


Carbomycin: isobutyric acid ester

Tylosin



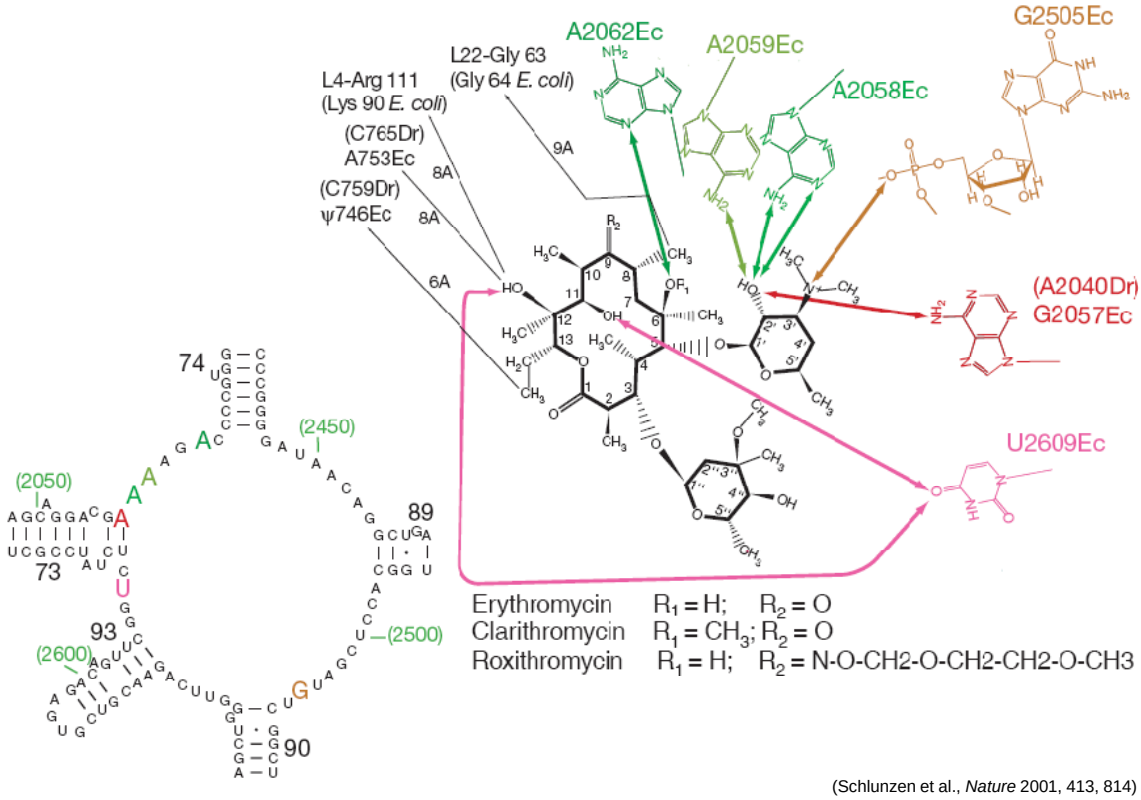
Erythromycin



- Carbomycin: reaches to PTC and occupies A-site amino acid binding pocket -> any peptide bond formation blocked
- Tylosin: 2 sugar extensions at 5-position -> allows dipeptide formation
- Erythromycin: 1 sugar extension at 5-position -> allows tetrapeptide formation

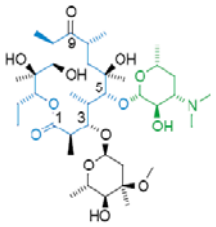
(Tu et al., *Cell* 2005, 121, 257)

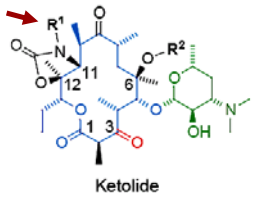
Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides

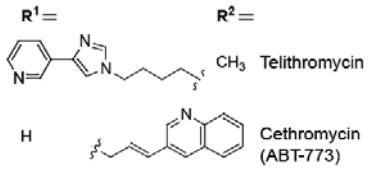


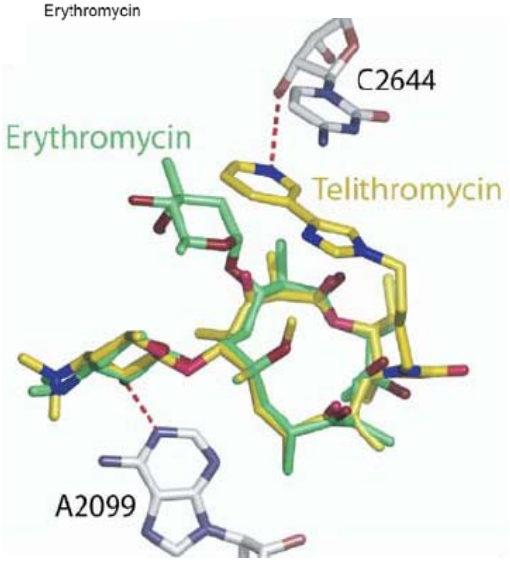
(Schlunzen et al., *Nature* 2001, 413, 814)

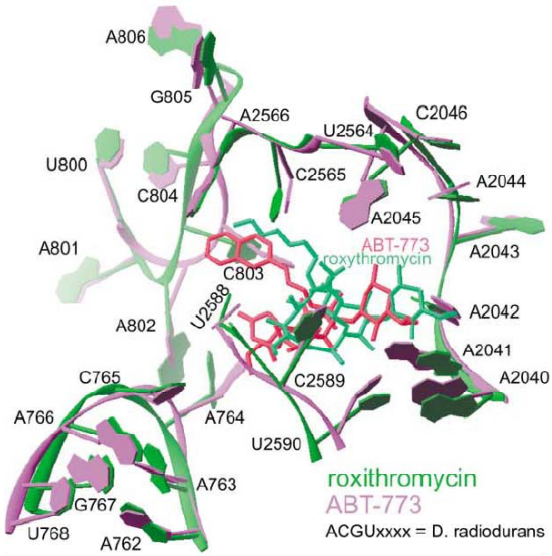
Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides - Ketolides



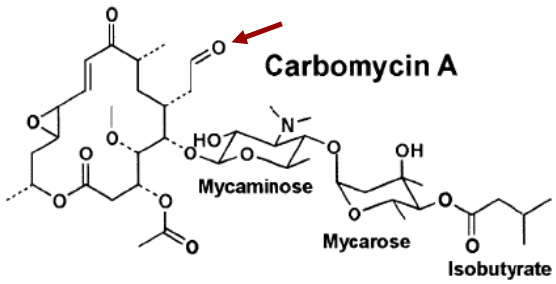


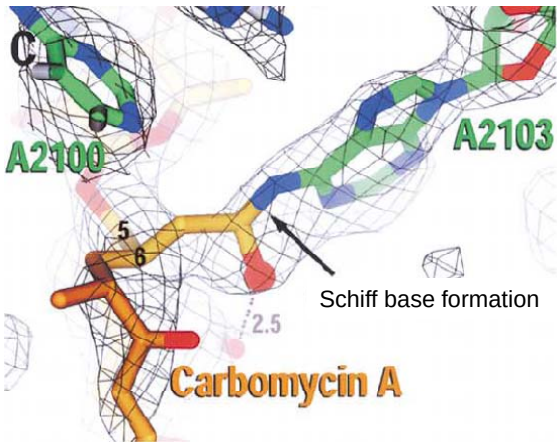






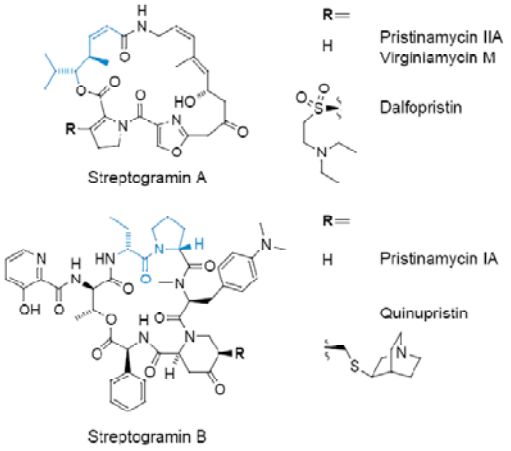
Bacterial Protein Synthesis: 50S Subunit as a Target: Macrolides - Ketolides





(Hansen et al., *Mol. Cell.* 2002, 10, 117)

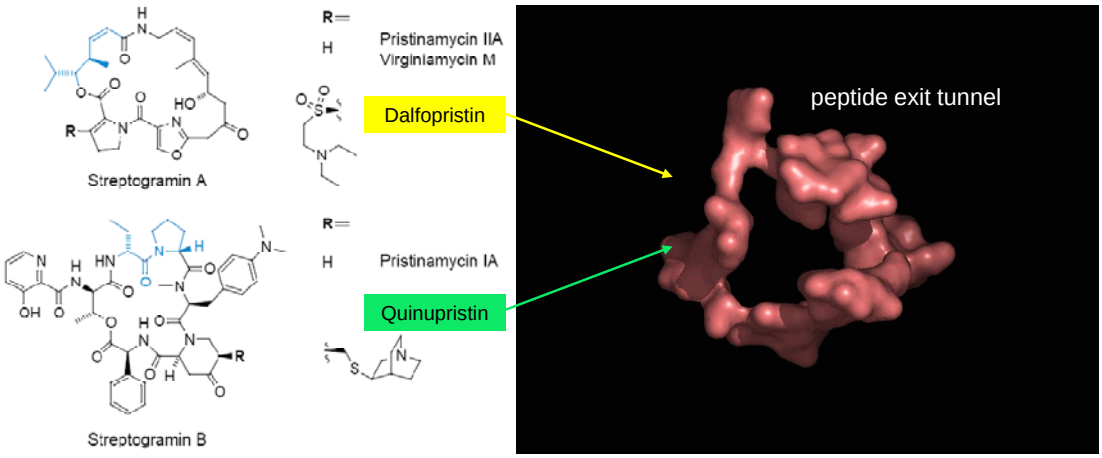
Bacterial Protein Synthesis: 50S Subunit as a Target: Streptogramins



- Isolated in 1950s from *Streptomyces pristina spiralis*
- Obtained by fermentation and semi-synthesis
- Co-synthesized in the same organism (pristinamycin IA/IIA = 7:3 ratio)
- Individual compounds are bacteriostatic, combination acts synergistically and is bactericidal
- Natural products (pristinamycins) have limited water solubility
- Semi-synthetic compounds (dalfopristin, quinupristin) have improved solubility
- 7:3 mixture of quinupristin/dalfopristin (Synercid) approved 1999 in the US (earlier in Europe)
- Active against resistant Gram-positives, *Staphylococci* (inc. MRSA, VISA), *Streptococci, Enterococci* (inc. VRE)
- Used by injection
- Resistance by compound and target site modification (same as for macrolides)

- Bind to the peptidyl transferase center at the peptide exit tunnel and block egress of the nascent peptide
- Presence of streptogramin A stimulates binding of streptogramin B component
- Hydrophobic interaction between A and B components (blue fragments)
- Streptogramin B binding site coincides with macrolide binding site at the entrance of the peptide exit tunnel

Bacterial Protein Synthesis: 50S Subunit as a Target: Streptogramins



(Harms et al., *BMC Biology* 2004, 2)

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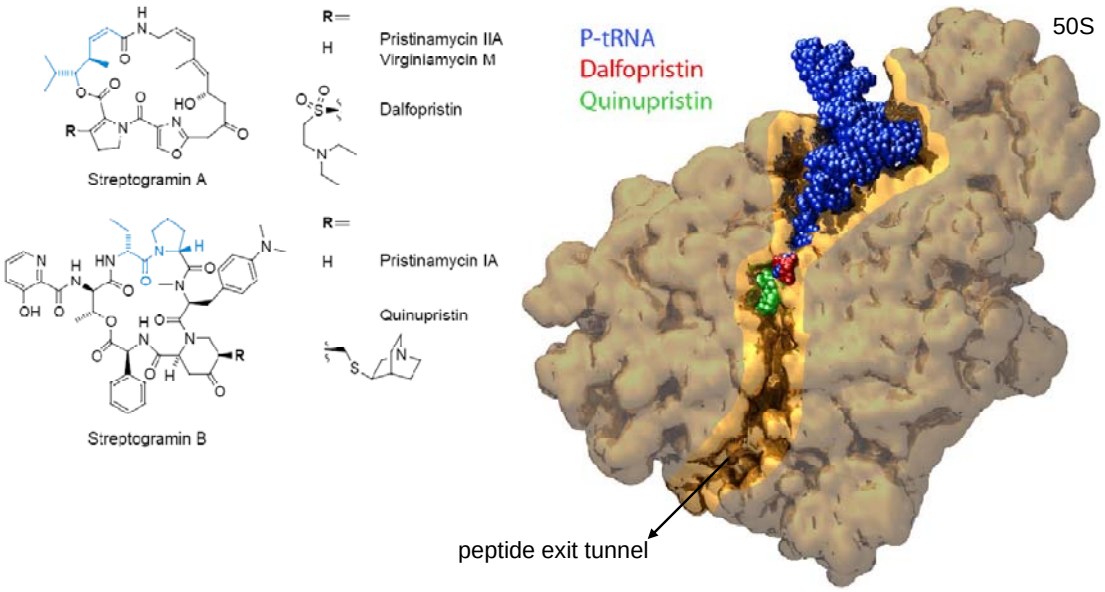
Bacterial Protein Synthesis: 50S Subunit as a Target: Streptogramins



(Harms et al., *BMC Biology* 2004, 2)

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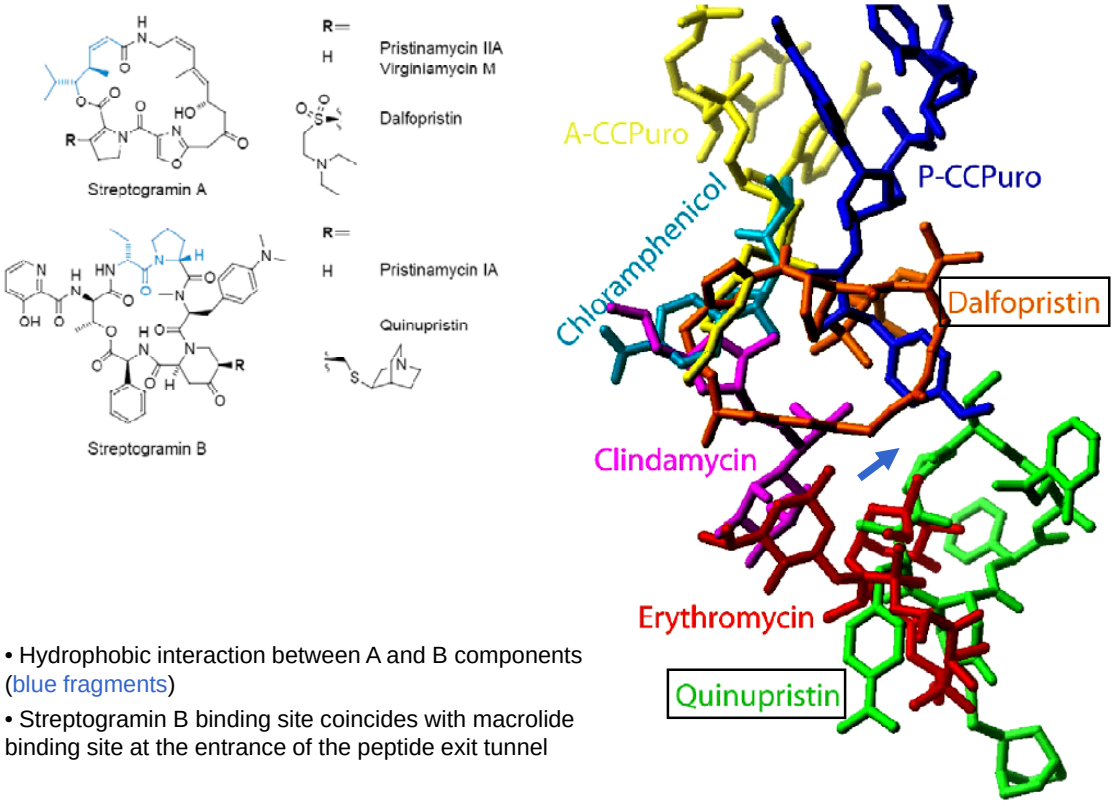
Bacterial Protein Synthesis: 50S Subunit as a Target: Streptogramins



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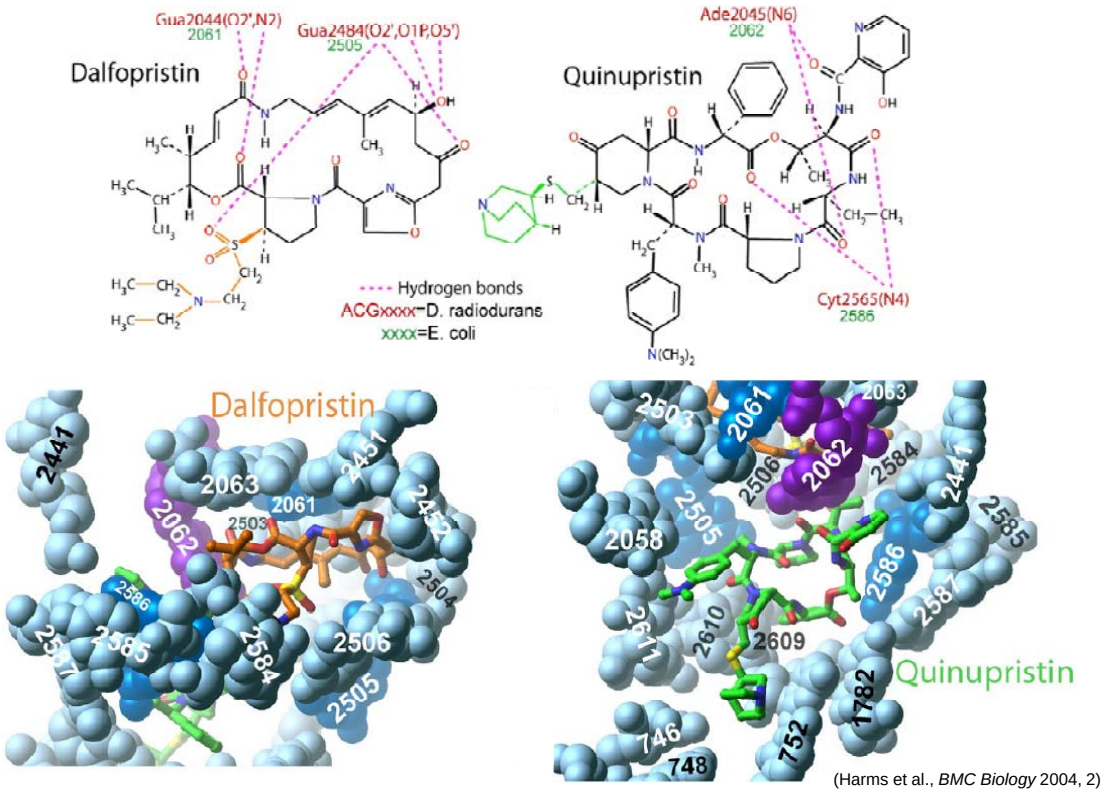
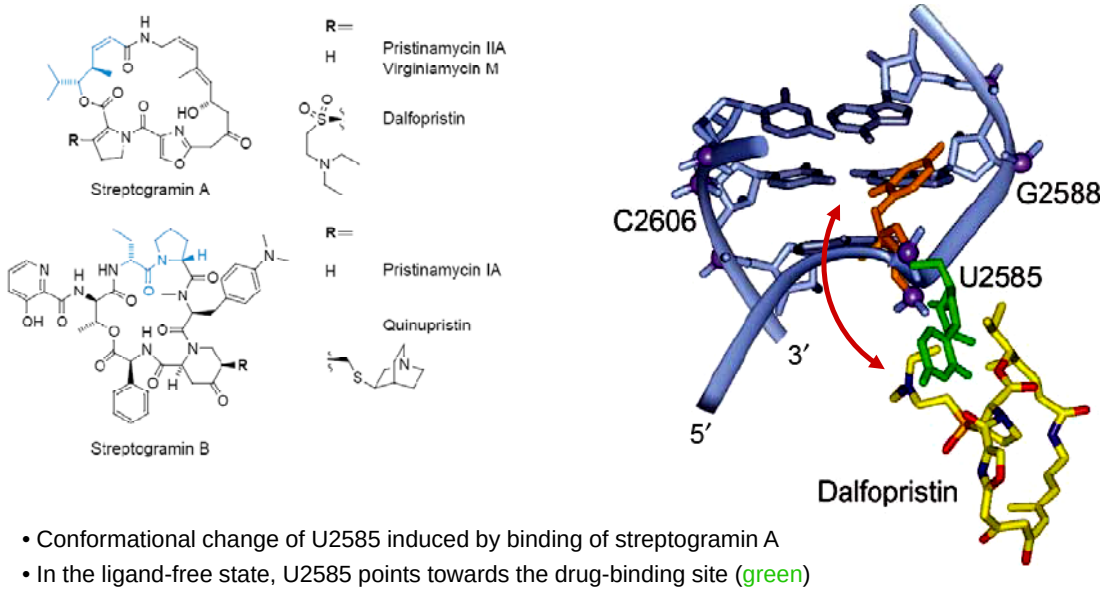
Bacterial Protein Synthesis: 50S Subunit as a Target: Streptogramins



(Harms et al., *BMC Biology* 2004, 2)

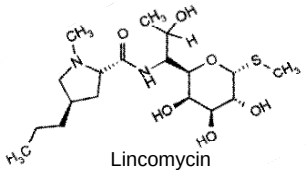
Bacterial Protein Synthesis: 50S Subunit as a Target: Streptogramins



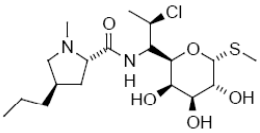
- Conformational change of U2585 induced by binding of streptogramin A
- In the ligand-free state, U2585 points towards the drug-binding site (green)
- In complex with dalfopristin, U2582 is flipped towards the G2588-C2606 base pair (orange) and forms hydrogen bonds
- Might lock the PTC in a pseudo-stable conformation that returns to the native state only slowly after removal of the drug
- Hysteresis of an RNA conformational switch
- Time-delayed return of the ribosomal machinery to a productive state?

(Harms et al., *BMC Biology* 2004, 2)

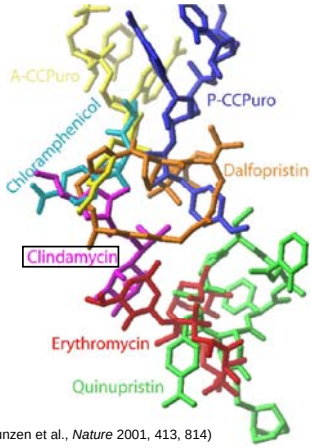
Bacterial Protein Synthesis: 50S Subunit as a Target: Lincosamides



Lincomycin

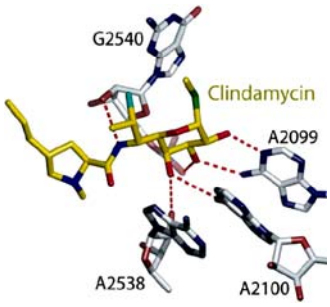


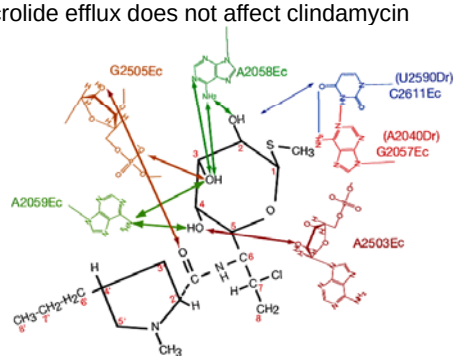
Clindamycin



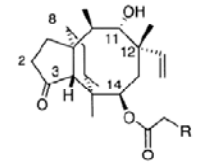
(Schlunzen et al., *Nature* 2001, 413, 814)

- Isolated in 1962 from *Streptomyces lincolnesis* (lincomycin)
- Obtained by fermentation and semi-synthesis
- Clindamycin (semi-synth.) is absorbed better and has broader spectrum
- Bacteriostatic; may be bactericidal at high concentrations
- Active against resistant Gram-positives, *Staphylococci* (some MRSA), *Streptococci (pneumoniae,* etc.*)*
- Used by injection or orally; for surgical prophylaxis, neck infections
- Rapid absorption (90% bioavailability); good distribution and tissue penetration
- Binds competitively at the macrolide binding site of 23S rRNA
- Resistance by target site modification (same as for macrolides and streptogramins -> *MLS antibiotics* = macrolide, lincosamide, streptogramin)
- Resistance by increased macrolide efflux does not affect clindamycin





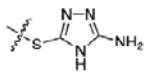
Bacterial Protein Synthesis: 50S Subunit as a Target: Pleuromutilins

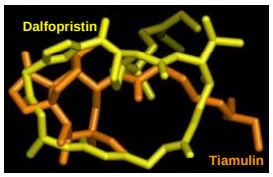


1 Pleuromutilin R = OH

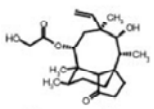
2 Tiamulin R = $SCH_2CH_2N(CH_2CH_3)_2$

3 Azamulin R =

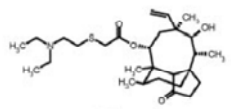




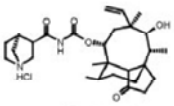
- Tricyclic diterpenoid metabolite isolated in 1951 from *Pleurotus mutilus* (pleuromutilin); semi-synthetic tiamulin introduced in 1970s
- Obtained by fermentation and semi-synthesis (tiamulin, valnemulin)
- Water soluble and readily absorbed but poor PK
- Rapid metabolism; short $t_{1/2}$; CYP450-mediated hydroxylation at C-2 and C-8
- Active against resistant Gram-positives
- Used orally or by IP injection; exclusively in animals so far (pigs)
- Binds at the PTC of 23S rRNA, almost exactly at the same site as streptogramin A (dalfopristin); overlaps with the binding site of the A-site substrate and thereby blocks peptide synthesis
- Resistance develops slowly; several mutations in ribosomal L3 protein and 23S rRNA required for full resistance



Pleuromutilin

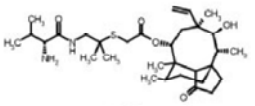


Tiamulin



SB-264128

(in Phase III testing; SKB)



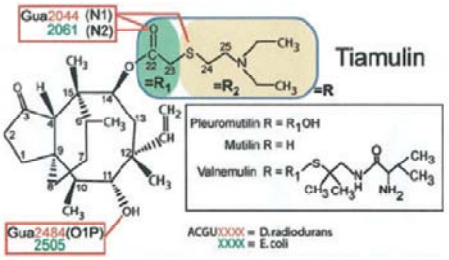
Valnemulin

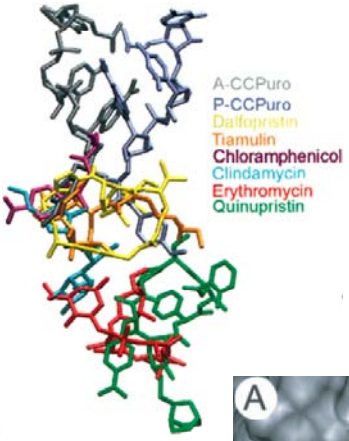
Vol 348 • August 31, 1996 THE LANCET

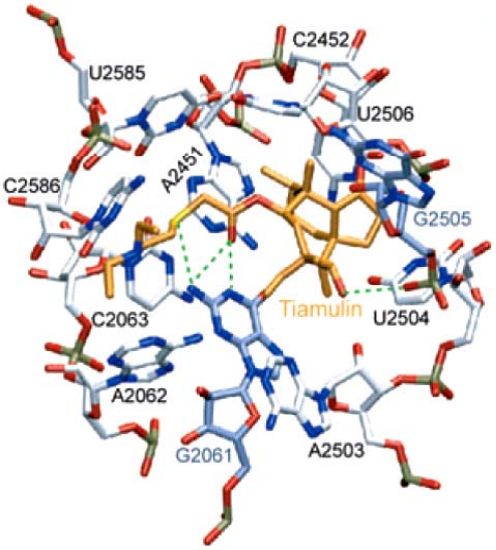
Time to ban all antibiotics as animal growth-promoting agents?

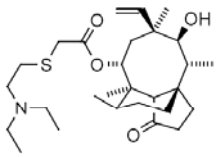
SIR—The UK was the first country to respond to the threat of antimicrobial resistance due to inclusion of antibiotics in animal feeds and the possible risks to public health. In 1968, the Swann Committee recommended that use of therapeutically prescribed antibiotics as growth promoting additives in animal feeds should be prohibited. In 1970 the ban was put into effect in the UK, and other member states of the EC soon followed. The assumption behind the Swann recommendations was that use of other molecules with coincidental antimicrobial properties would not cause pathogens to develop resistance against therapeutically-used antibiotics. This assumption was wrong, as has clearly been shown for avoparcin and vancomycin.[1] New developments—especially increasing problems with multiresistant bacteria and the search for new antibiotics against them—make it necessary to reconsider the Swann recommendations.

Bacterial Protein Synthesis: 50S Subunit as a Target: Pleuromutilins

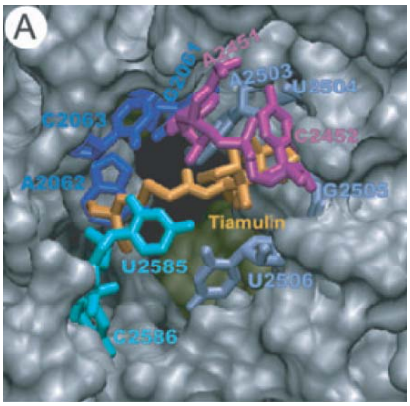






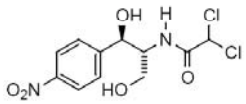


Tiamulin

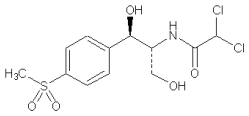


(Schlunzen et al., *Mol. Microbiol* 2004, 54, 1287)

Bacterial Protein Synthesis: 50S Subunit as a Target: Chloramphenicol

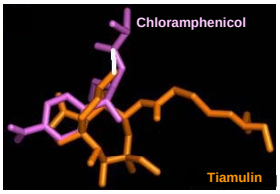


Chloramphenicol



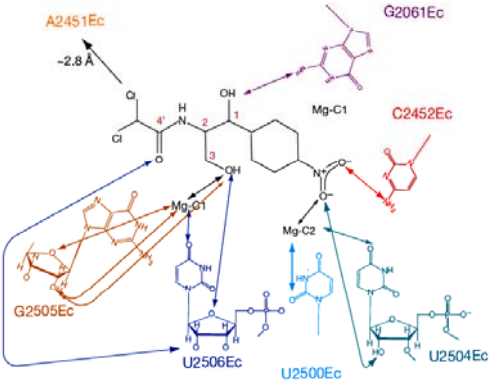
Thiamphenicol

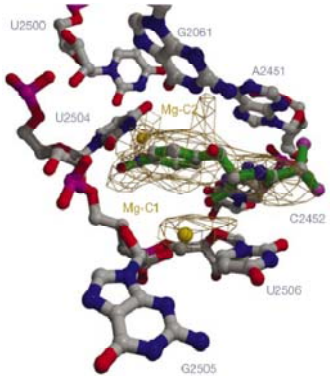
- Isolated in 1947 from *Streptomyces venezuleae*
- Obtained by fermentation
- Broad activity against Gram+/- bacteria; for serious infections: typhoid fever, meningitis, brucellosis, anaerobes
- Bacteriostatic
- Used orally, by injection or topically
- Good distribution and bioavailability ($t_{1/2}$=4h); readily penetrates bacterial and human cells
- Eukaryotic mitochondrial (but not cytoplasmic) ribosomes are susceptible; erythropoietic cells are particularly sensitive
- High toxicity in newborns due to slow metabolism of neonate liver ("gray baby syndrome" when chloramphenicol was widely used in newborns)
- Now rarely used in humans due to serious side-effects caused by mitochondrial toxicity (aplastic anemia, leukopenia, bone marrow damage)
- Thiamphenicol has not been found to cause aplastic anemia and is used in Europe (not approved in the US)

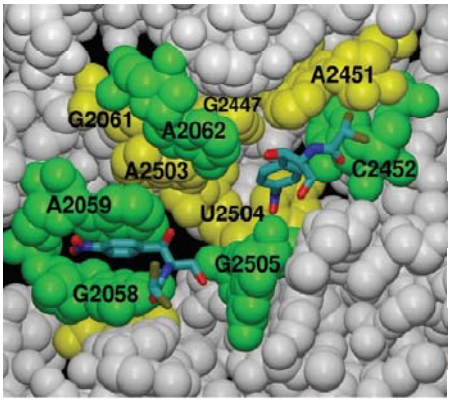


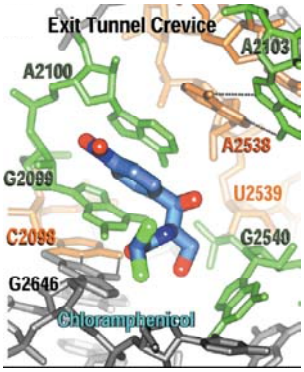
- Binds at the PTC of 23S rRNA, probably at two sites; one of the sites overlaps with pleuromutilin and other PTC-binding antibiotics
- Interaction with 23S rRNA involves Mg^{++} ion
- Blocks peptide synthesis by sterical interference with the aminoacyl moiety in the A site and prevention of the transition state
- Resistance develops by compound modification (acetylation)

Bacterial Protein Synthesis: 50S Subunit as a Target: Chloramphenicol









(Schlunzen et al., *Nature* 2001, 413, 814; Hansen et al., *JMB* 2003, 330, 1061)

Bacterial Protein Synthesis: 50S Subunit as a Target: Oxazolidinones

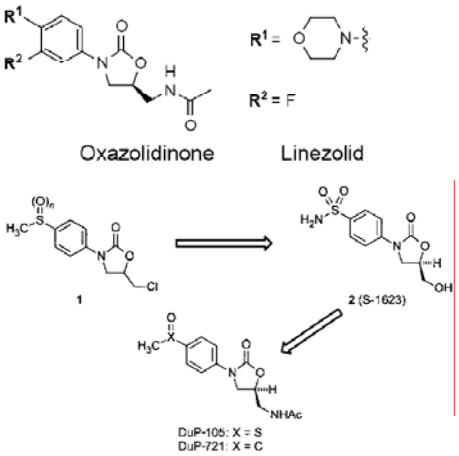
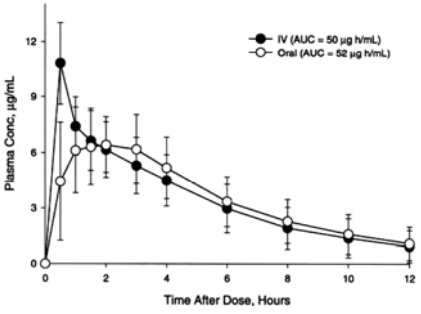
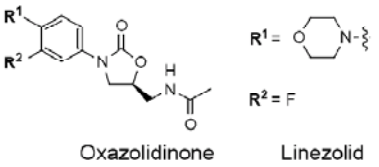


Figure 2. Emergence of the oxazolidinones at DuPont.



- Fully synthetic compounds; discovered at DuPont in an effort to develop oxazolidinones to treat plant diseases; first disclosed 1987 (DuP-105, DuP-721)
- Stopped at DuPont due to toxicity concerns; continued at Upjohn (~1987) -> bought by Pharmacia (1995; approved as Zyvox=linezolid in 2000) -> bought by Pfizer (2003)
- Excellent activity against resistant Gram+ bacteria (*S. aureus*, MRSA, methicillin and vancomycin-resistant *S. aureus*, *Streptococci*, *Enterococci*, VRE, *Bacilli*); inactive against G-
- Bacteriostatic for *Staphylococci, Enterococci*; bactericidal for *Streptococci*
- Used orally and IV as "antibiotic of last resort" for serious G+ infections (in the hospital); superior to vancomycin in MRSA
- Good distribution and extreme bioavailability (100%, $t_{1/2}$=5h)
- Binds at the PTC of 23S rRNA and prevents formation of the initiation complex by interfering with association of the initiator fMet-tRNA at the P site (unique mechanism)
- Resistance develops by target modification of 23S rRNA (G2576->U mutation; very rare: in 1998-2000, only 8 out of 40,000 tested clinical pathogen strains had MIC >=8µg/ml); no cross-resistance to any other antibiotic

Bacterial Protein Synthesis: 50S Subunit as a Target: Oxazolidinones



Oxazolidinone Linezolid

Table 3. Mean (+/-SD) multiple-dose pharmacokinetics of linezolid q12h [2].

Parameter	600 mg Tablet	600 mg iv.	400 mg Tablet
C_{max} (mcg/ml)	21.2 (±5.78)	15.1 (±2.52)	11.0 (±4.37)
C_{min} (mcg/ml)	6.15 (±2.94)	3.68 (±2.36)	3.08 (±2.25)
T_{max} (h)	1.03 (±0.62)	0.51 (±0.03)	1.12 (±0.47)
AUC (mcgh/ml)	138.0 (±42.1)	89.7 (±31.0)	73.40 (±33.5)
Half-life (h)	5.40 (±2.06)	4.80 (±1.70)	4.69 (±1.70)
Clearance (ml/min)	80 (±29)	123 (±40)	110 (±49)

Organism	Antibacterial agent	MIC_{90} [μg mL^{-1}][a]
Staphylococcus aureus (methicillin-susceptible)	linezolid	4
	vancomycin	1
S. aureus (methicillin-resistant)	linezolid	4
	vancomycin	2
Staphylococcus epidermidis (methicillin-sensitive)	linezolid	2
	vancomycin	2
S. epidermidis (methicillin-resistant)	linezolid	2
	vancomycin	2
Enterococcus faecalis (methicillin-sensitive)	linezolid	4
	vancomycin	2
E. faecalis (VanB)	linezolid	4
	vancomycin	>16
Enterococcus faecium	linezolid	2
	vancomycin	≤0.5
E. faecium (VanA)	linezolid	4
	vancomycin	>16
E. faecium (VanB)	linezolid	4
	vancomycin	>16
Streptococcus pneumoniae	linezolid	1
	vancomycin	≤0.25
S. pneumoniae (penicillin-sensitive or -resistant	linezolid	1
	vancomycin	≤0.25
Streptococcus pyogenes	linezolid	2
	vancomycin	0.5
Haemophilus influenzae[b]	linezolid	8
	vancomycin	>16
Moraxella catarrhalis[b]	linezolid	4
	vancomycin	>16
Gram-negative bacilli[c]	linezolid	>64
	vancomycin	>16
Bacteroides fragilis[d]	linezolid	≥16
	vancomycin	>16
Clostridium spp.[d]	linezolid	2
	clindamycin	4
Peptostreptococcus spp.[d]	linezolid	2
	clindamycin	2

(Barbachyn & Ford, *Angew. Chem. Int. Ed.* 2003, 42, 2010)

Bacterial Protein Synthesis: 50S Subunit as a Target: Oxazolidinones

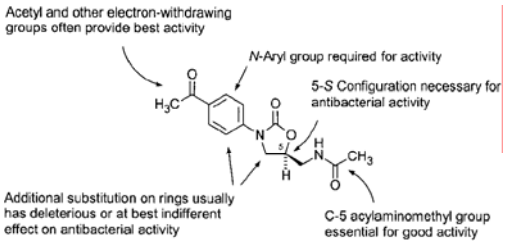


Figure 3. Early structure–activity relationships of oxazolidinones determined at DuPont.

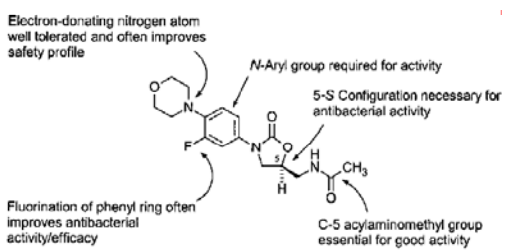
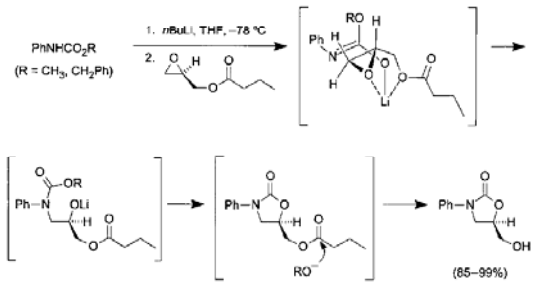
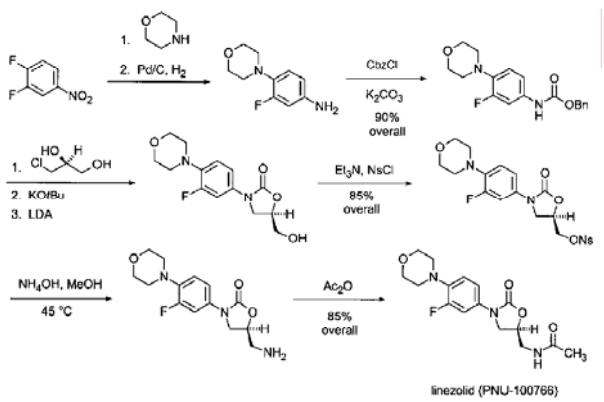


Figure 5. Revised structure–activity relationships identified at Pharmacia.



Scheme 1. Enantiomeric synthesis of phenyloxazolidinones (Manninen reaction).



Scheme 3. Synthesis of linezolid (PNU-100766) on a process scale. LDA = lithium diisopropylamide, Ns = *meta*-nitophenylsulphonyl.

(Barbachyn & Ford, *Angew. Chem. Int. Ed.* 2003, 42, 2010)

Bacterial Protein Synthesis: 50S Subunit as a Target: Oxazolidinones

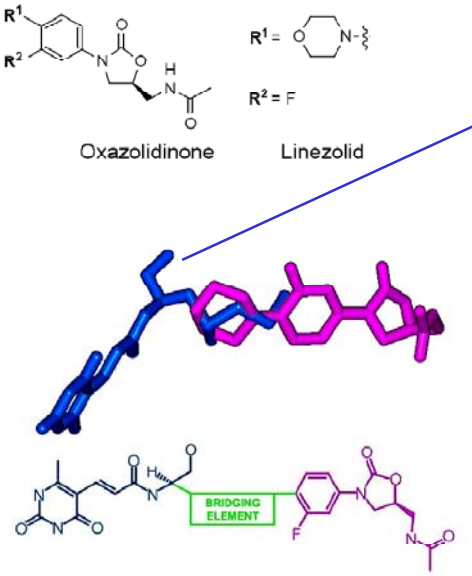
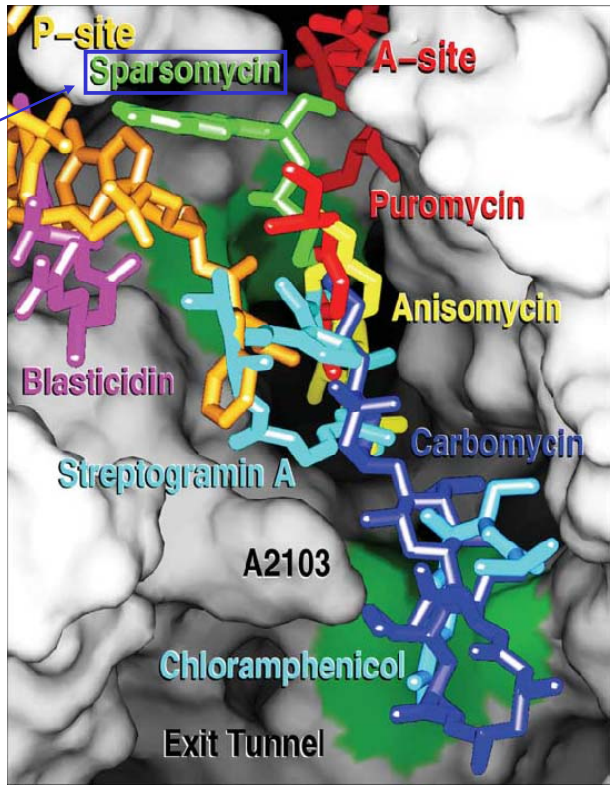
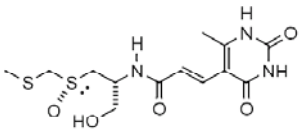


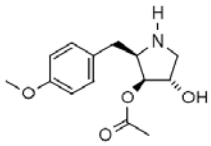
Fig. 3 – (Top) Relative binding orientations of sparsomycin (magenta) and linezolid (blue) in the 50S ribosomal subunit, with the rRNA stripped away for clarity. (Bottom) Original design hypothesis.

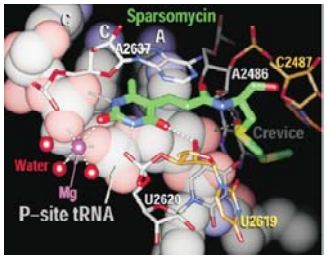


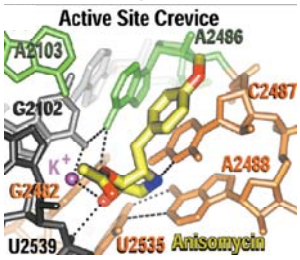
(Franceschi & Duffy, *Biochem. Pharmacol.* 2006, 71, 1016)

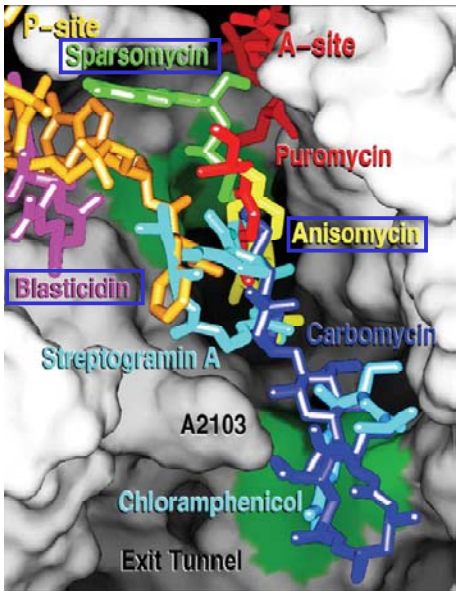
Bacterial Protein Synthesis: 50S Subunit as a Target: Other PTC-Binders







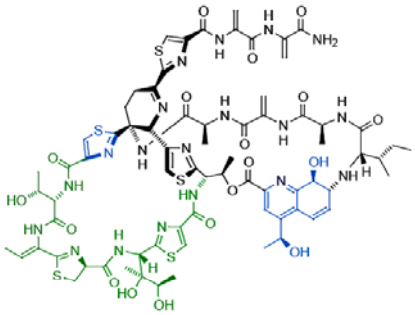


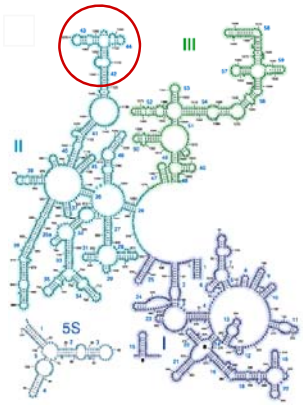


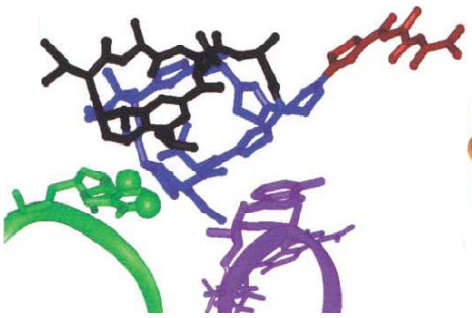
- Sparsomycin binds to the PTC in the presence of peptidyl–tRNA in the P site, which in turn is stabilized by the bound antibiotic and prevented from proceeding into the peptidyl-transfer step
- Anisomycin inhibits peptide bond formation by sterical interference with the aminoacyl–tRNA 3' acceptor
- All lack specificity for the bacterial ribosome -> not used as antibiotics

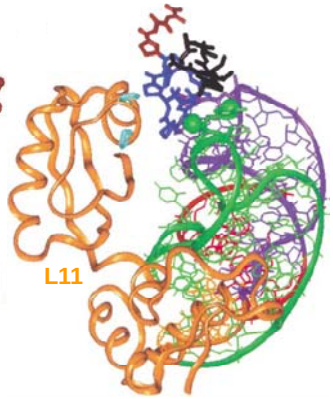
(Hansen et al., *JMB* 2003, 330, 1061)

Bacterial Protein Synthesis: 50S Subunit as a Target: Thiopeptides





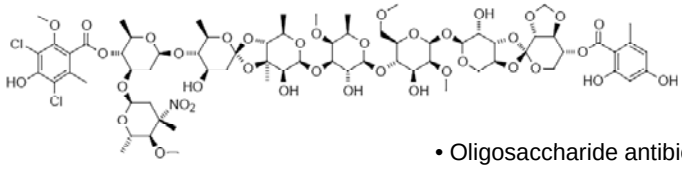


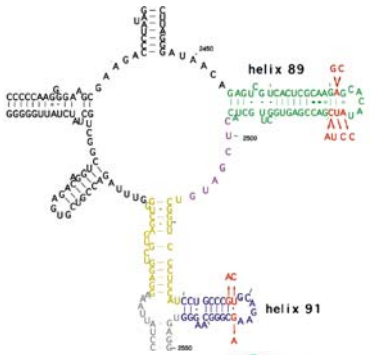


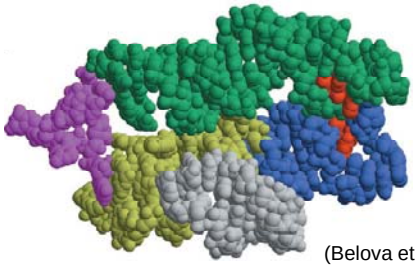
- First thiazole-peptide antibiotic isolated in 1948 from *Streptomyces* (micrococcin), followed 1954 by thiostrepton; today, ~ 29 families of antibacterial thiopeptides known
- Obtained by fermentation and semi-synthesis
- Very low water solubility and bioavailability
- Inactive against Gram- (no membrane permeability); active against resistant Gram-positives, *Staphylococci* (inc. MRSA)
- Binds at the L11 protein-binding domain of 23S rRNA, which is involved in stimulating GTPase action of elongation factors
- Binds at the interface of rRNA and L11 protein and prevents a conformational transition required for GTPase activation
- Resistance develops by complete loss of ribosomal protein L11 (cells still viable)

(Lentzen et al., *Chem.Biol.* 2003, 10, 769)

Bacterial Protein Synthesis: 50S Subunit as a Target: Orthosomycins







- Oligosaccharide antibiotics; first isolated 1964 from *Micromonospora carbonaceae* (everni(no)micin)
- Obtained by fermentation and semi-synthetic modification (SCH-27899 = Ziracin, Schering-Plough)
- Development of SCH-27899 voluntarily discontinued after Phase III (in 2000; toxicity? economic reasons?)
- Used by IV only
- High protein binding (96-99%); $t_{1/2}$=8-18h
- Broadly active against resistant Gram-positives, *Staphylococci* (inc. MRSA), *Enterococci* (inc. VRE), and *Streptococci;* better potency than vancomycin
- Binds at two hairpins (H89 & H91) of 23S rRNA and L16 protein, thereby prevents binding of initiation factor 2 (IF2)
- Resistance develops very slowly by mutation of ribosomal protein L16; no cross-resistance to any other antibiotic

(Belova et al., *PNAS* 2001, 98, 3726)

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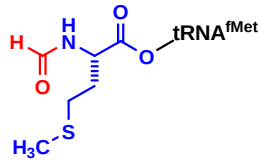
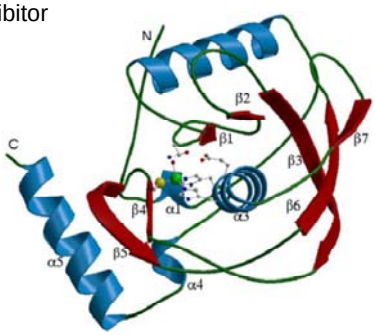
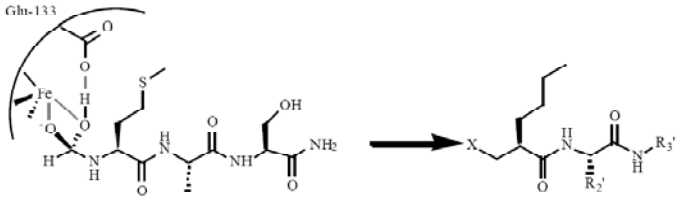
- In bacteria, translation initiation begins with formyl-methionine-tRNA
- Formyl group is removed co-translationally by ribosome-associated peptide deformylase (PDF)
- PDF is an iron-containing metallo hydrolase
- PDF is essential for bacteria
- Hydroxamates inhibit function of PDF by chelating the active-site Fe(II)
- Actinonin, isolated in 1960 from *Streptomyces*, is a natural PDF inhibitor (identified in 1999)
- Since 1999, numerous attempts to find improved inhibitors
- Enzyme is instable and difficult to handle

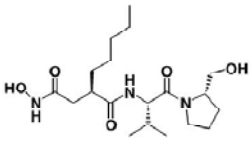






Transition State of fMAS

General Inhibitor Structure



Actinonin

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Table 3. List of Published PDF Inhibitors, Reported IC_{50} Against *E. Coli* PDF and MIC Against Various Bacteria. Refer to Fig. 3-6 for the Corresponding Structures

	1st Author (Year first published)	IC_{50} (nM)	MIC (μg/ml)	Comments
1	Hu [41] (1998)	37,000	NA	H-phosphonate: designed inhibitor based on its best substrate formyl-MA-p-nitrophenylalanine.
2	Meunnel [42] (1999)	2,500	NA	Thiol peptide: designed based on the best substrate identified.
3	Durand [44] (1999)	10,000	NA	Aldehyde peptide: identified through screening existing library.
4	Chen [39] (1999)	0.3 (Ki)	8-16[1]	Actinonin: naturally occurring PDF inhibitor
5	Jayasekera [45] (2000)	1,000	>64[1]	Thyropropic acid derivatives: non-peptidic PDF inhibitor.
6	Huntington [43] (2000)	19	10[2] 25[3]	Thiol peptide: thiol chelating group combined with the best substrate motif.
7	Green [46] (2000)	3900	NA	Biaryl acid: non-peptidic PDF inhibitor
8	Apfel [36] (2000)	16	256[1]	β-Sulfonylhydroxamic acid: identified by screening existing chemical library.
9	Clements [34, 53, 55, 56, 58, 72] (2001)	7	4-16[1]	*N*-formyl hydroxylamine with t-butylglycine at P_2' site (BB3497): active *in vivo*; P_1' analog BB-83698 has better PK profile and is in clinical development. SAR explored on chelator, P_1', P_3', and P_1 position. Patent: WO 99/39704
10	Chu [47, 54] (2001)	30 (Ki)	32[2]	Pseudopeptide metabolites: isolated from fermentation broth of *Streptomyces sp.*
11	Chen [11, 52, 73] (2000)	4-10	0.13-4[1]	Alkyl-succinate-proline hydroxamate (VRC3375): active *in vivo*; analogs with α-flouro or α-hydroxy substitution improve antibacterial activity >8-fold *in vitro*, and >3-fold *in vivo*. Patent: WO 01/44179
12	Grant [74] (2001)	200,000	ND	*N*-substituted methionine hydrazide: identified by screening compound collection.
13	Apfel [48] (2001)	120	16[4]	Quinazoline hydroxamic acid: identified by screening compound collection.
14	Thorarensen [49] (2001)	2200	32[1]	*N*-Hydroxy urea: identified by screening compound collection.
15	Harckbarth [35] (2002)	3	0.25-1[1]	*N*-alkyl urea hydroxamate: active *in vivo*. Patent: WO 01/44178
16	Xiang, *et al.* (2002)	ND	ND	Carbamate *N*-formyl hydroxylamine: Patent only: WO 02/70653
17	Xiang, *et al.* (2002)	ND	ND	Hydrazide *N*-formyl hydroxylamine: Patent only: WO 02/70541
18	Xiang, *et al.* and Aubart, *et al.* (2002)	ND	ND	2-Oxo-pyrrolidine with chelators Patent only: WO 02/70654, WO02/70540
19	Beckett, *et al.* (2002)	ND	4[1]	Benzimidazole *N*-formyl hydroxylamine: Patent only: WO 02/41886.
20	Bhat, *et al.* (2002)	ND	ND	*N*-Hydroxy-2-(substituted phenyl) acetamide Patent only: WO 02/081426, WO 03/002522
21	Chong, *et al.* (2002)	>200	ND	Hydantoin Patent only: WO 02/28829
22	Choi *et al.* (2002)	ND	ND	*N*-Sulfonyl-L-Val-L-Leu-hydroxamate Patent only: JP 02/322197
23	Christensen *et al.* and Karpinski, *et al.* (2002)	ND	ND	Benzimidazole derivative Patent only: WO 02/098901, WO 03/104209
24	Patel, *et al.* (2002)	ND	ND	Heteroarylaryl(P_2'/P_3') *N*-formyl hydroxylamine Patent only: WO 02/102790
25	Patel, *et al.* (2002)	ND	ND	Pyrrolidine bicyclic (P_2'/P_3')compounds Patent only: WO 02/102791
26	Hu [59] (2003)	7	~12[2]	Cyclization of the P_1' and P_3' side chain
27	Gane, *et al.* (2003)	ND	ND	Urea analogs (P_2'/P_3') of *N*-formyl hydroxylamine Patent only: WO 03/048115
28	Takayama [75] (2003)	1040	10[3]	Benzothiazolylidene hydroxamic acid derivatives
29	Aubart, *et al.* (2003)	ND	ND	*N*-Hydroxy-*N*-(3-hydrazino-3-oxopropyl)formamide Patent only: WO 03/101442
30	Molteni [50] (2004)	5	8-128	Benzothiazinone derivatives
31	Howard [51] (2004)	1660	ND	2-Thioxo-4-thiazolidinone N-hexanoic acid
32	Cali [10] (2004)	3400	>128	Isoxazole-3-hydroxamic acid derivatives

In the table, NA=Not active; ND=No data; MICs against various organisms: 1=*S. aureus*; 2=*E. coli*; 3= *B. subtilis*; 4=*H. influenzae*. Activities for *in vivo* active compounds are in bold.

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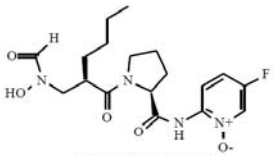
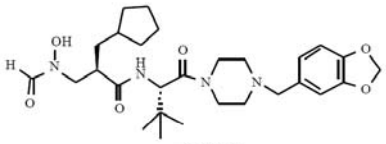


VIC-104959 (LBM415)



BB-83698

Structures of two PDF inhibitors, **VIC-104959 (LBM415)** and **BB-83696**, that went into human Phase I clinical trials.

Table 5. MIC_{90} (μg/mL) of VIC-104959 (LBM415) and BB-83698 Against Key Pathogens (see Fig. 7 for Structures)

Organism	n	VIC-104959[a]	n	BB-83698[b]
S. aureus	56	2	30	4
S. pneumoniae	43	1	36	0.25
H. influenzae	33	4	50	16
M. catarrhalis	22	0.5	29	0.06
E. faecalis	23	8		
E. faecium	31	4		
H. pylori	19	0.5		
Mycoplasma	5	0.002-0.015		
Anaerobes (G-)	13	0.5		
Anaerobes (G+)	6	>128		
Enterobacteriaceae	80	>128		