Chemistry 259

Medicinal Chemistry of Modern Antibiotics

Spring 2012

Lecture 7: *Antibiotics Classes & Targets*

Part II: Drugs Targeting Fatty Acid and Folic Acid Biosynthesis, Cell Division

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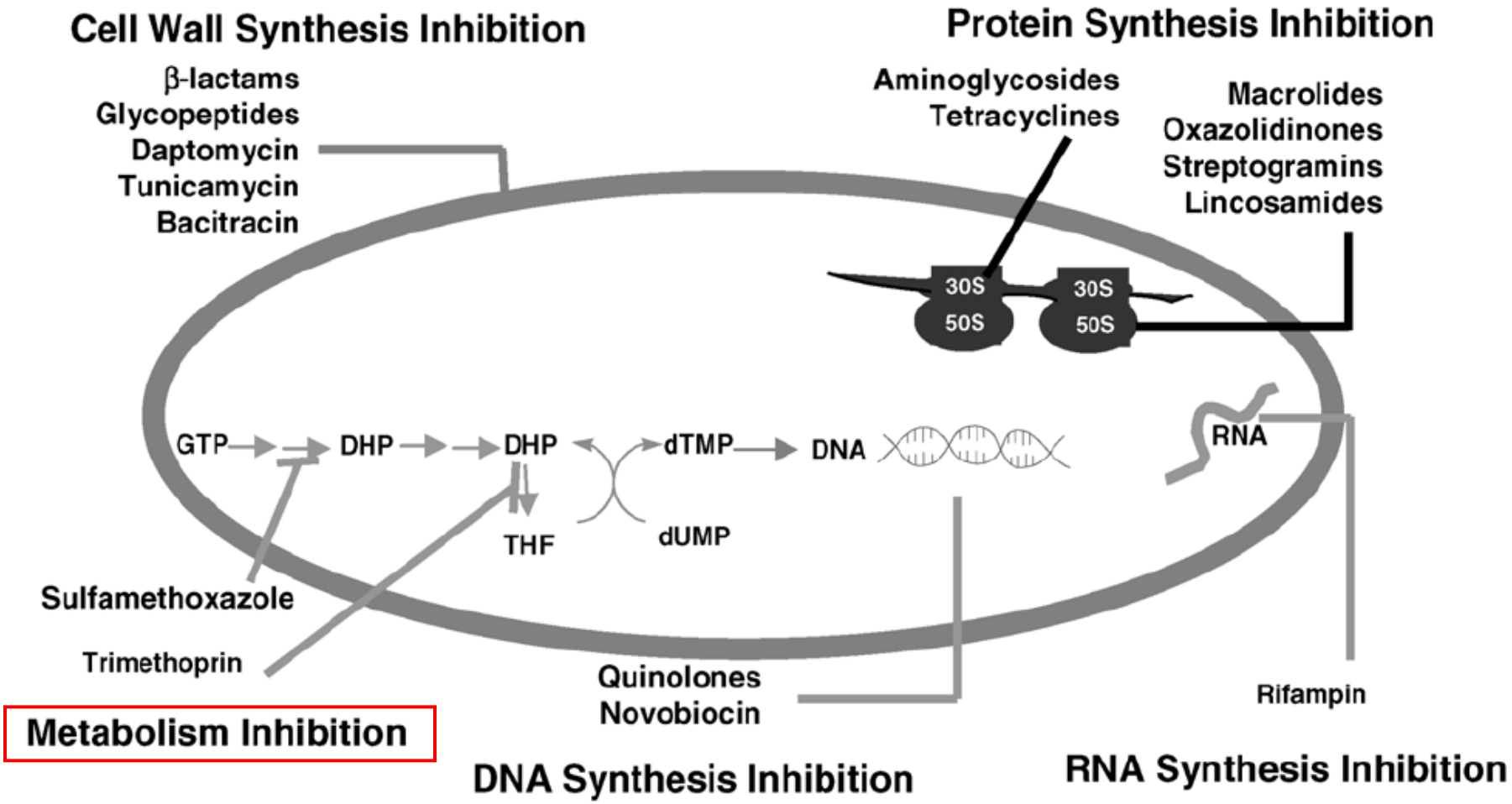
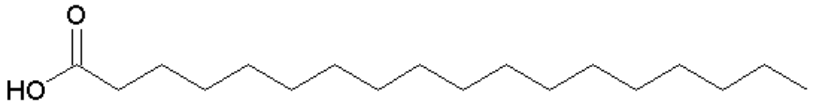
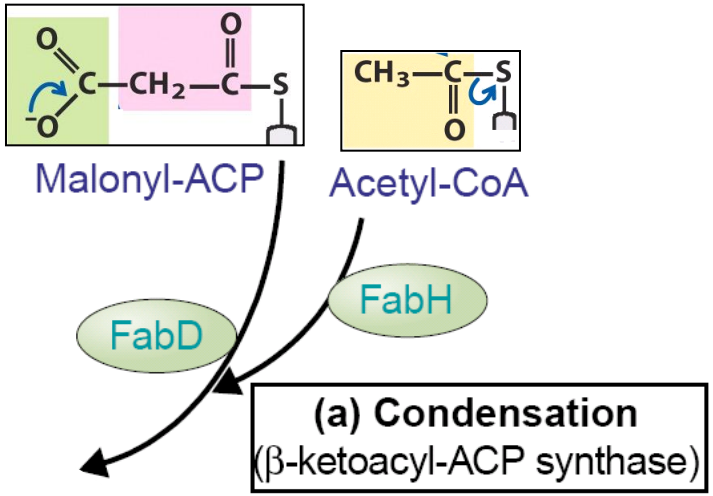
Antibacterial Targets: Overview

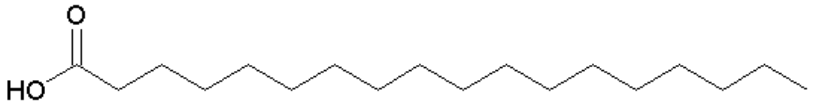


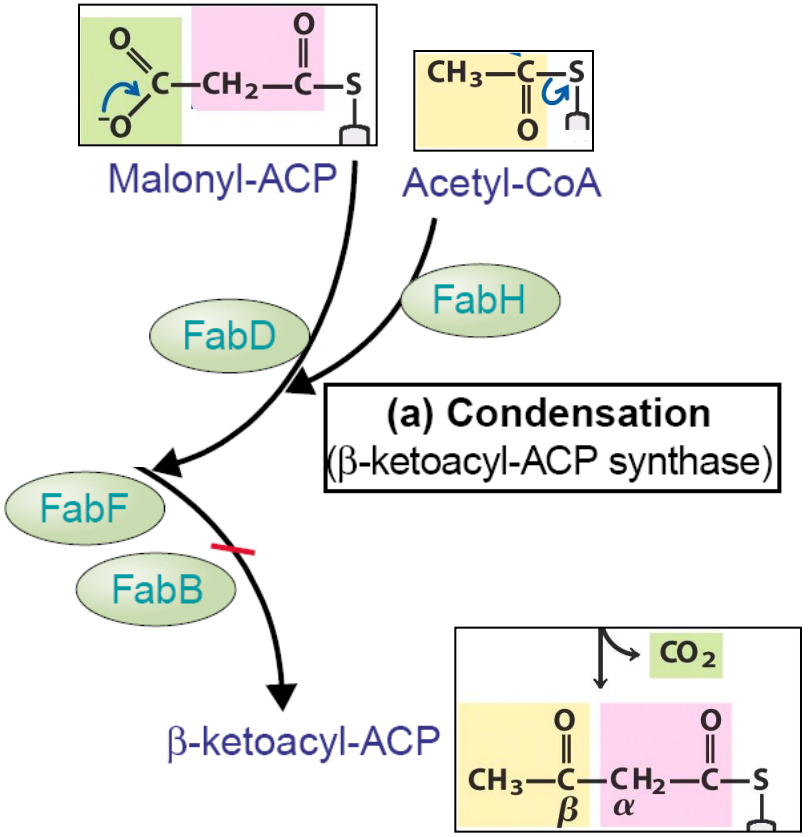
Bacterial Fatty Acid Biosynthesis



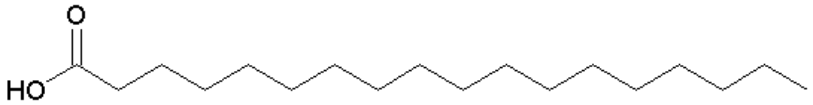


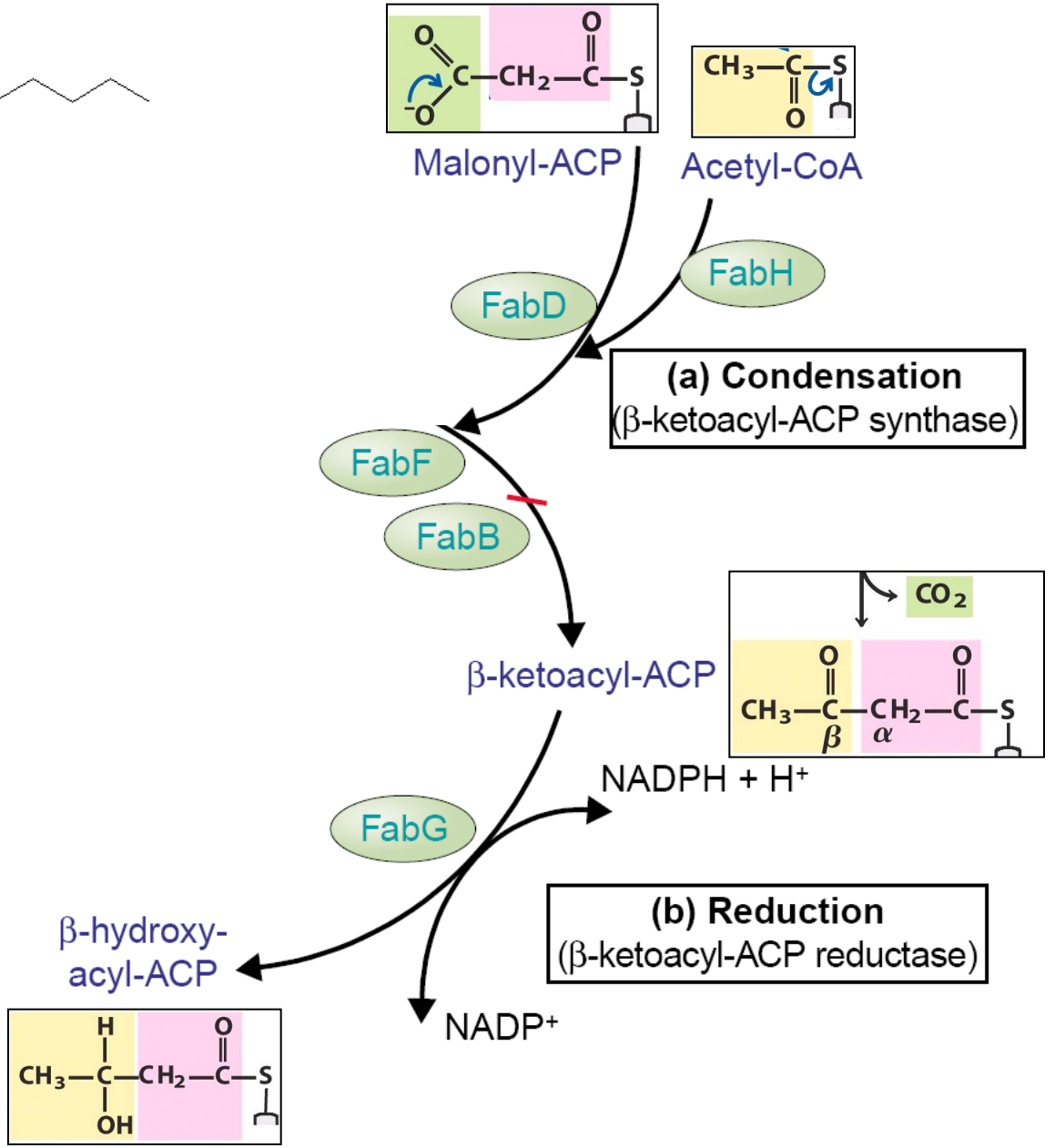
Bacterial Fatty Acid Biosynthesis



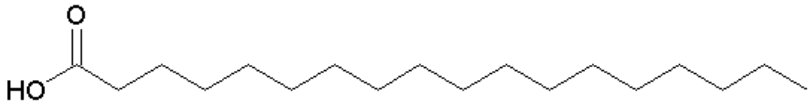


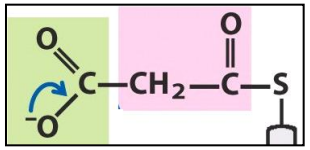
Bacterial Fatty Acid Biosynthesis

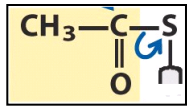




Bacterial Fatty Acid Biosynthesis







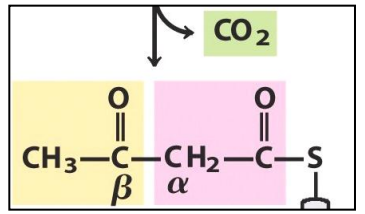
FabD

FabH

(a) Condensation
(β-ketoacyl-ACP synthase)

FabF

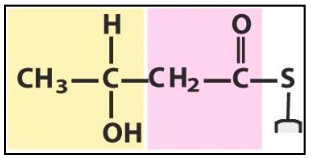
FabB



FabG

(b) Reduction
(β-ketoacyl-ACP reductase)

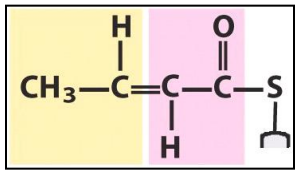
NADP+



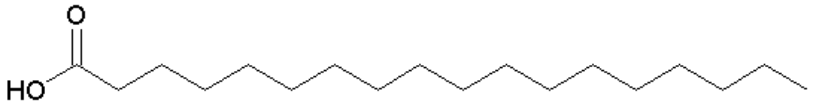
(c) Dehydration
(β-hydroxyacyl-ACP dehydrase)

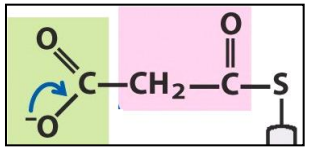
FabA

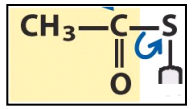
FabZ



Bacterial Fatty Acid Biosynthesis







FabD

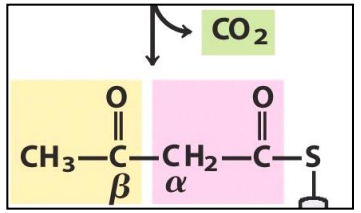
FabH

(a) Condensation
(β-ketoacyl-ACP synthase)

FabF

FabB

β-ketoacyl-ACP



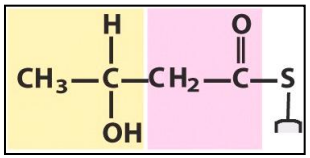
NADPH + H+

FabG

(b) Reduction
(β-ketoacyl-ACP reductase)

NADP+

β-hydroxy-
acyl-ACP



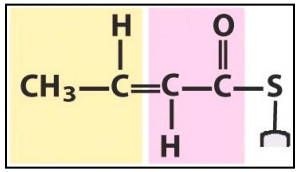
(c) Dehydration
(β-hydroxyacyl-ACP dehydrase)

FabA

FabZ

H_2O

Enoyl-ACP



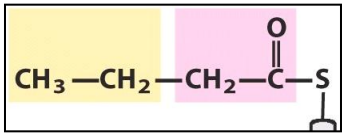
NADPH + H+

FabI

FabK

FabL

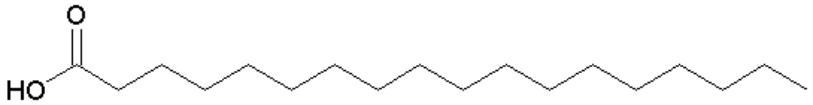
Acyl-ACP (n=2)

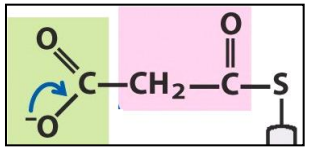


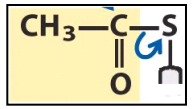
NADP+

(d) Reduction
(enyol-ACP reductase)

Bacterial Fatty Acid Biosynthesis







(d) Reduction
(enyol-ACP reductase)

Bioavailable fatty acid

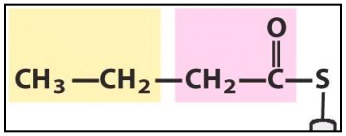
NADP+

FabD

FabH

(a) Condensation
(β-ketoacyl-ACP synthase)

Acyl-ACP (n=2)



FabI

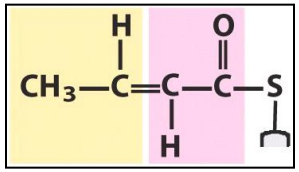
FabK

FabL

FabF

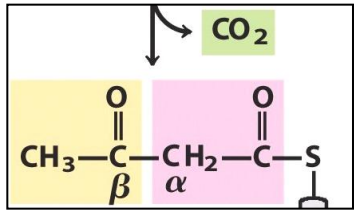
FabB

NADPH + H+



Enoyl-ACP

β-ketoacyl-ACP



H_2O

FabA

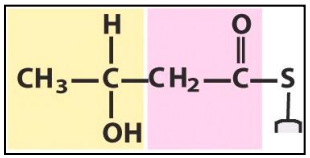
FabZ

NADPH + H+

FabG

(b) Reduction
(β-ketoacyl-ACP reductase)

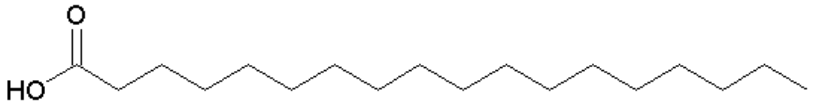
β-hydroxy-
acyl-ACP

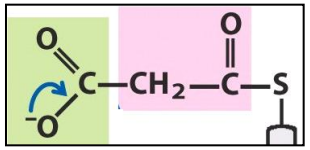


NADP+

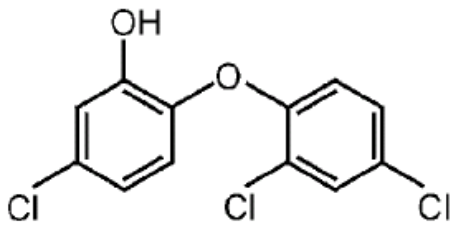
(c) Dehydration
(β-hydroxyacyl-ACP dehydrase)

Bacterial Fatty Acid Biosynthesis: Inhibitors



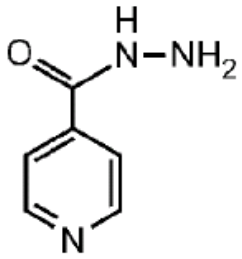


Bacterial Fatty Acid Biosynthesis: FabI Inhibitors



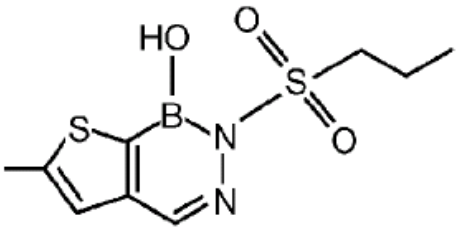
Triclosan

(broadspectrum antibacterial; used in soap, disinfectant)



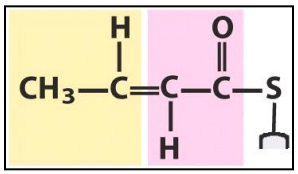
Isoniazid

(used to treat *M. tuberculosis* for over 50 years)

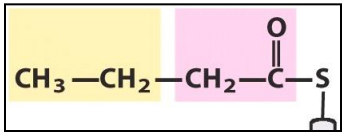


Thioenodiazaborine

(Gram -, *M. tuberculosis;* not used in human; toxic)



FabI



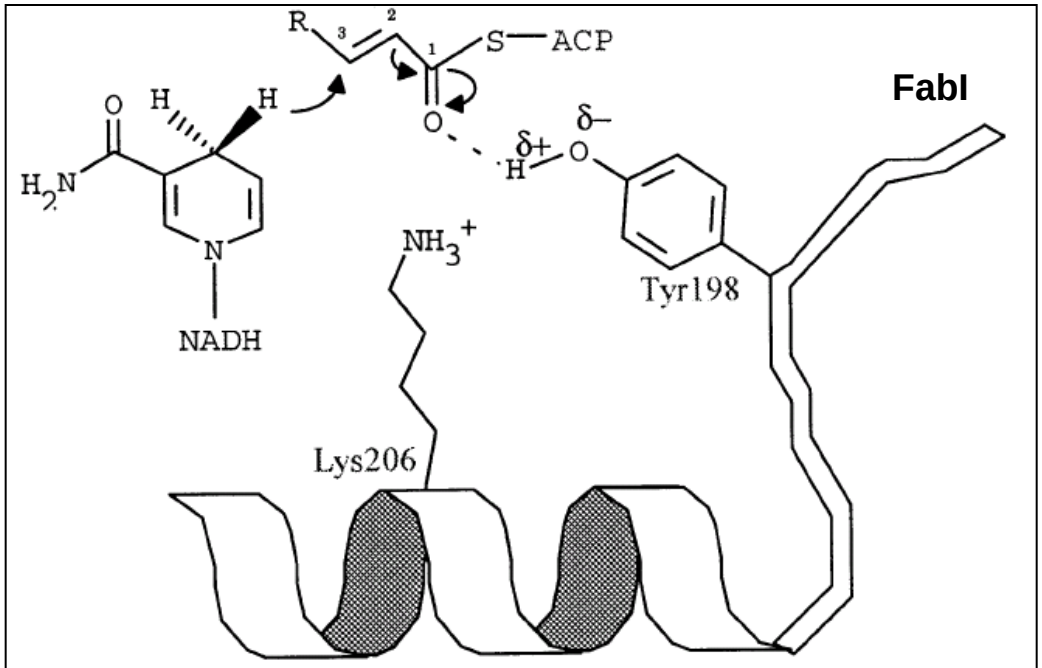
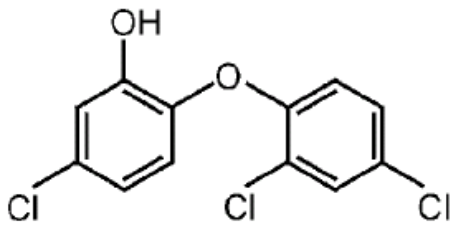


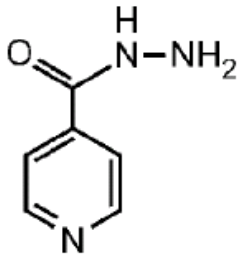
FIG. 7. Proposed catalytic mechanism of reduction of the double bond in an enoyl substrate by ENR.

Bacterial Fatty Acid Biosynthesis: FabI Inhibitors



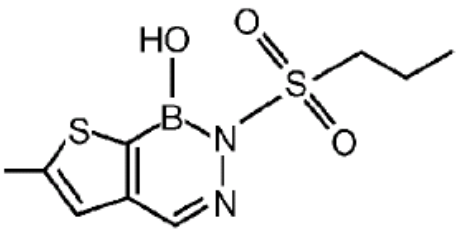
Triclosan

(broadspectrum antibacterial; used in soap, disinfectant)



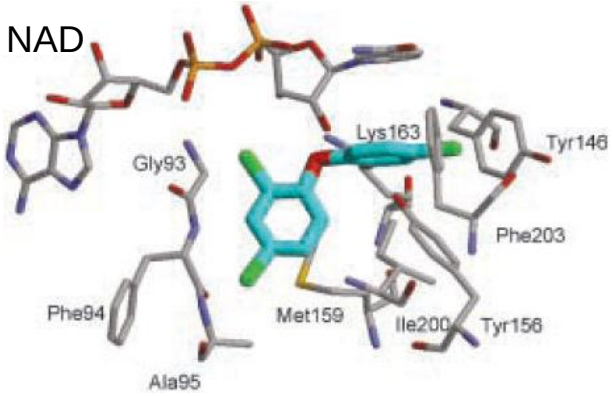
Isoniazid

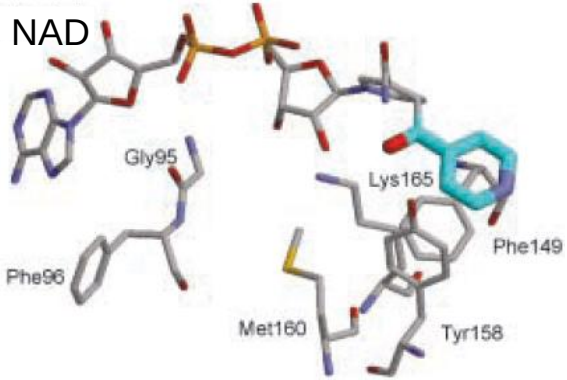
(used to treat *M. tuberculosis* for over 50 years)

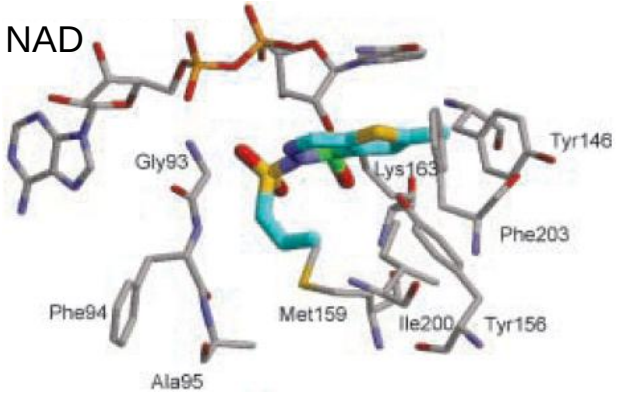


Thioenodiazaborine

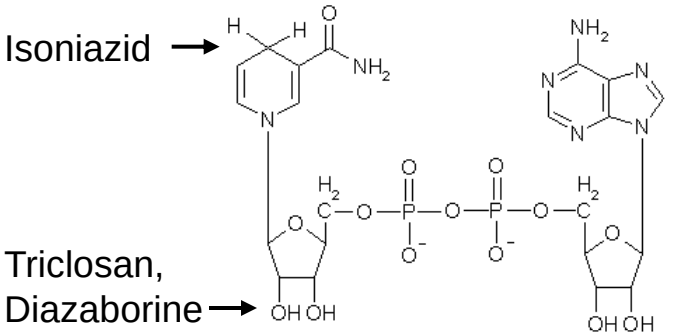
(Gram -, *M. tuberculosis;* not used in human; toxic)



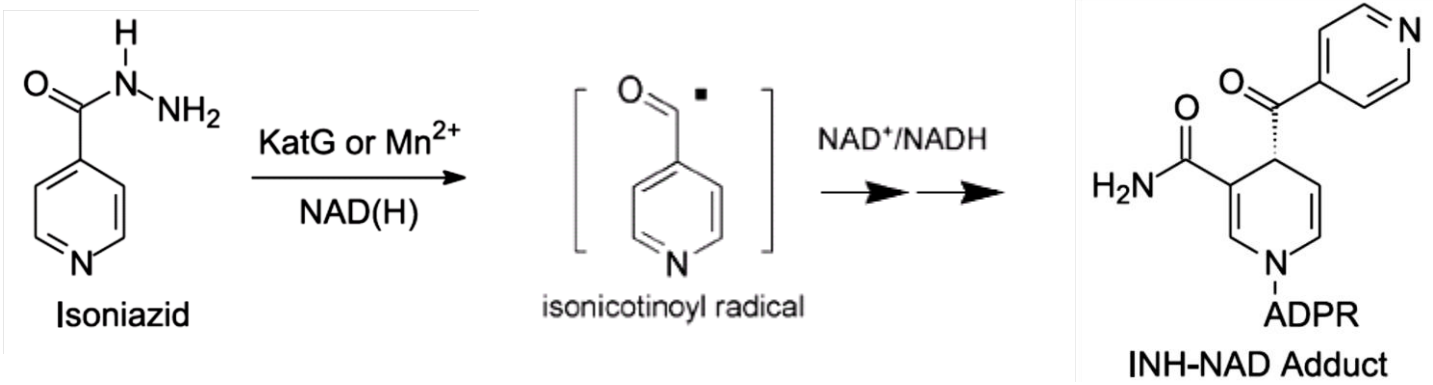


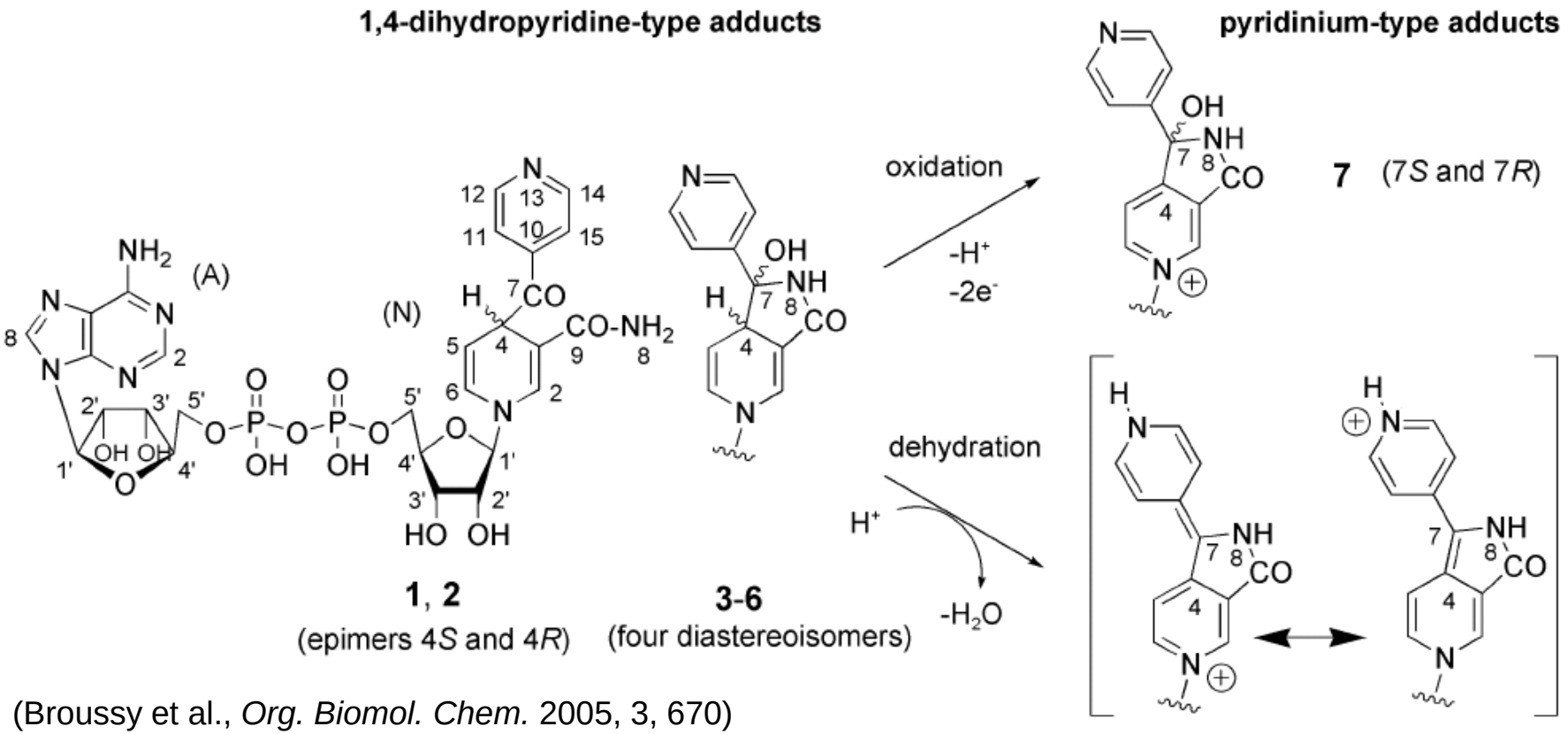


FabI inhibitors bind to the active site of the reductase and form a tight complex with the NAD cofactor.



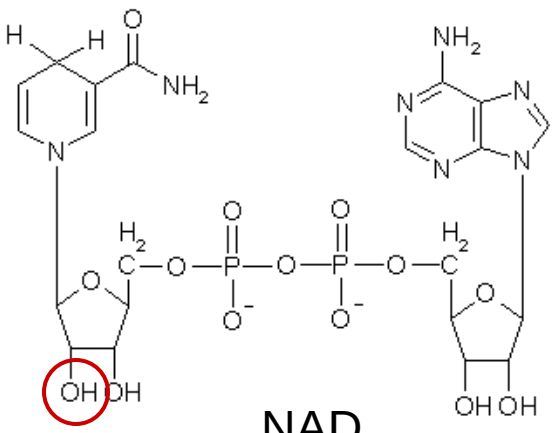
Bacterial Fatty Acid Biosynthesis: FabI Inhibitors: Isoniazid



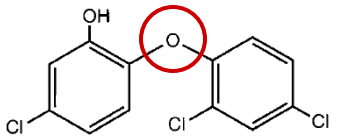


(Broussy et al., *Org. Biomol. Chem.* 2005, 3, 670)

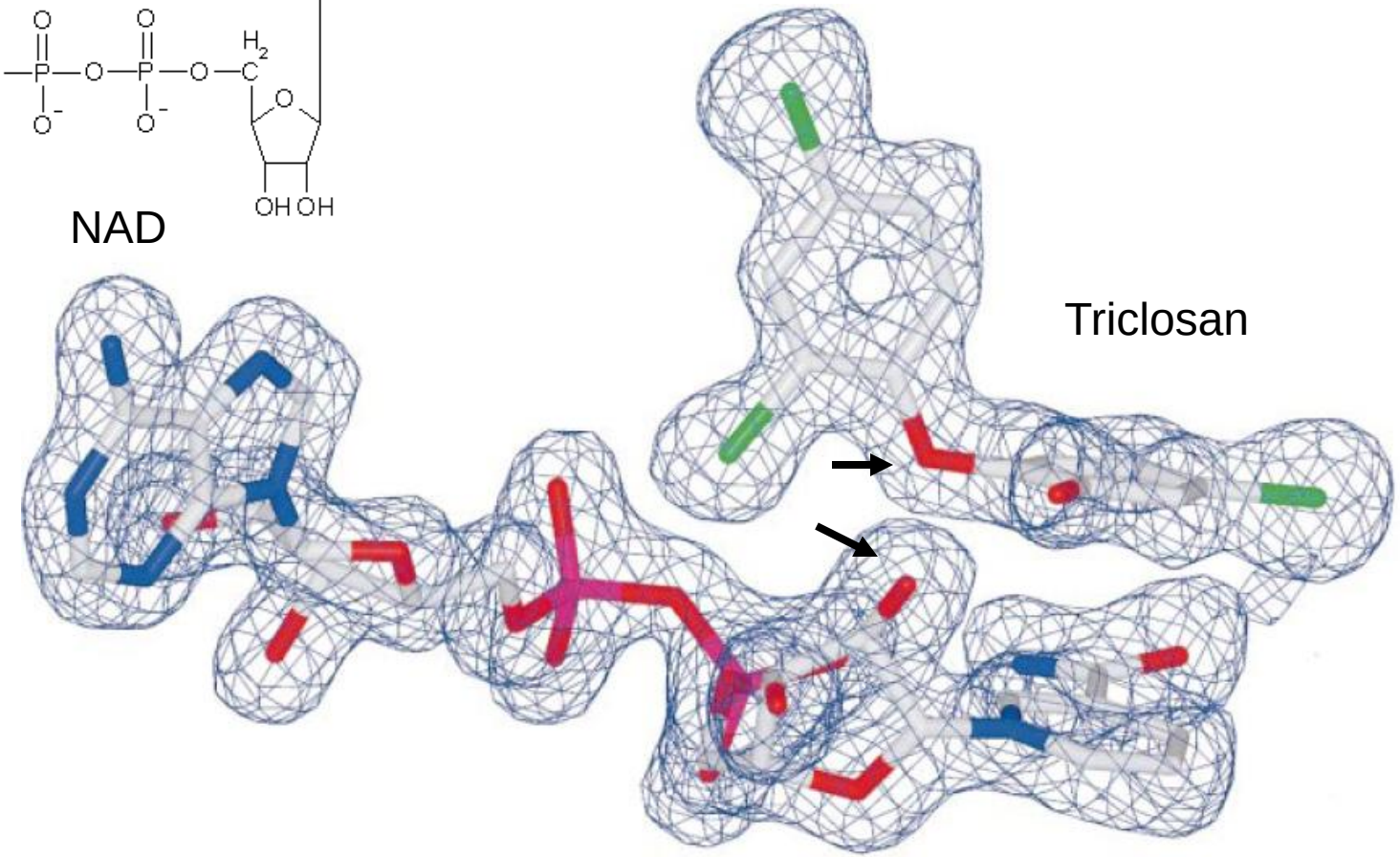
Bacterial Fatty Acid Biosynthesis: FabI Inhibitors: Triclosan



NAD

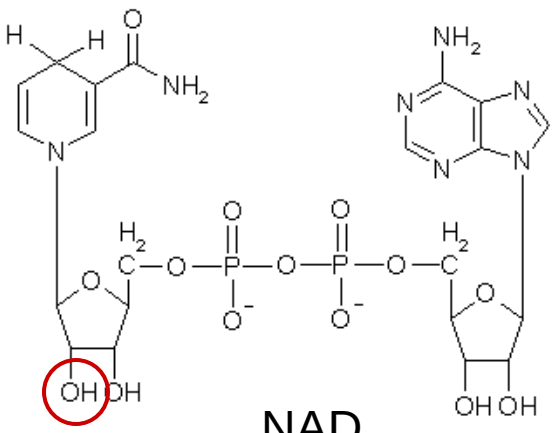


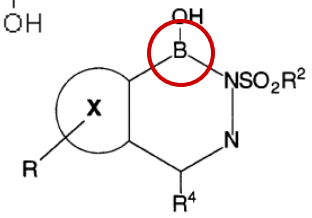
Triclosan

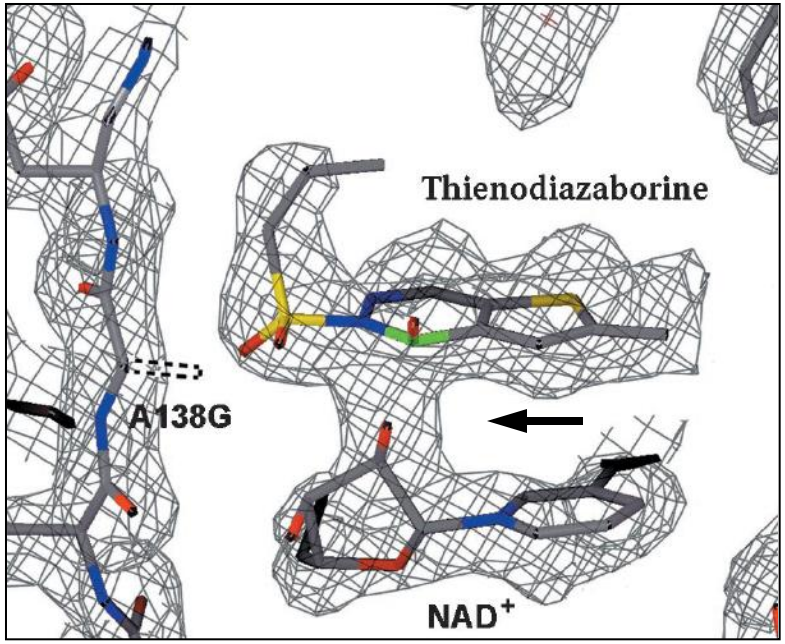


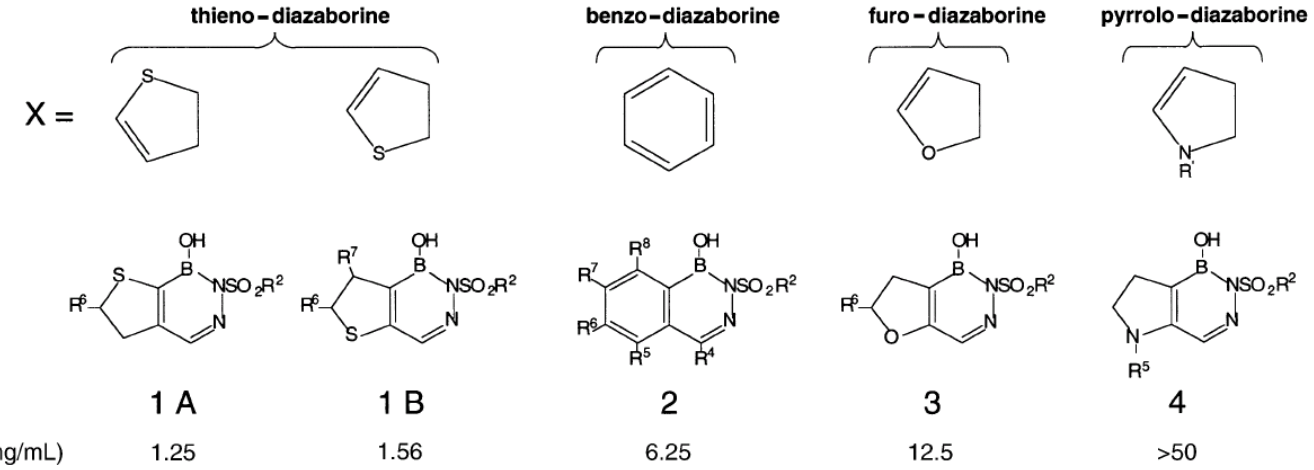
(Stewart et al., *JMB* 1999, 290, 859)

Bacterial Fatty Acid Biosynthesis: FabI Inhibitors: Diazaborines



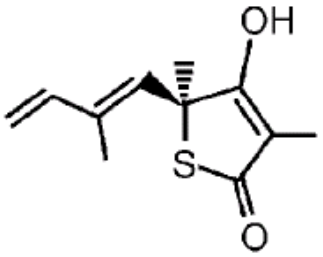






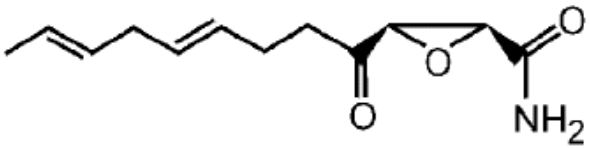
(Levy et al., *JMB* 2001, 309, 171)

Bacterial Fatty Acid Biosynthesis: FabB Inhibitors



Thiolactomycin

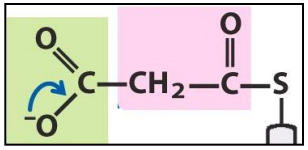
(isolated from fungus *Nocardia sp.*; efficacious against G+/- in mouse models)

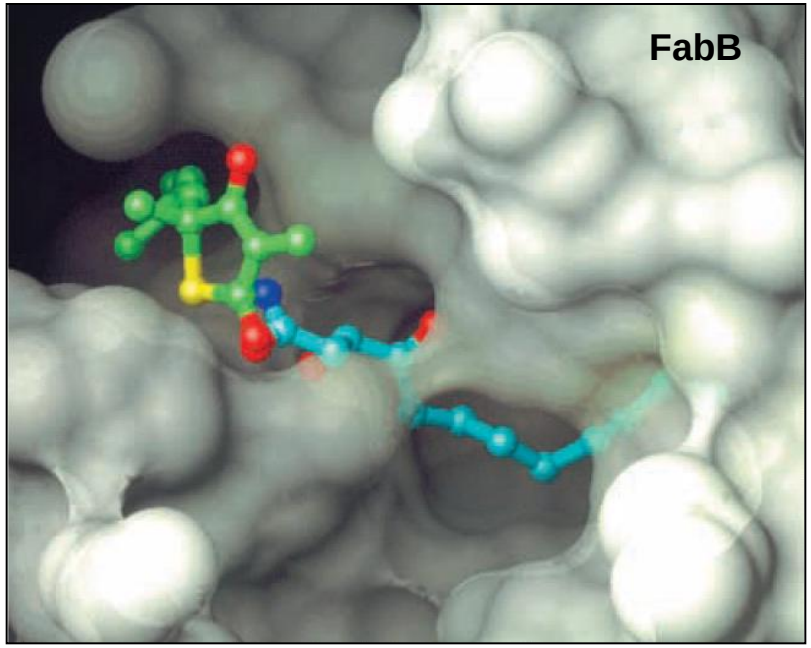


Cerulenin

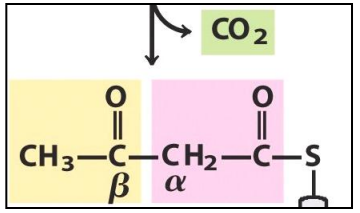
(isolated from fungus *Cephalosporium ceruleans*; inhibits also mammalian fatty acid synthases -> not used)

Thiolactomycin occupies malonate portion of the substrate binding site in FabB.



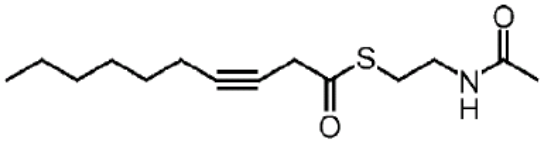


Cerulein mimics the covalently bound condensation intermediate; nonpolar tail is located in a hydrophobic tunnel.



(Heath et al., *Appl. Microbiol. Biotechnol.* 2002, 58, 695)

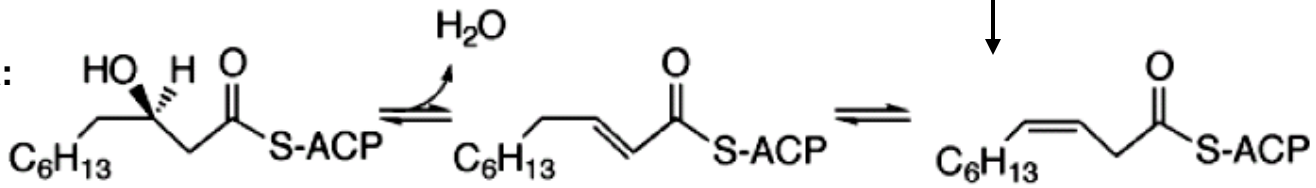
Bacterial Fatty Acid Biosynthesis: FabA Inhibitors: Decynoyl-NAC



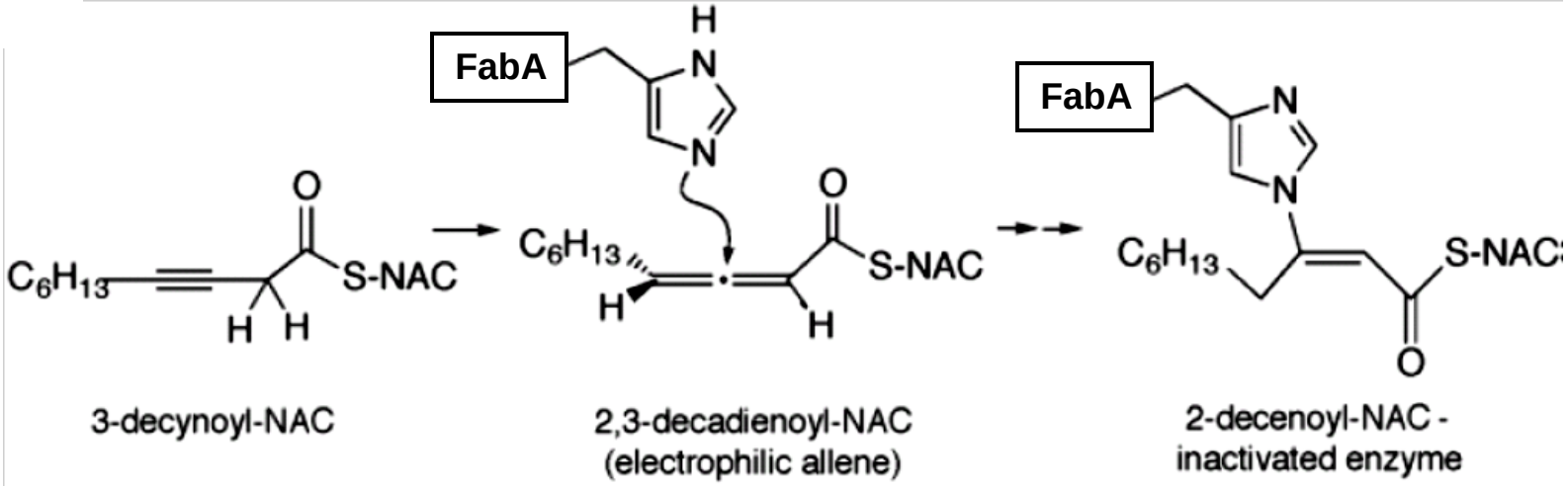
cis-3-decynoyl-NAC

Analog of cis-3-decenoyl-ACP

FabA:



(*R*)-3-hydroxydecanoyl-ACP (*E*)-2-decenoyl-ACP (*Z*)-3-decenoyl-ACP



3-decynoyl-NAC

2,3-decadienoyl-NAC (electrophilic allene)

2-decenoyl-NAC - inactivated enzyme

Historical importance: First example of a suicide enzyme inhibitor (Helmkamp et al., *JBC* 1969, 244, 6014)

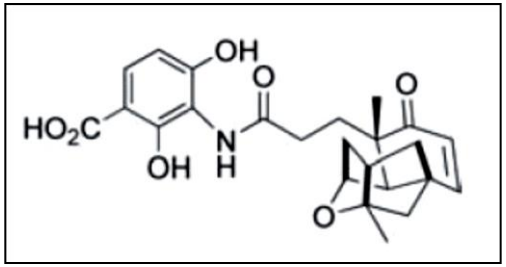
Bacterial Fatty Acid Biosynthesis: FabF Inhibitors: Platensimycin

nature Vol 441|18 May 2006|doi:10.1038/nature04784

LETTERS

Platensimycin is a selective FabF inhibitor with potent antibiotic properties

Jun Wang[1]*, Stephen M. Soisson[1]*, Katherine Young[1], Wesley Shoop[1]†, Srinivas Kodali[1], Andrew Galgoci[1], Ronald Painter[1], Gopalakrishnan Parthasarathy[1], Yui S. Tang[1], Richard Cummings[1], Sookhee Ha[1], Karen Dorso[1], Mary Motyl[1], Hiranthi Jayasuriya[1], John Ondeyka[1], Kithsiri Herath[1], Chaowei Zhang[1], Lorraine Hernandez[1], John Allocco[1], Ángela Basilio[1], José R. Tormo[1], Olga Genilloud[1], Francisca Vicente[1], Fernando Pelaez[1], Lawrence Colwell[1], Sang Ho Lee[1], Bruce Michael[1], Thomas Felcetto[1], Charles Gill[1], Lynn L. Silver[1]†, Jeffery D. Hermes[1], Ken Bartizal[1], John Barrett[1]‡, Dennis Schmatz[1], Joseph W. Becker[1], Doris Cully[1] & Sheo B. Singh[1]



Bacterial Fatty Acid Biosynthesis: FabF Inhibitors: Platensimycin

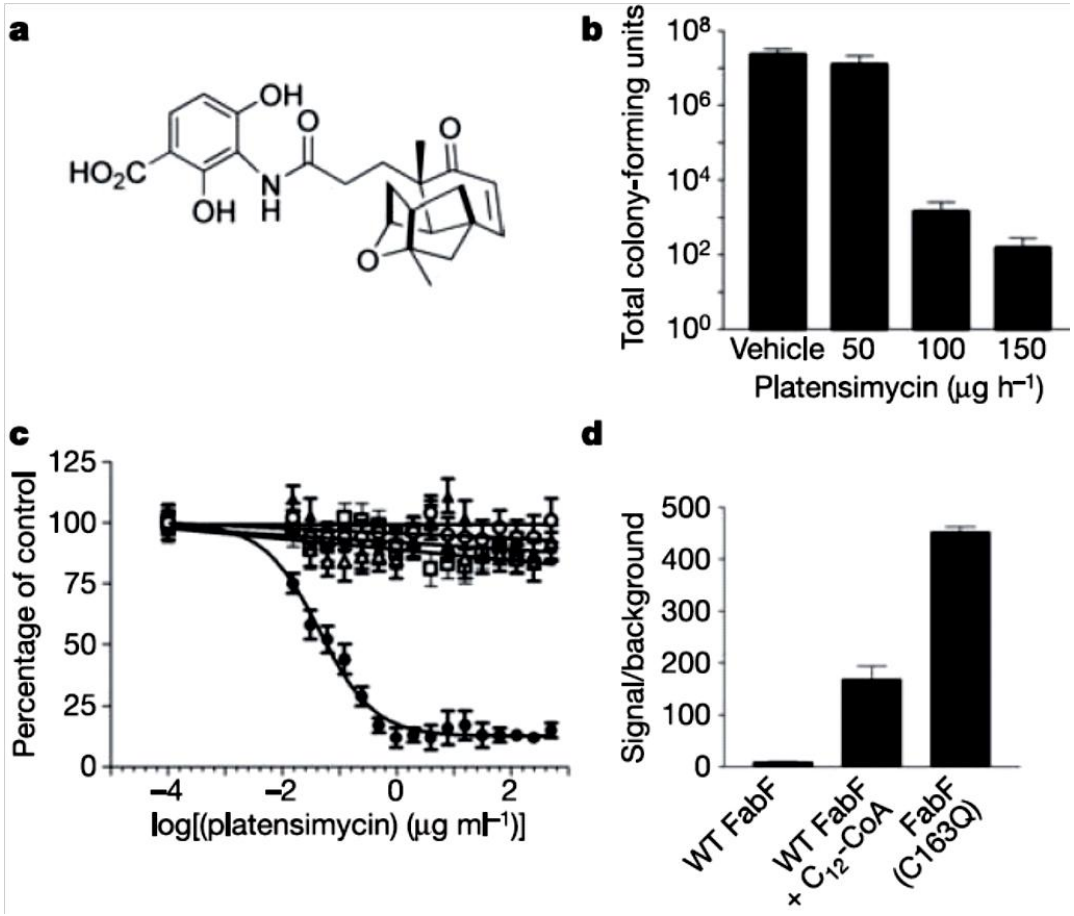
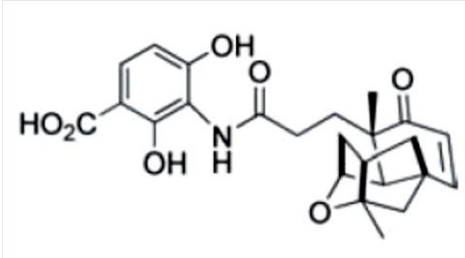


Figure 1 | Characterization of platensimycin. **a**, Structure of platensimycin. **b**, *In vivo* studies on platensimycin. Dosing at 50 $\mu g\,h^{-1}$ showed small decrease in viable *S. aureus* cells from the infected kidney. However, a 10^4–10^5 fold decrease (4 and 5 log reduction) were achieved with 100 and 150 $\mu g\,h^{-1}$, respectively. Dosing at 150 $\mu g\,h^{-1}$ showed 40% of the kidneys with no viable *S. aureus*, whereas dosing at 100 $\mu g\,h^{-1}$ showed 20% of the kidneys without detectable viable *S. aureus*. Error bars indicate s.d. observed with five infected mice. The results were confirmed by a repeat experiment. **c**, Whole-cell labelling assay[16] with platensimycin. The assay was performed with a serial dilution of platensimycin, starting at 500 $\mu g\,ml^{-1}$. Platensimycin showed no significant inhibition against syntheses of DNA (open circles), cell wall (filled triangles), protein (open squares) and RNA (open triangles) but greatly inhibited phospholipid synthesis (filled circles), providing an IC_{50} value of 0.1 $\mu g\,ml^{-1}$. Error bars indicate s.d. for three individual experiments. **d**, Direct binding assay results of [^{3}H]dihydroplatensimycin and *E. coli* FabF (ecFabF) in the presence and absence of n-dodecanoyl coenzyme A (lauroyl-CoA; C_{12}-CoA) and the C163Q mutant protein. Error bars indicate s.d. observed with six replicate

Bacterial Fatty Acid Biosynthesis: FabF Inhibitors



Systematic screening of 250,000 natural product extracts (83,000 strains in three growth conditions), with the use of a combination of target-based whole-cell and biochemical assays, led to the identification of a potent and selective small molecule from a strain of *Streptomyces platensis* recovered from a soil sample collected in South Africa. This molecule, platensimycin ($C_{24}H_{27}NO_7$, relative molecular mass 441.47), comprises two distinct structural elements connected by an amide bond (Fig. 1a).

Table 1 | Microbiological profiles and toxicity of platensimycin and linezolid

Organism and genotype	Platensimycin	Linezolid
Antibacterial activity (MIC, $\mu g\ ml^{-1}$)*		
S. aureus (MSSA)	0.5	4
S. aureus + *serum*	2	4
S. aureus (MRSA)	0.5	2
S. aureus (MRSA, macrolide^R)	0.5	2
S. aureus (MRSA, linezolid^R)	1	32
S. aureus (VISA, vancomycin^I)	0.5	2
Enterococcus faecalis (macrolide^R)	1	1
Enterococcus faecium (VRE)	0.1	2
S. pneumoniae†	1	1
E. coli (wild-type)	>64	>64
E. coli (tolC)	16	32
Toxicity ($\mu g\ ml^{-1}$)		
HeLa MTT (IC_{50})	>1,000	>100
Candida albicans (MIC)	>64	>64

*A concentration of 1 $\mu g\ ml^{-1}$ equals 2.27 μM for platensimycin and 2.96 μM for linezolid.
†Cells were inoculated at 10^5 colony-forming units followed by incubation overnight at 37 °C with a serial dilution of compounds in Todd–Hewitt broth.
Linezolid is a synthetically derived agent that has been in clinical use since 2000. MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; VISA, vancomycin-intermediate *S. aureus*; VRE, vancomycin-resistant *Enterococcus*.

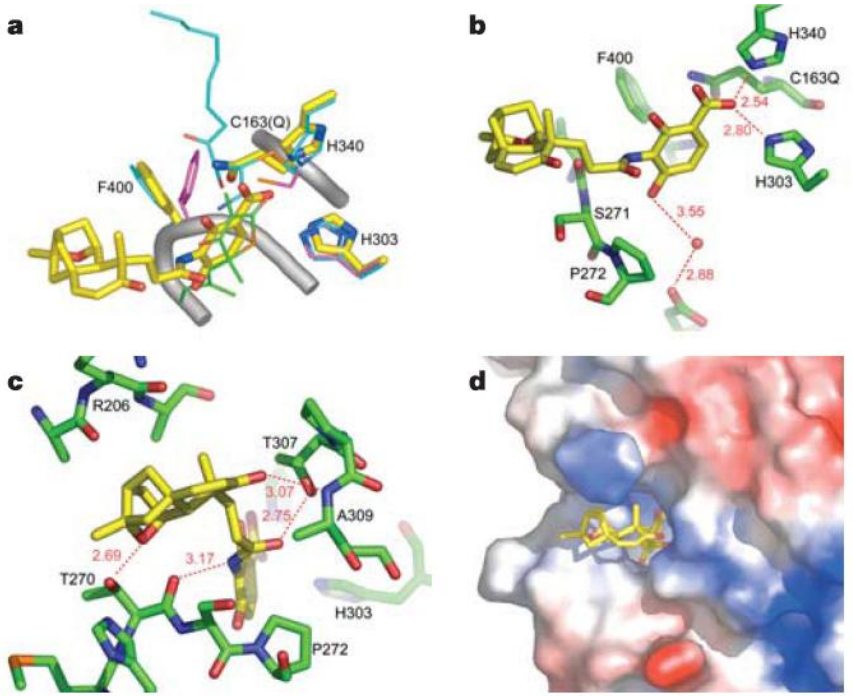
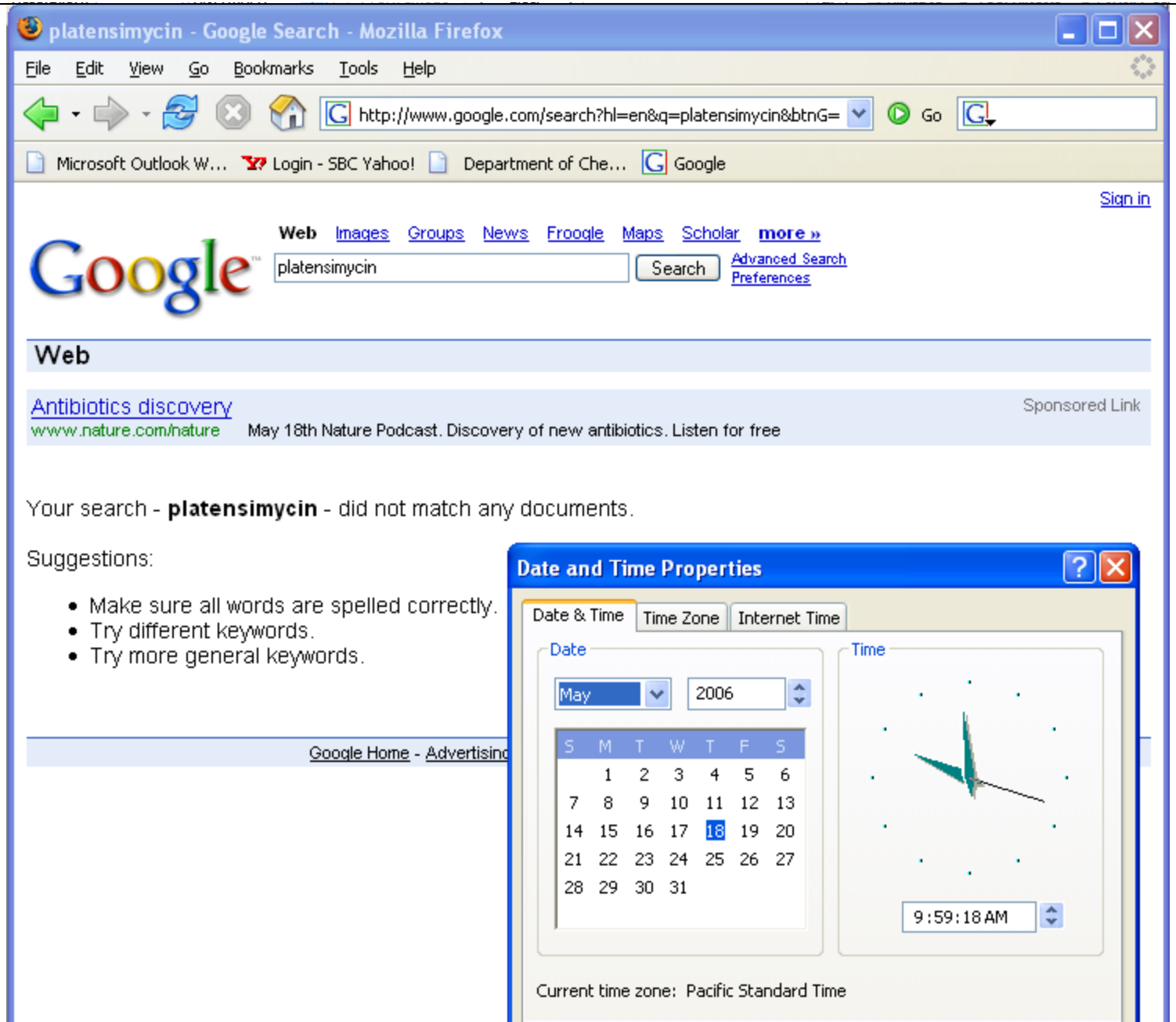


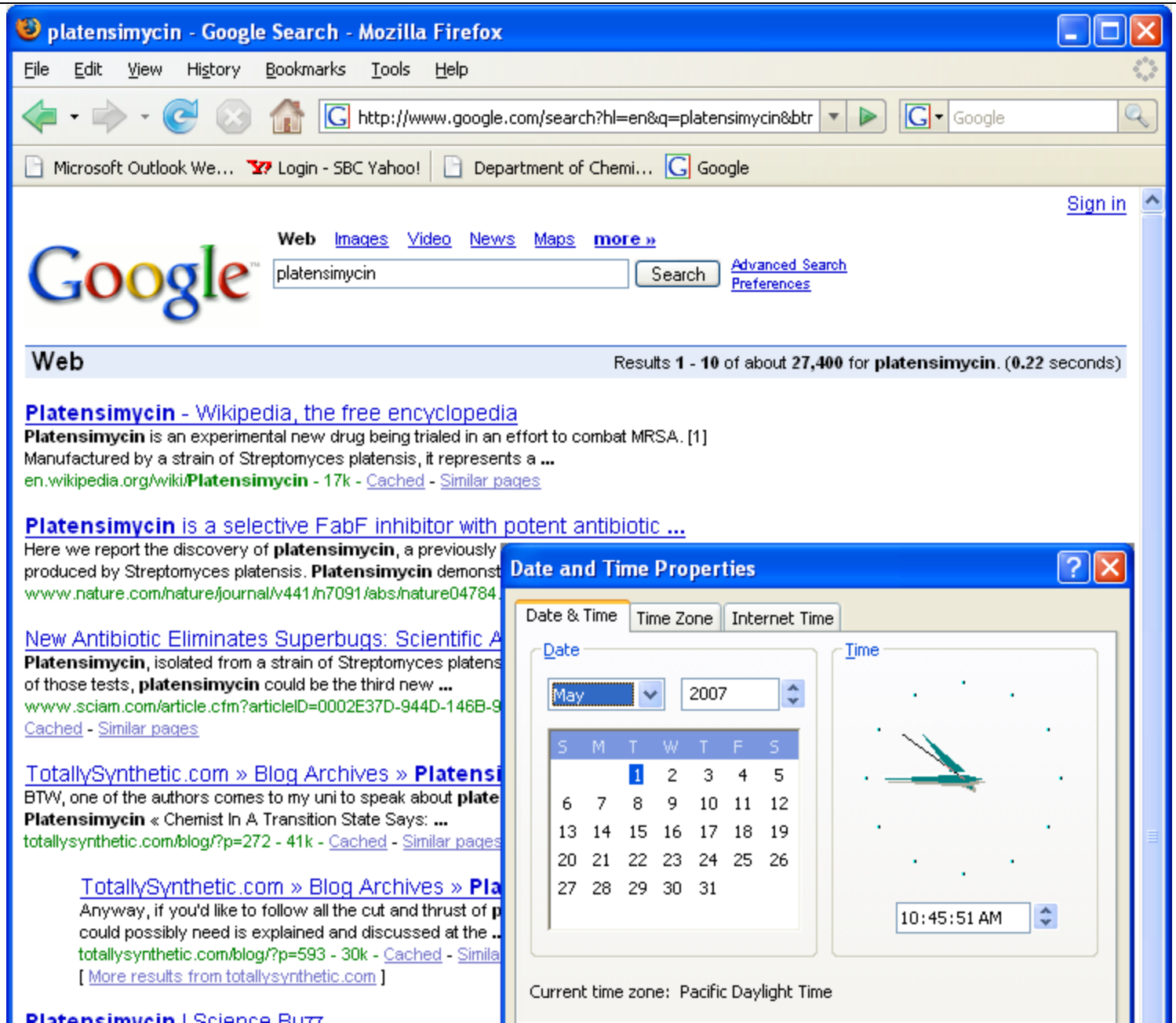
Figure 2 | Interactions of platensimycin with ecFabF(C163Q) and comparison with the apo structure. a, Superposition of platensimycin (yellow, thicker sticks) on ecFabF, with thiolactomycin (green) and cerulenin (cyan) shown for reference. Side chains discussed in the text are labelled and

The 2.6-Å structure of ecFabF(C163Q) in complex with platensimycin shows that the antibiotic binds in the malonyl subsite of FabF (Fig. 2a), with its benzoic acid ring in roughly the same orientation as

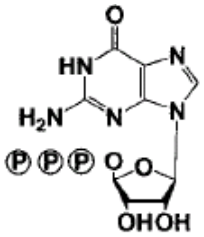
Bacterial Fatty Acid Biosynthesis: FabF Inhibitors



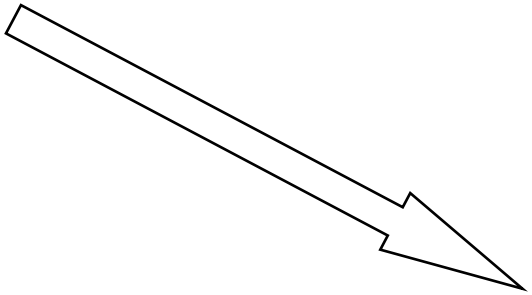
Bacterial Fatty Acid Biosynthesis: FabF Inhibitors



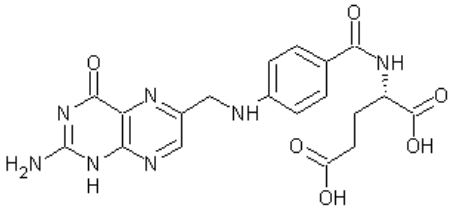
Bacterial Folic Acid Biosynthesis



GTP

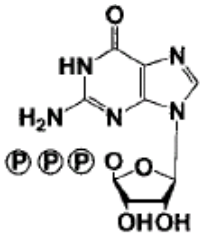


- Methylation of dUMP
- Purine/pyrimidine biosynthesis
- Met-tRNA transformylase
- Met and Gly synthesis
- Pantothenate biosynthesis



Folic acid

Bacterial Folic Acid Biosynthesis

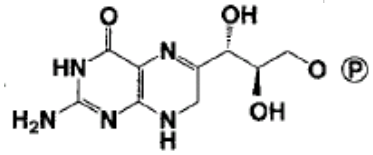


GTP

↓ *mtrA*

H_2-neopterin-P3

↓



H_2-neopterin-P

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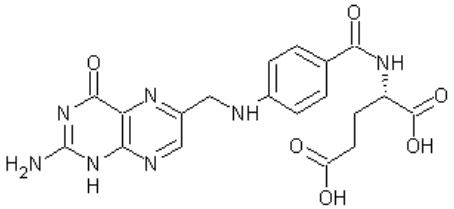
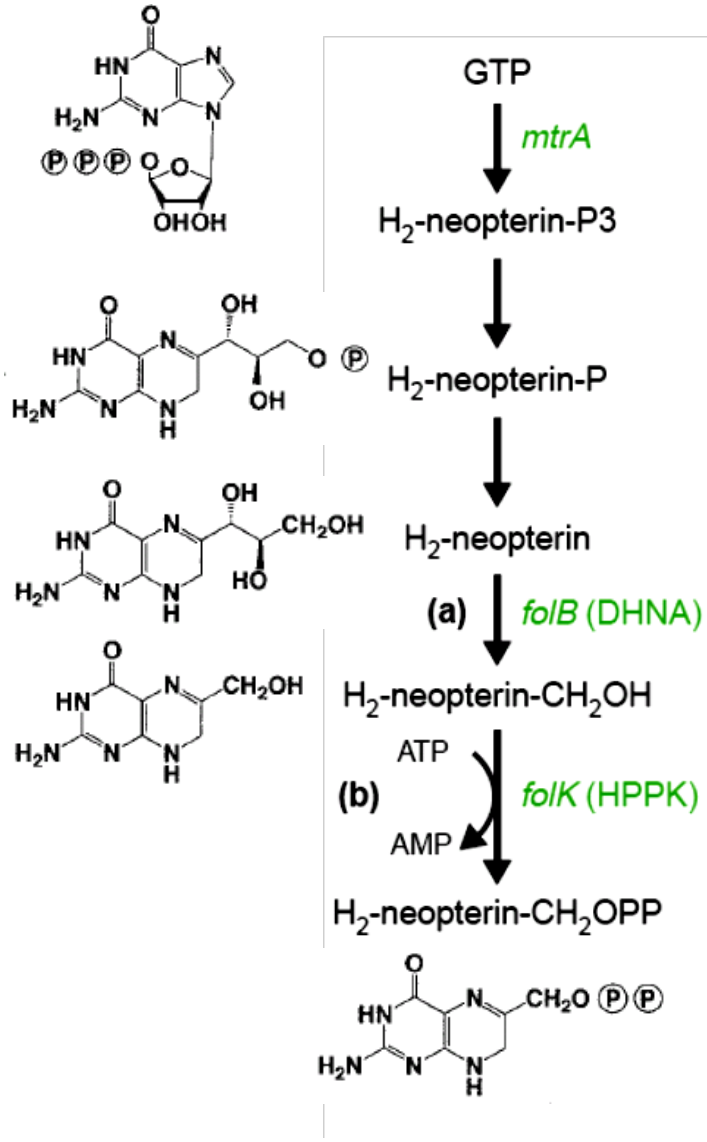


Folic acid

Bacterial Folic Acid Biosynthesis



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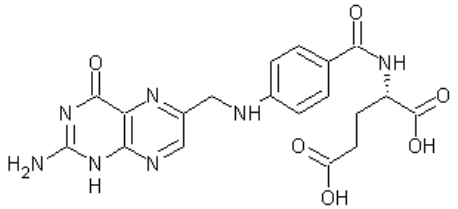
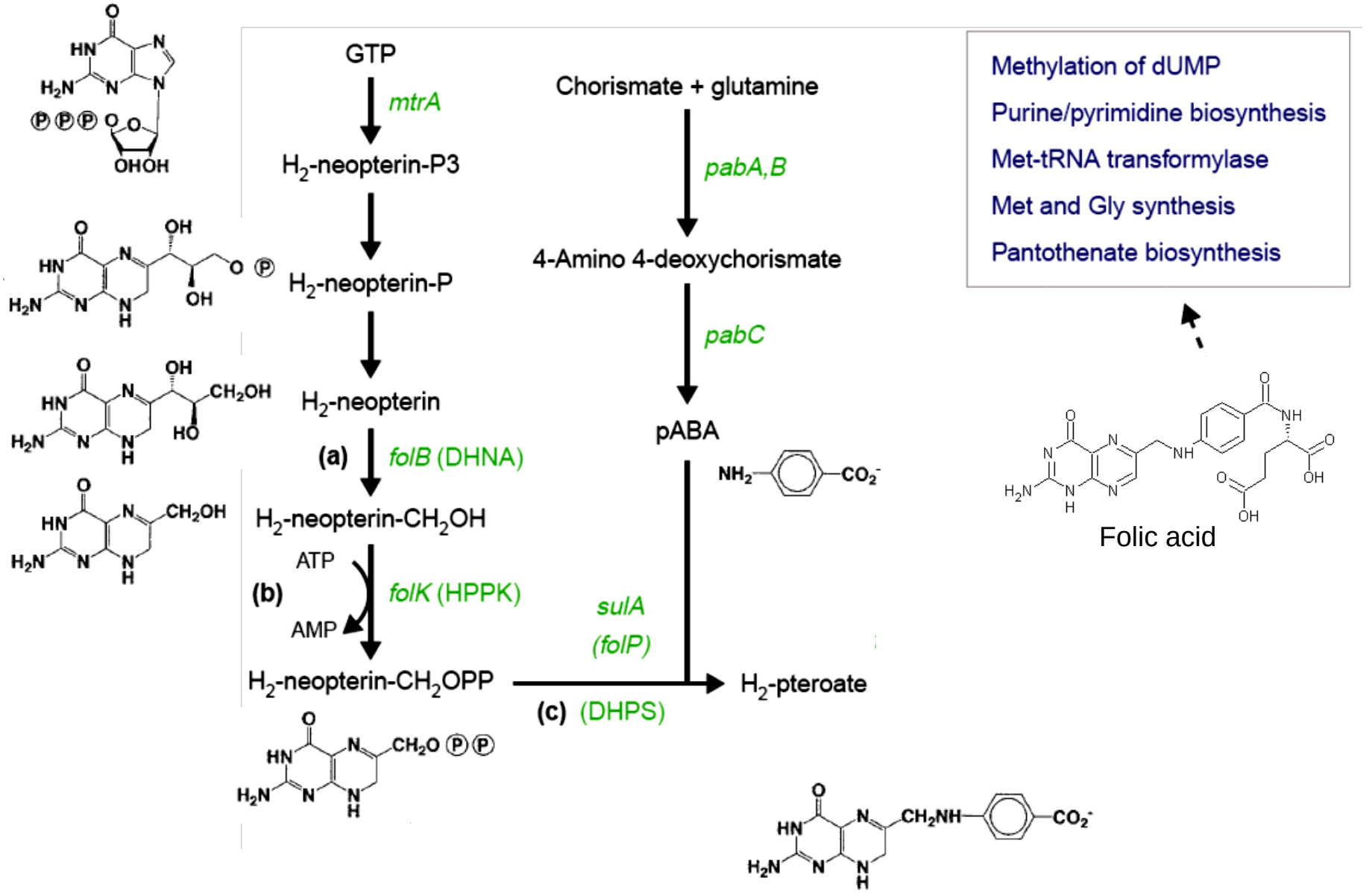
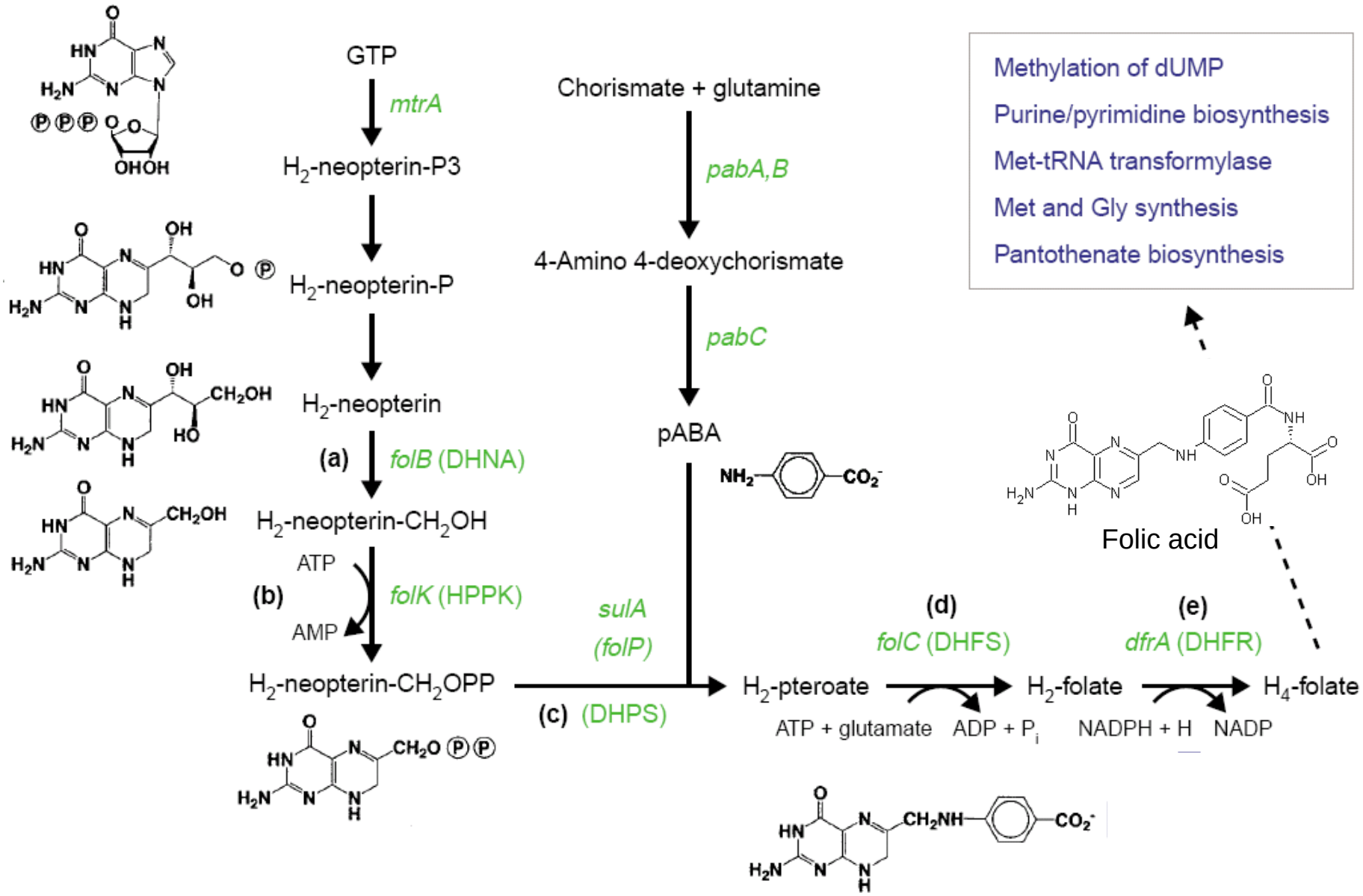


Folic acid

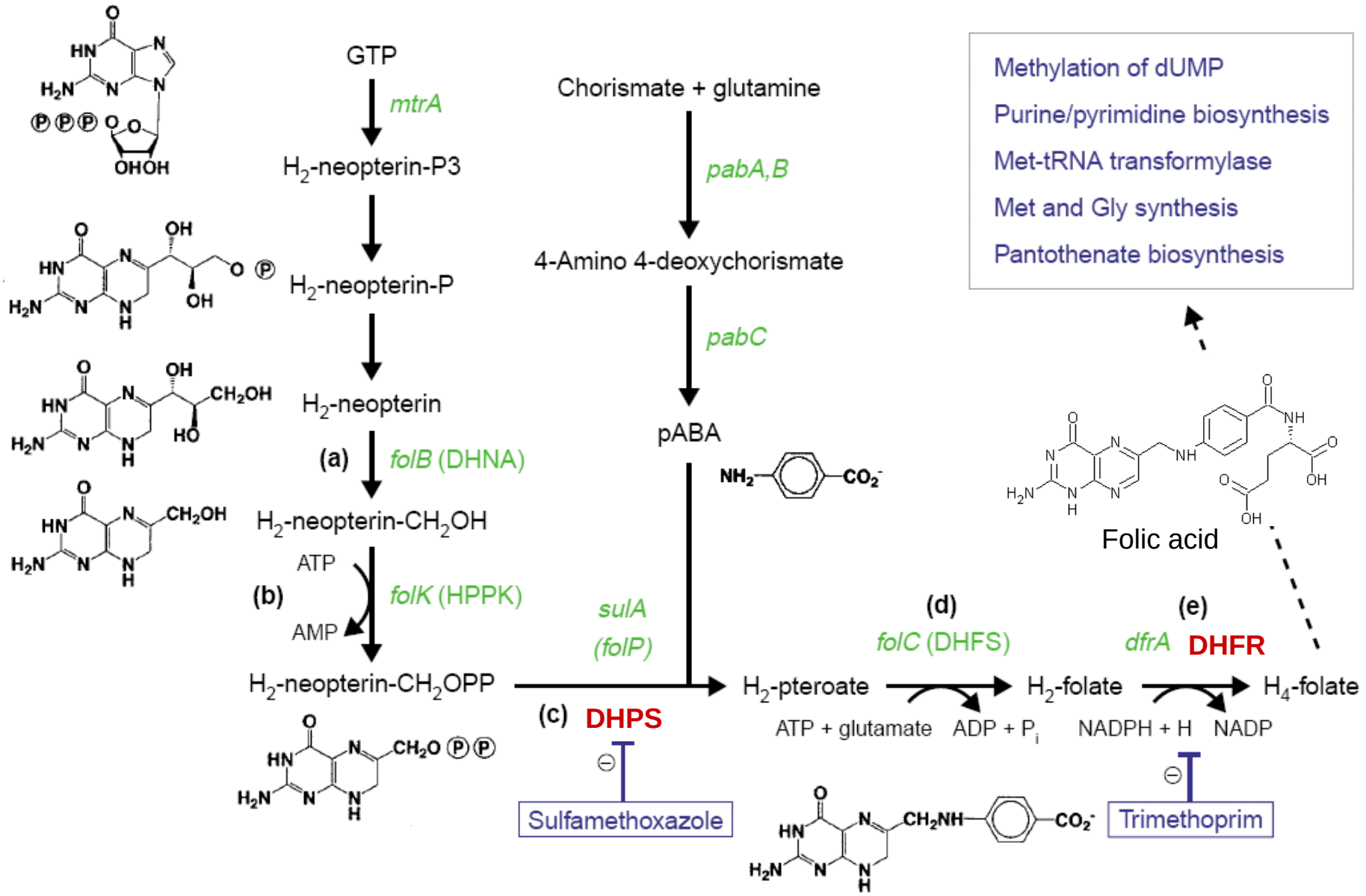
Bacterial Folic Acid Biosynthesis



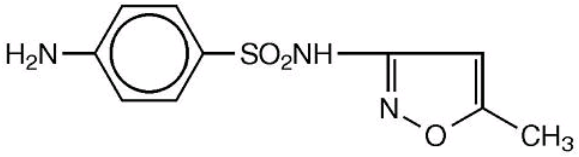
Bacterial Folic Acid Biosynthesis



Bacterial Folic Acid Biosynthesis: Inhibitors

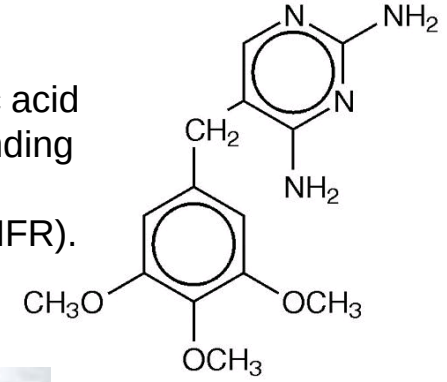


Bacterial Folic Acid Biosynthesis: Inhibitors – Combination Therapy

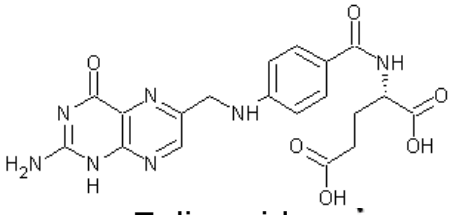


Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with *para* - aminobenzoic acid (PABA) at the 7,8-dihydropteroate synthase (DHPS).

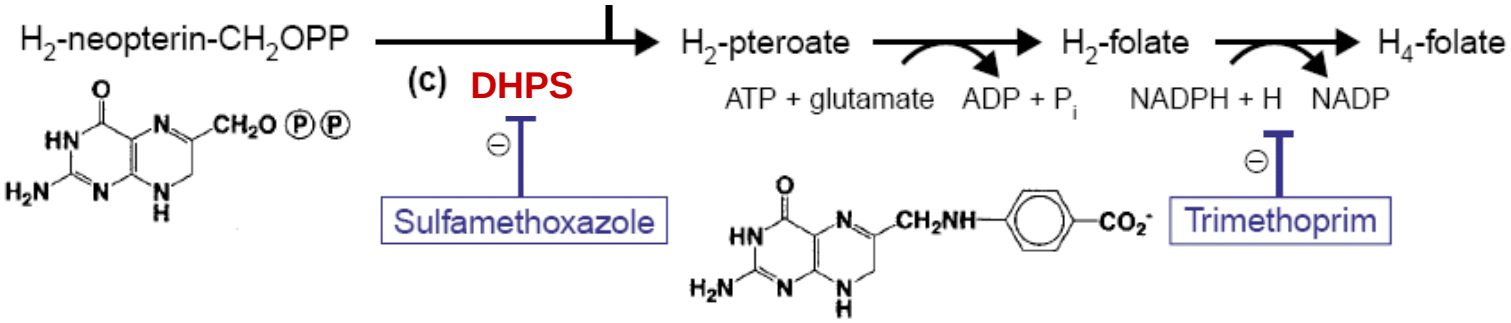
Trimethoprim blocks the production of tetrahydrofolic acid from dihydrofolic acid by binding to and reversibly inhibiting dihydrofolate reductase (DHFR).



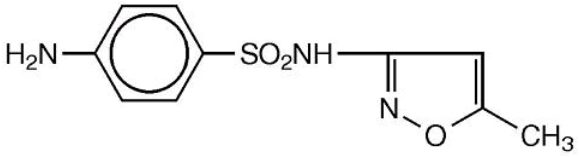
- Pathway inhibition by synergistic use of two drugs that act on different targets within the same pathway
- Resistance develops much more slowly than with either of the components alone
- *H. influenzae*, *S. pneumomoniae*, *Neisseria* species, *S. aureus;* urinary tract infections (Bactrim; $0.15/day)



Folic acid

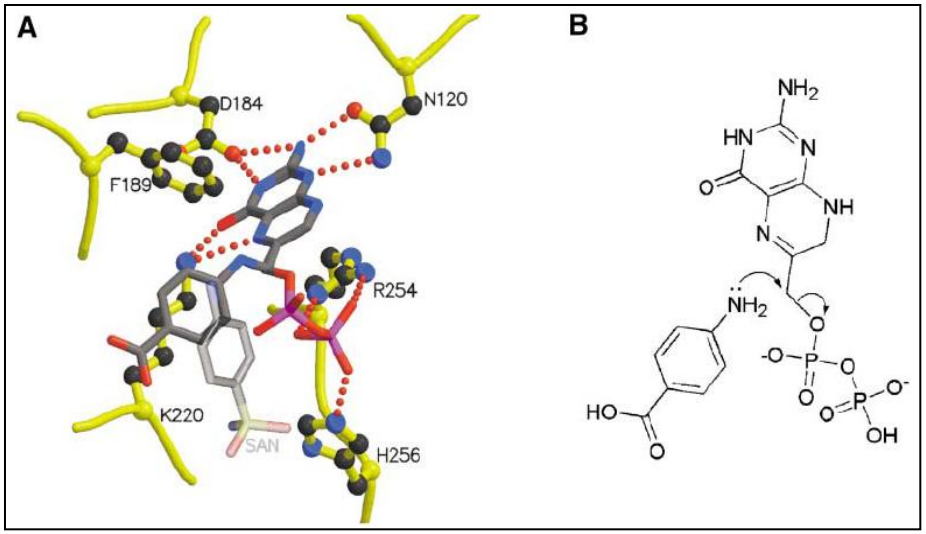


Bacterial Folic Acid Biosynthesis: Sulfamethoxazole

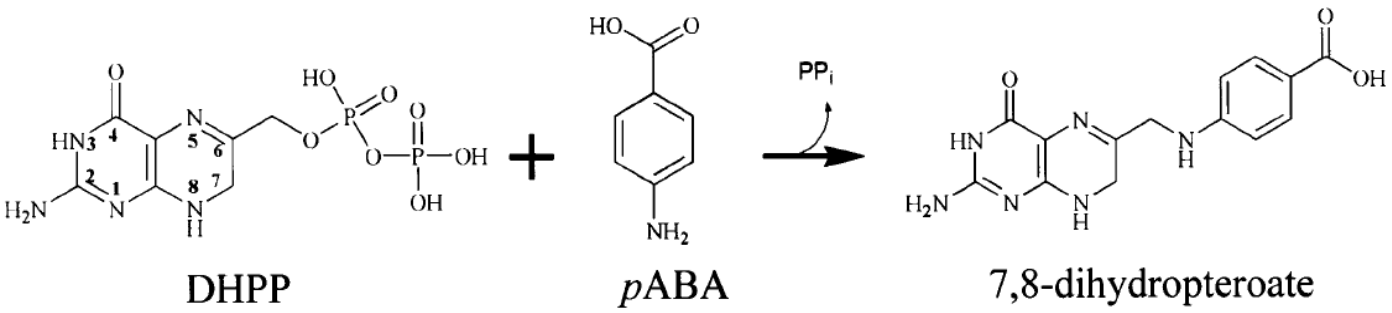


Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with *para* - aminobenzoic acid (PABA) at the 7,8-dihydropteroate synthase (DHPS).

• DHPS is unique to bacteria, not found in mammalian cells



(Babaoglu et al., *Structure* 2004, 12, 1705)



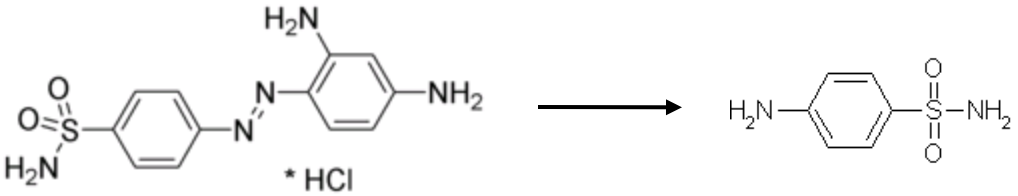
DHPP *p*ABA 7,8-dihydropteroate

First Antibiotics: Domagk Discovers Sulfonamides ("Sulfa-Drugs")

Gerhard J. P. Domagk
(Wuppertal, 1895-1964)

Worked at Bayer (IG Farben) where he discovered and developed sulfonamides (Prontosil), the first drugs effective against bacterial infections.

Nobel Price in Medicine 1939 for discovery of sulfonamides.

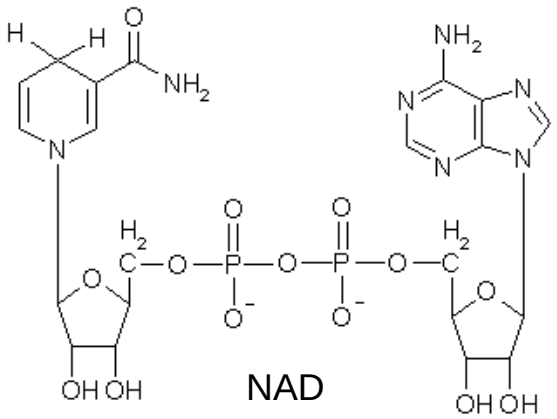


Prontosil (red azo dye)
(Bayer 1935)

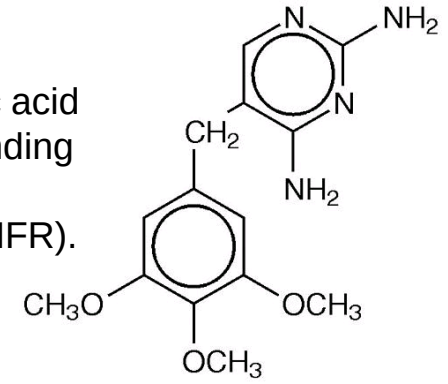
Sulfanilamide
(1936)

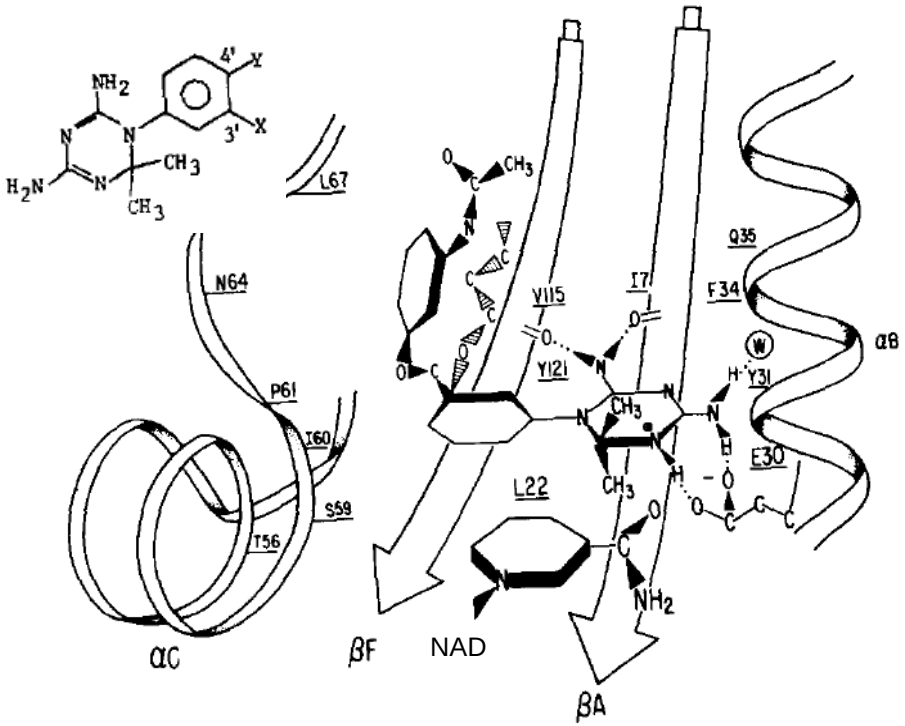
Prontosil is a prodrug that is not active *in vitro*. Cleavage in the gastrointestinal tract leads to the active compound sulfanilamide which is competes with *p*-aminobenzoic acid, the substrate of dihydropteroate synthetase in the bacterial synthetic pathway to folic acid.

Bacterial Folic Acid Biosynthesis: Trimethoprim



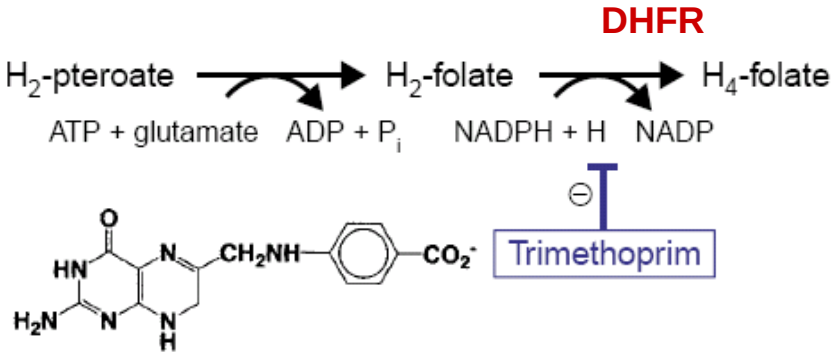
Trimethoprim blocks the production of tetrahydrofolic acid from dihydrofolic acid by binding to and reversibly inhibiting dihydrofolate reductase (DHFR).





(Matthews et al., *JBC* 1985, 260, 392)

- DHFR is essential to both bacteria and eukaryotes but trimethoprim is selective for the bacterial target



Bacterial Folic Acid Biosynthesis: Iclaprim

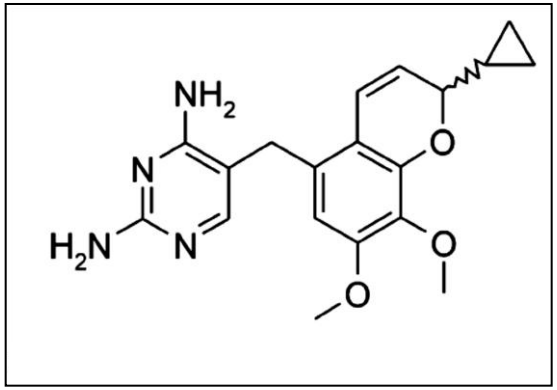
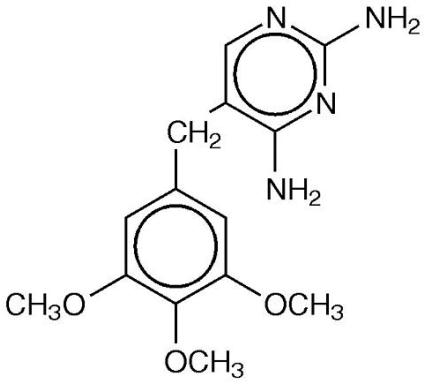


Table 1. Inhibition of bacterial and human DHFR enzymes by Iclaprim and TMP[28]

Enzyme	Iclaprim IC_{50} (μM)	Trimethoprim IC_{50} (μM)
Human	**>300**	>300
E. coli	**0.007**	0.007
S. aureus	**0.007**	0.007
S. pneumoniae	**0.008**	0.075
P. carinii	**2.4**	43

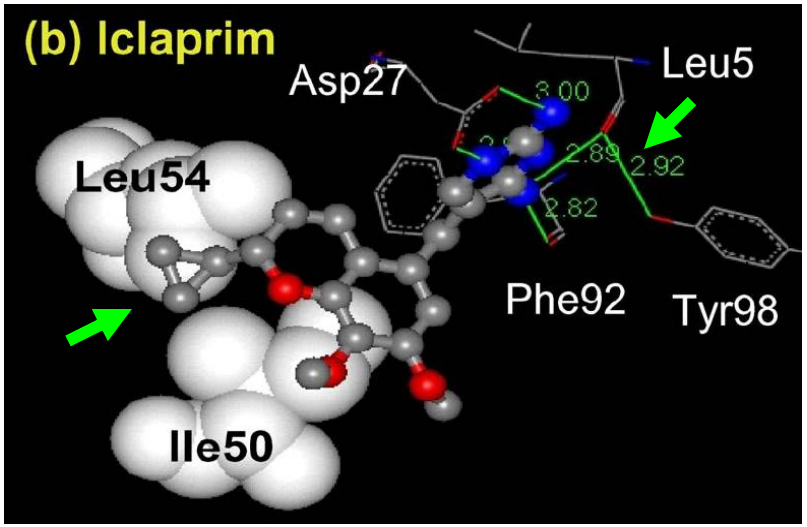
Table 3. Efficacy of Iclaprim and TMP in murine models of septicaemia and pneumonia[39]

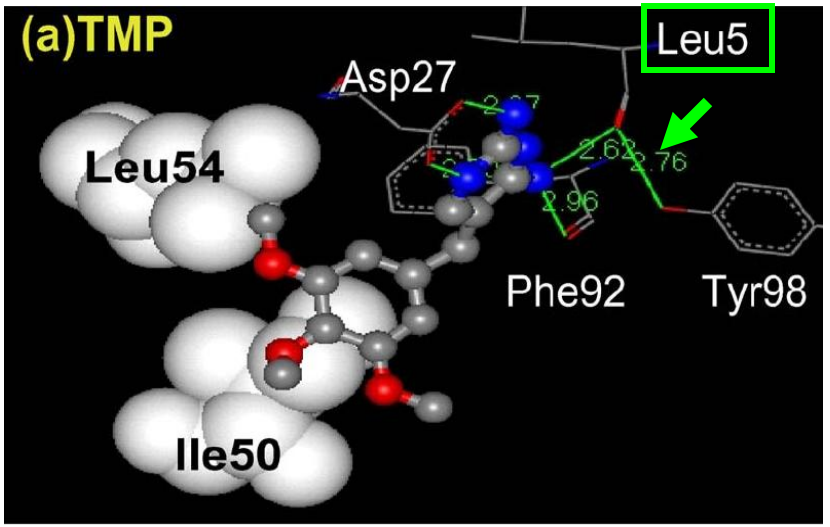
Pathogen	Drug	ED_{50} (mg/kg)
MRSA (septicaemia)	Iclaprim (iv)	4.3
	Iclaprim (po)	17
	Trimethoprim (iv)	15
S. pneumoniae (lung infection)	Iclaprim (sc)	20
	Trimethoprim (sc)	60



Iclaprim

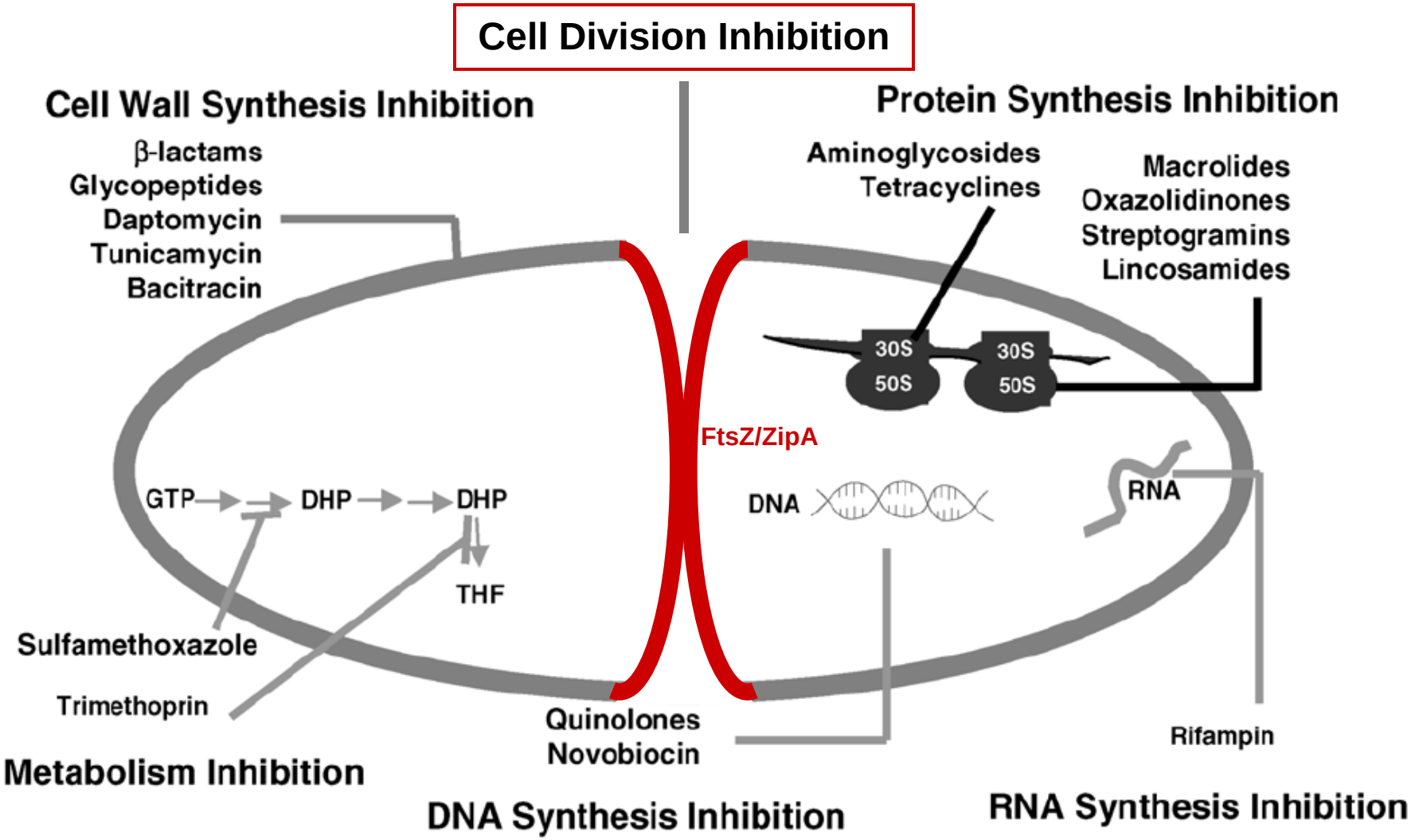
- currently in Phase III testing
- active against trimethoprim-resistant *S. aureus* (Phe98->Tyr98)



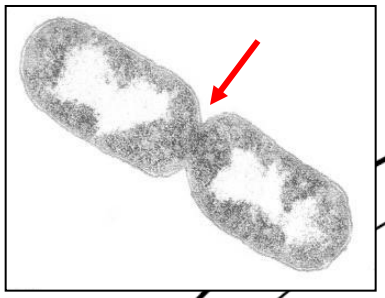


(Hawser et al., *Biochem. Pharmacol.* 2006, 71, 941)

Antibacterial Targets: Overview

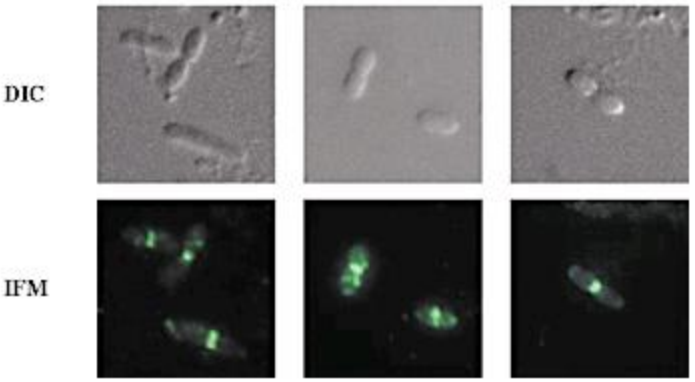


Bacterial Cell Division



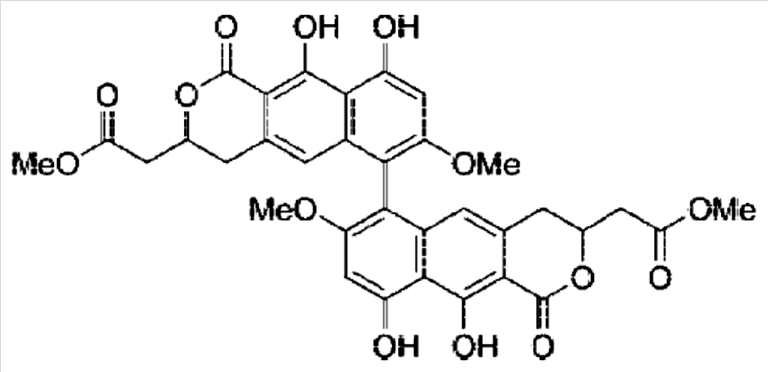
Septal ring model for cell division in *E. coli*:

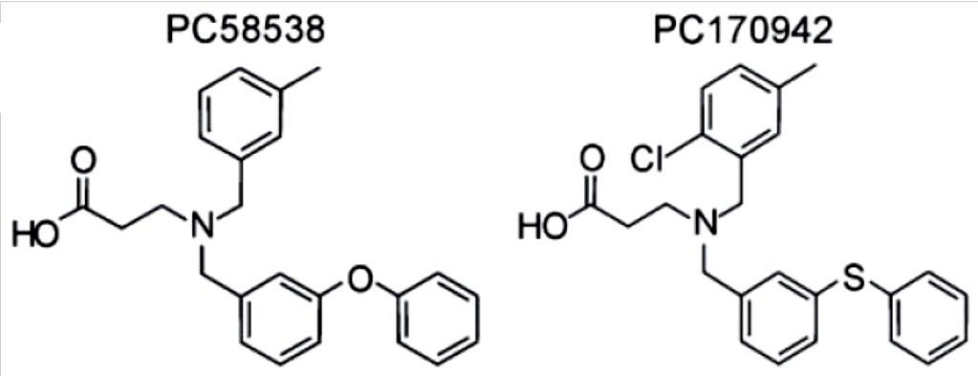
- • FtsZ polymerizes and forms a ring in the cytoplasm.
- • The ring becomes associated with the cell membrane via the ZipA protein and FtsA.
- • Penicillin-binding protein 3 (PBP3) is required for the production of the murein layer at the site of septal division.
- • FtsK is required for localization of the complex.
- • The roles for FtsL, N, Q and W are not clear.
- • TG, transglycosylase.



(Hate & DeBoer, *J. Bact.* 1999, 181, 167)

Bacterial Cell Division: FtsZ Polymerization Inhibitors





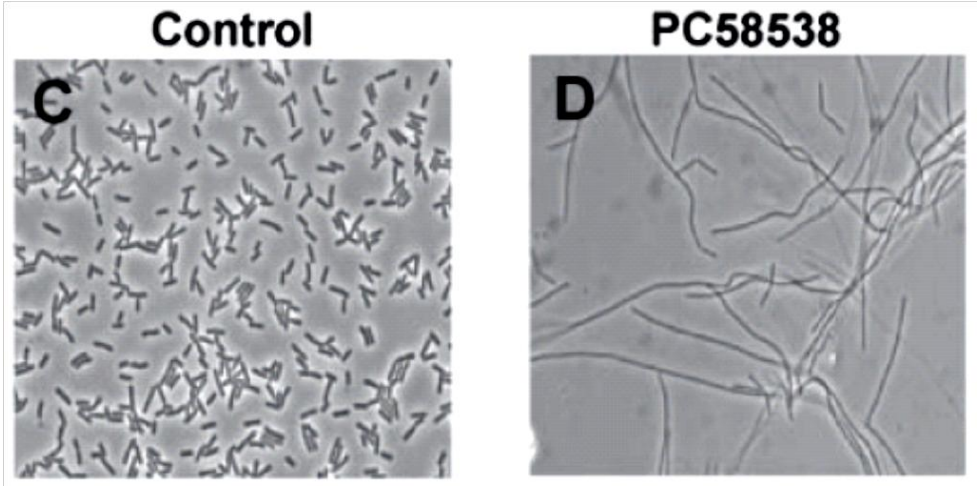
Viriditoxin from *Aspergillus sp.* fermentation broth.

Discovered in high-throughput screen of >100,000 microbial and plant extracts at Merck & Co.

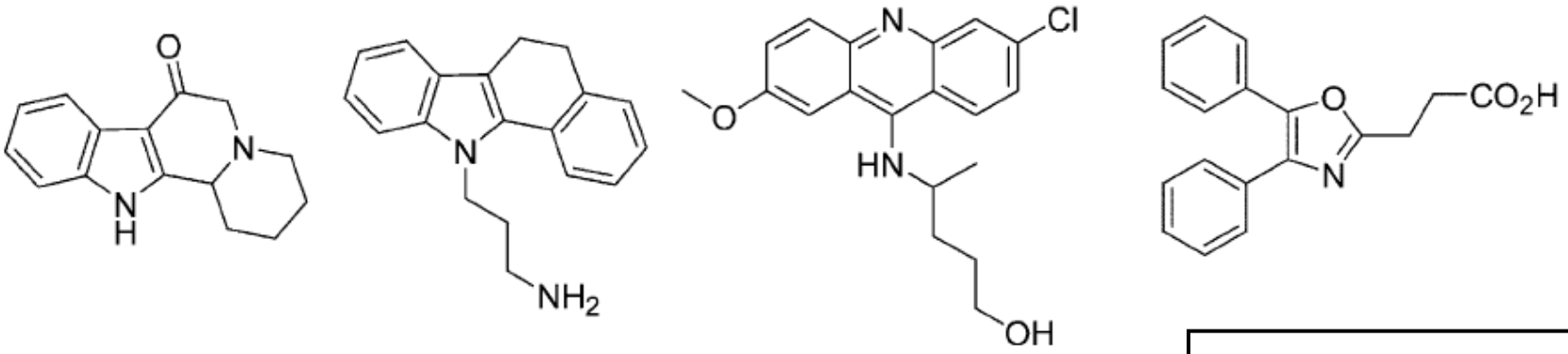
(Jennings et al., *JBC* 2003, 278, 44424)

FtsZ inhibitors discovered in high-throughput screen of ~100,000 synthetic compounds at Prolysis, Ltd. (UK)

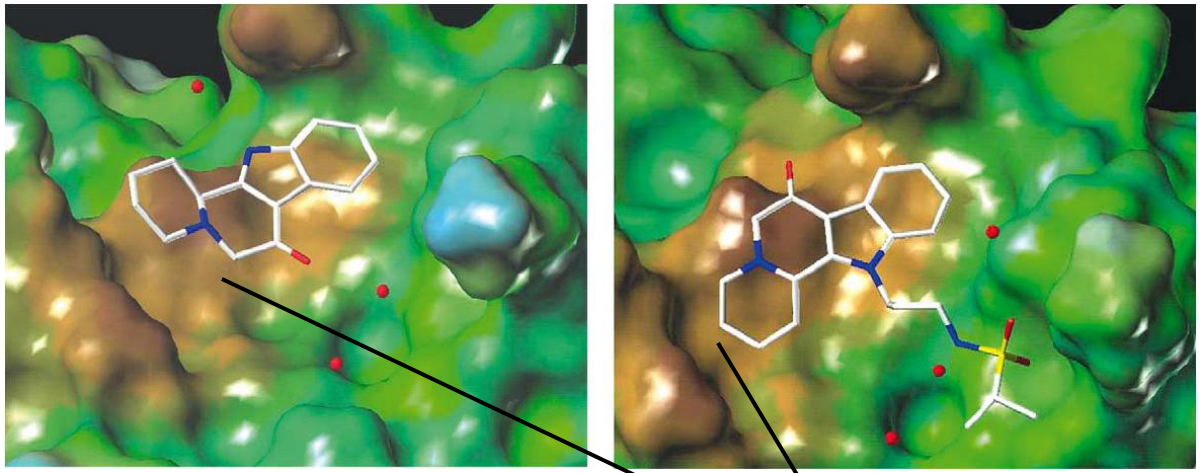
(Stokes et al., *JBC* 2005, 280, 39709)



Bacterial Cell Division: Inhibitors of FtsZ – ZipA Interaction



ZipA-binding inhibitors of FtsZ-ZipA interaction discovered in high-throughput screen of >250,000 synthetic compounds at Wyeth.



Hydrophobic binding region of FtsZ on ZipA

(Jennings et al., *BMCL* 2004, 14, 1427)

Cell division process is difficult to target:

Most potential targets are protein-protein interactions, which are difficult to inhibit with small molecules.

(But:

Related proteins of the eukaryotic cytoskeleton are validated targets of cytotoxic agents.)