

Radezolid (RX-1741), a Novel Oxazolidinone, Accumulates Extensively within Human Macrophages and PMNs and Shows Activity towards Intracellular Linezolid-Sensitive and Linezolid-Resistant *Staphylococcus aureus*

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Intracellular *S. aureus*: is it important ?



Intracellular *Staphylococcus aureus*. A mechanism for the indolence of osteomyelitis.

Ellington et al. J. Bone Joint Surg Br. (2003) 85:918-21



Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease.

Von Eiff et al. Clin Infect Dis. (2001) 32:1643-7



Phagocytosis of *Staphylococcus aureus* by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections.

Hamill et al. Infect Immun. (1986) 54:833-6.

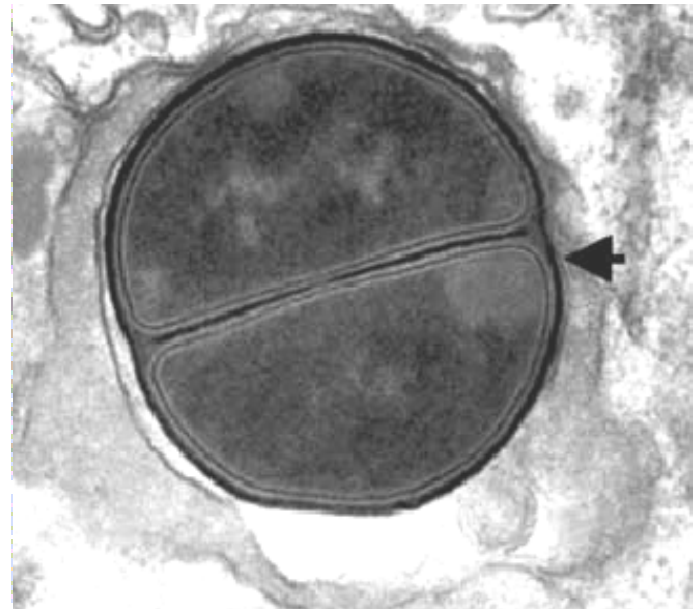
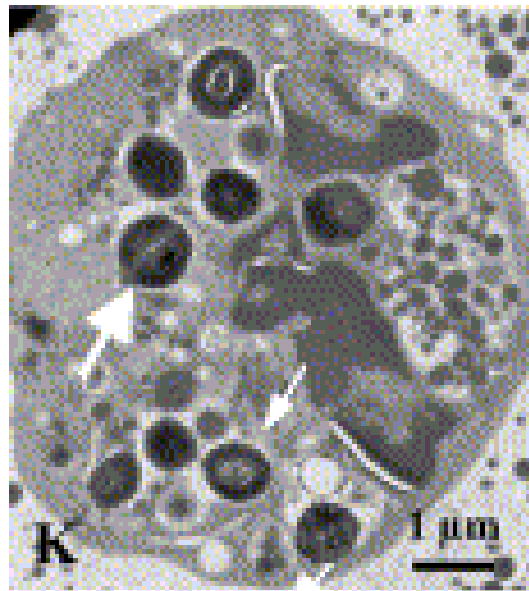


Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis.

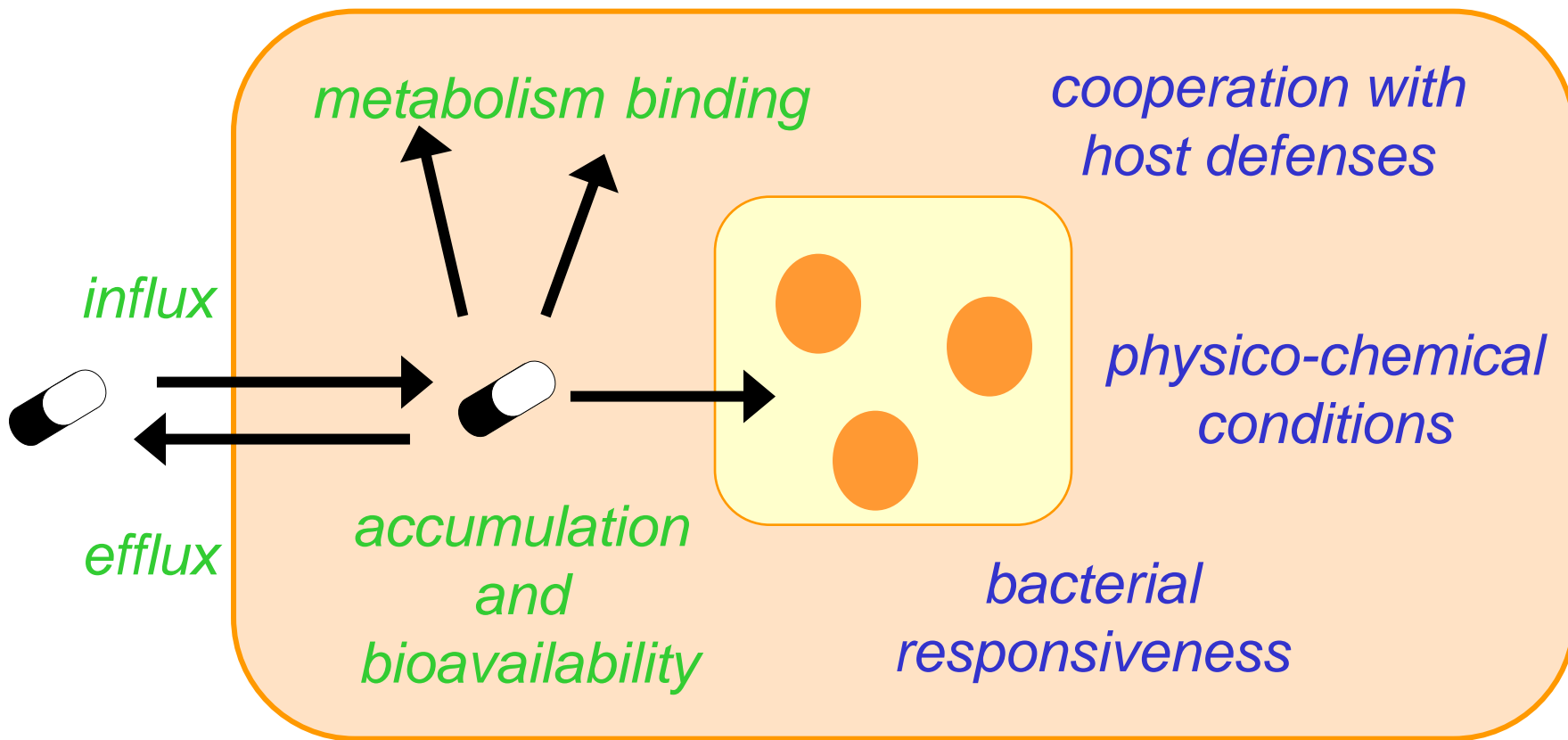
Clement et al., J Infect Dis. (2005) 192:1023-8

S. aureus can also survive and multiply in phagocytic cells

PMN and macrophages: *S. aureus* found in vesicles



Intracellular vs extracellular activity of antibiotics : PK – PD in action



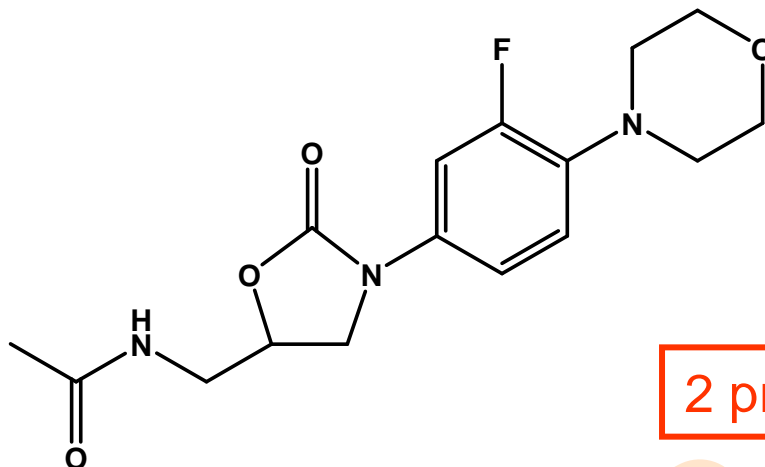
→ activity of new antibiotics requires testing in appropriate models

From Linezolid to Radezolid

designed and
developed by

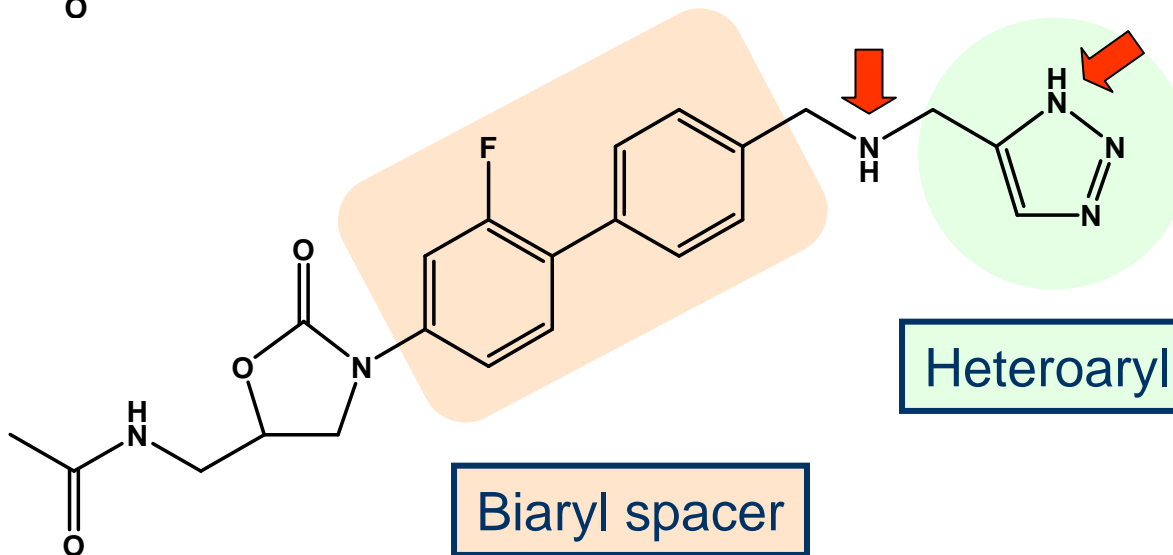
Rib-X
PHARMACEUTICALS

linezolid



2 protonable aminated functions

radezolid

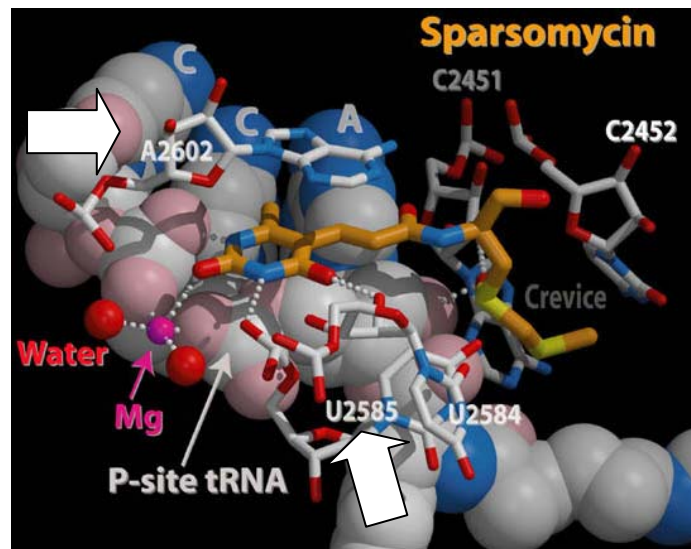
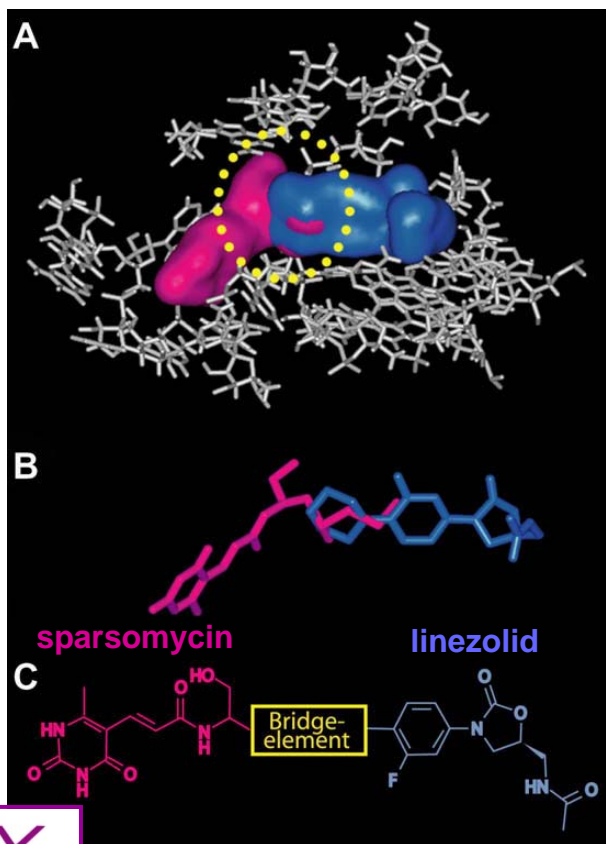


Biaryl spacer

Heteroaryl substituent

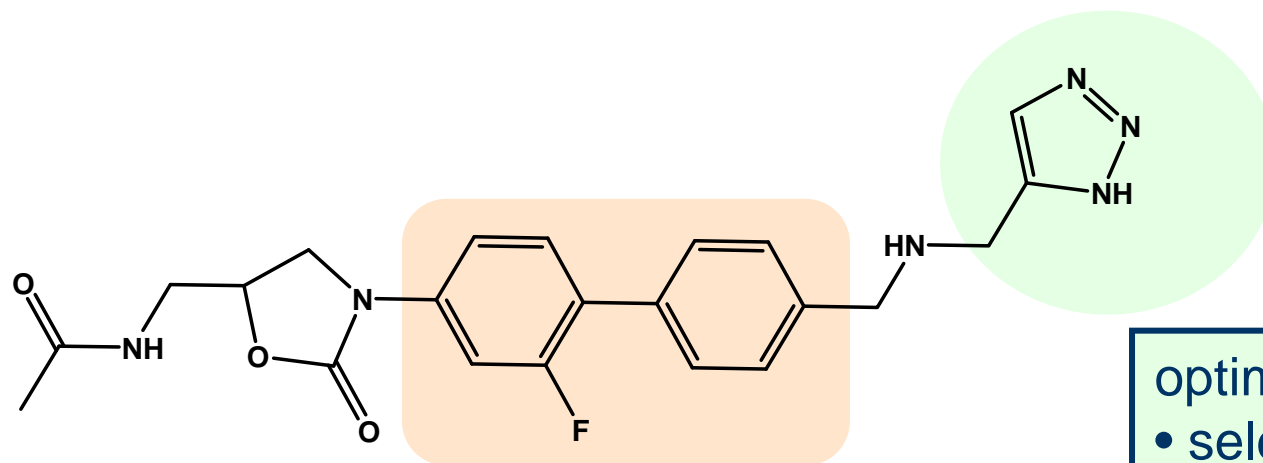
Structure-based design of biaryl-oxazolidinones

- derived from observations made using the crystal structure of the 50S ribosomal unit complexed with known drugs and antibiotics
- combines the most important interactions defined by sparsomycin and linezolid into a single molecular design



additional interaction
with A2602 and U2585
of the 50S ribosomal binding site

Structure-based design of radezolid



aromatic spacer (biaryl)
↓
 π stacking with ribosome

- optimized for
- selectivity
 - **potency**
 - **ADME**
 - **cell penetration**

In vitro activity of radezolid

Improved intrinsic activity against several organisms, including those capable of surviving within eukaryotic cells

bacteria	MICs in mg/L	
	Linezolid	Radezolid
<i>S. pneumoniae</i> ErmB	0.5-2	≤ 0.25
<i>S. pyogenes</i>	2-4	0.03-0.125
<i>E. faecalis</i> & <i>faecium</i>	1-16	≤ 0.25-4
<i>H. influenzae</i>	2-64	0.25-2
<i>M. catarrhalis</i>	2-16	≤ 0.25-1
<i>S. aureus</i> MSSA	2-4	0.5-4
<i>S. aureus</i> MRSA	2-8	≤ 0.25-8
<i>L. pneumophila</i>	4-16	1-4
<i>C. trachomatis</i>	8-16	0.5-1



Aim of the study

- to determine the cellular accumulation/distribution of **radezolid** in phagocytic cells (macrophages, PMN)
- to compare the intracellular activity of **radezolid** and **linezolid** against isogenic LZD-S and LZD-R strains of *Staphylococcus aureus*

Aim of the study

⇒ pharmacokinetics

- to determine the cellular accumulation/distribution of **radezolid** in phagocytic cells (macrophages, PMN)



General methodology: cellular accumulation

THP-1 human macrophages

PMNs from human volunteers

- incubated with linezolid or ^{14}C -radezolid
- washed in PBS and collected by low-speed centrifugation

• resuspended in water

cell prot.
(Lowry)

drug

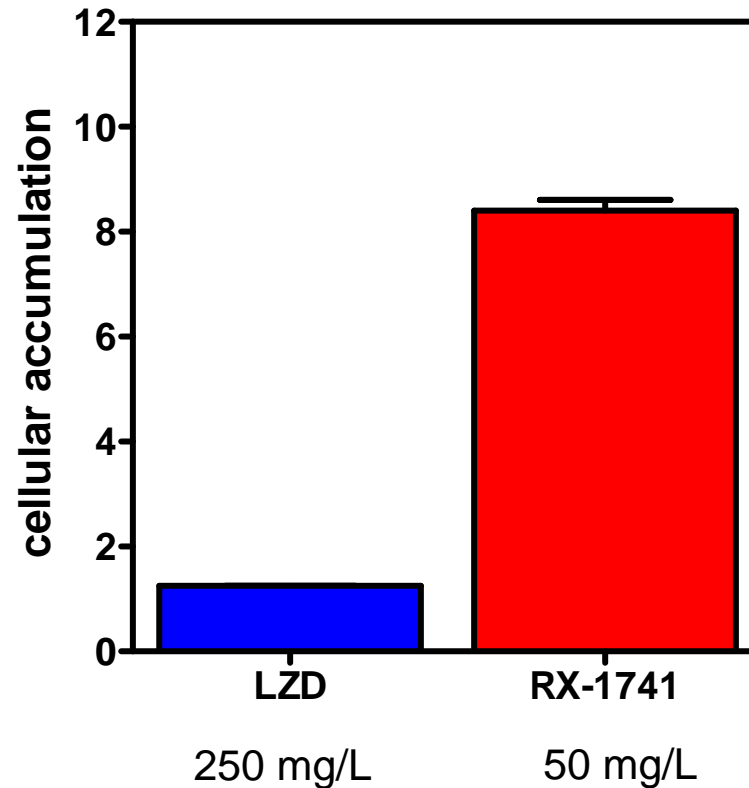
scintillation counting

microbiological assay
(*B. subtilis*)

accumulation calculated considering a cell volume of 5 $\mu\text{l}/\text{mg}$ prot.

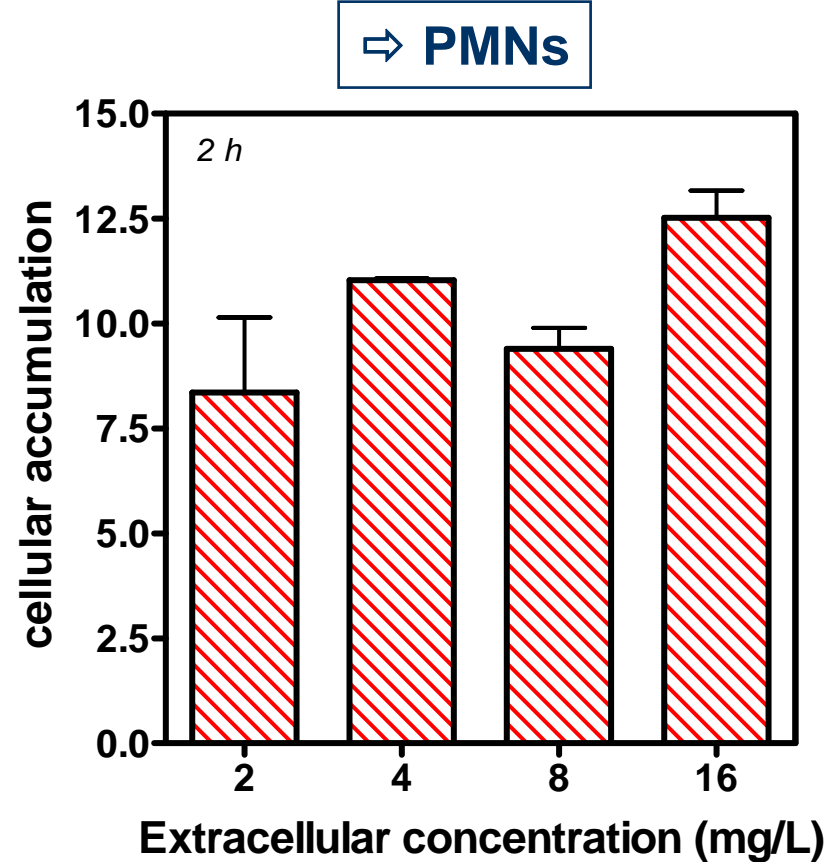
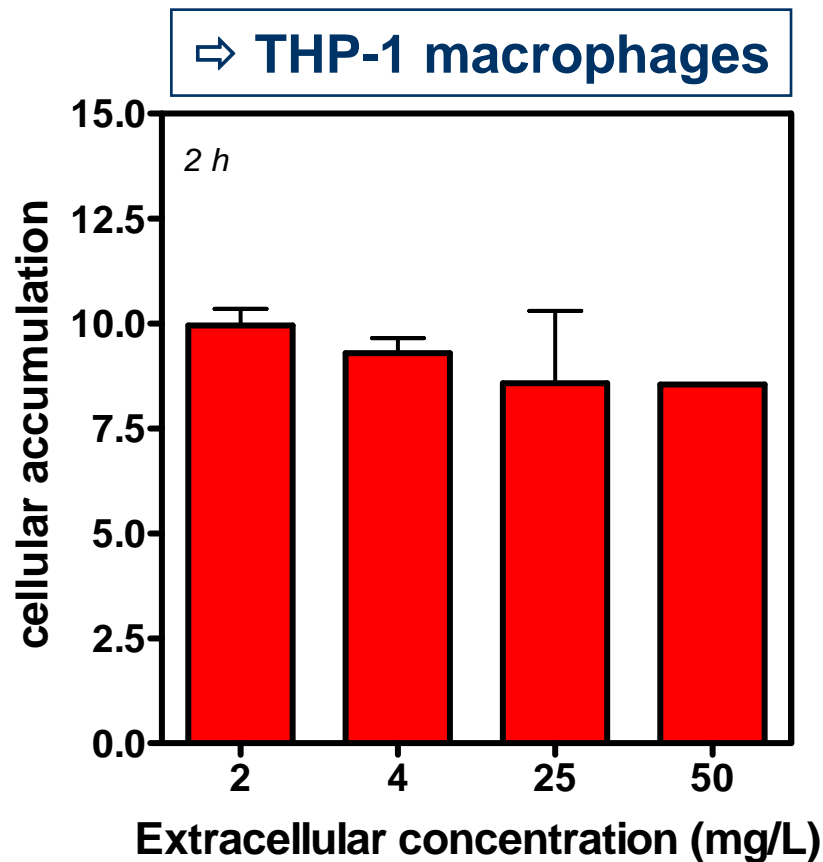
Comparative accumulation level at equilibrium

⇒ THP-1 macrophages



in contrast to linezolid, radezolid accumulates in eukaryotic cells !

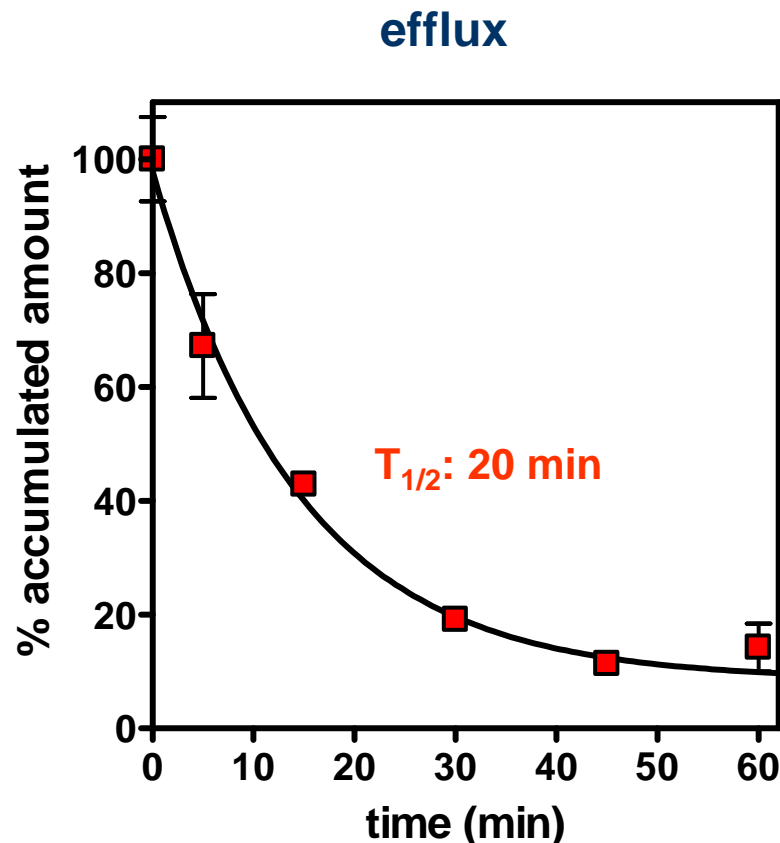
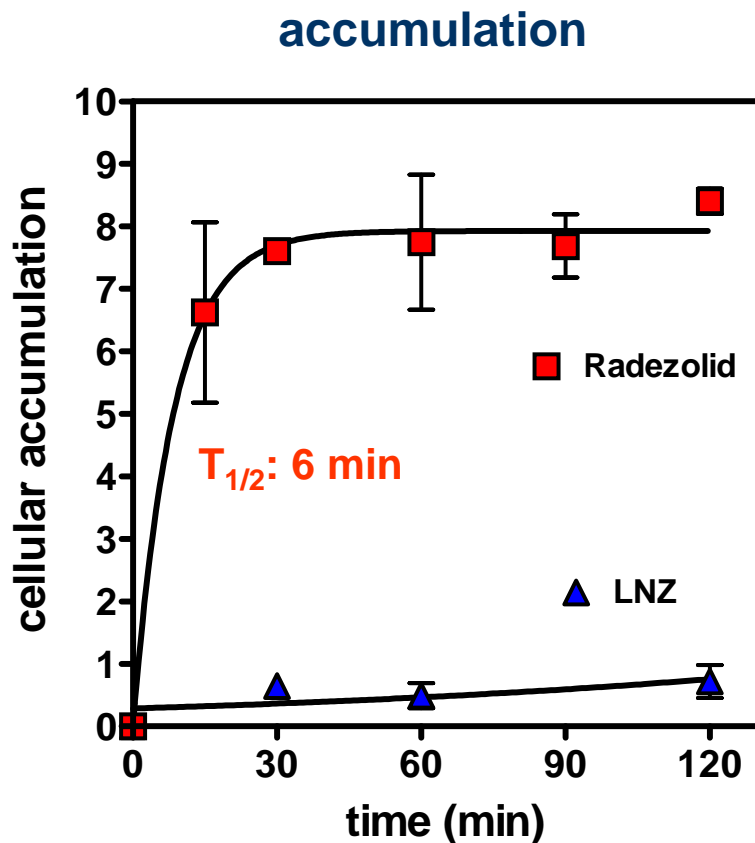
Radezolid accumulation is concentration-independent



- non-saturable accumulation in human phagocytic cells
- no influence of efflux pump inhibitors (verapamil; gemfibrozil) (*not shown*)

Kinetics of radezolid accumulation and efflux

⇒ THP-1 macrophages

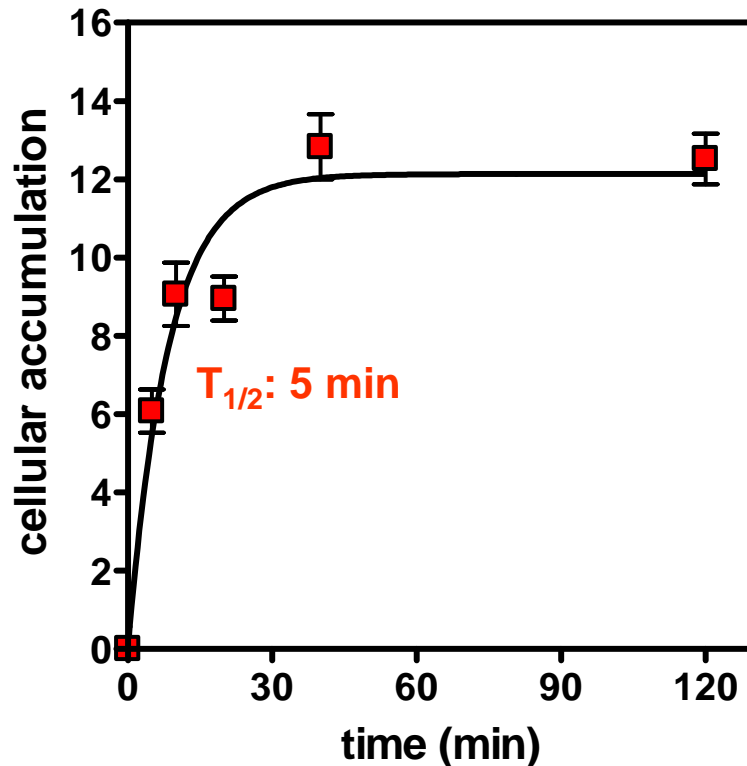


rapid accumulation and slightly slower efflux

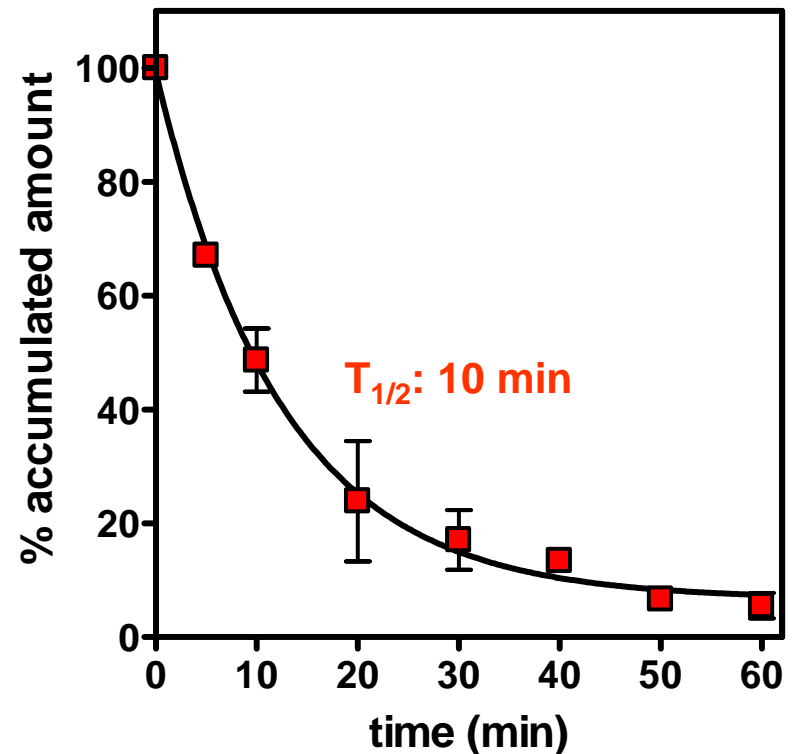
Kinetics of radezolid accumulation and efflux

⇒ PMNs

accumulation



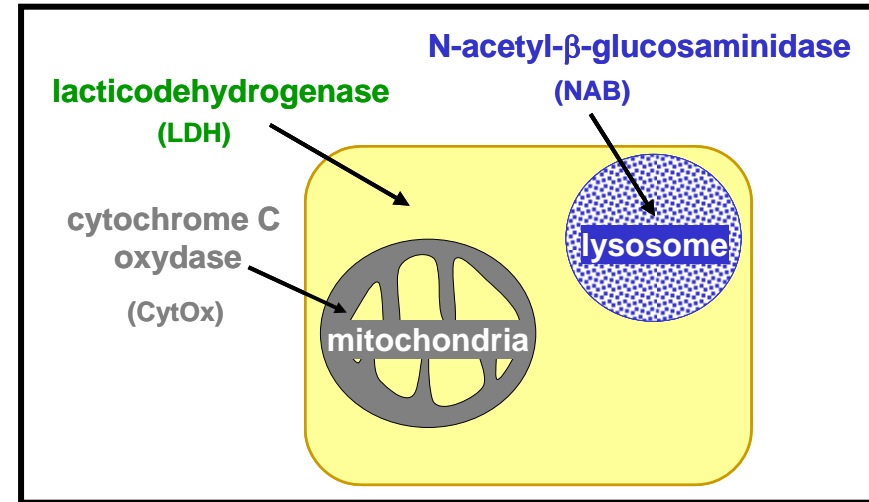
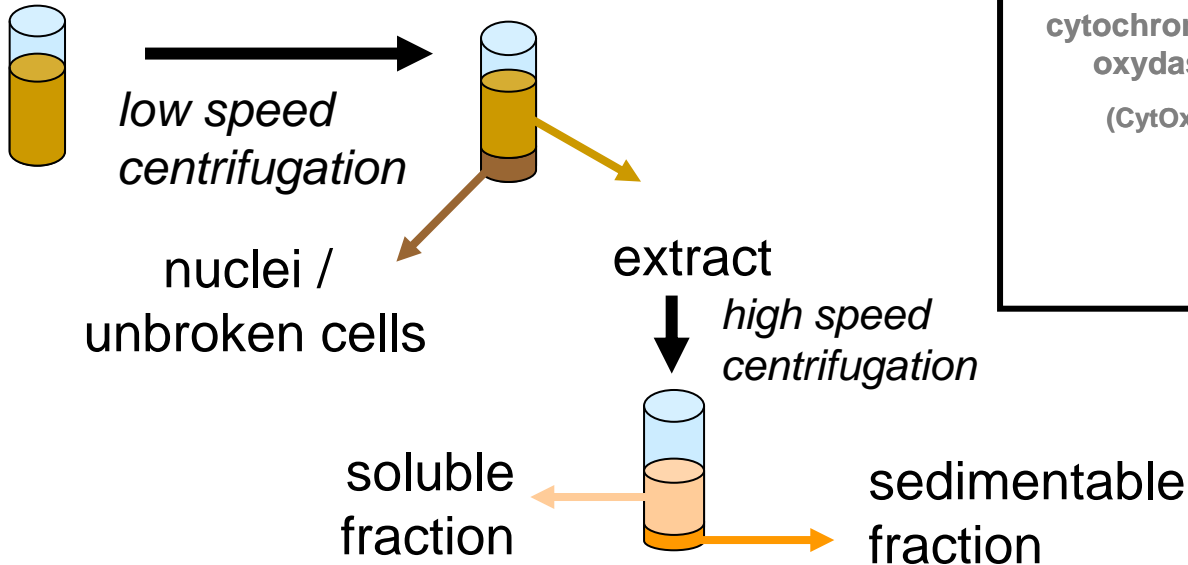
efflux



rapid accumulation and slightly slower efflux

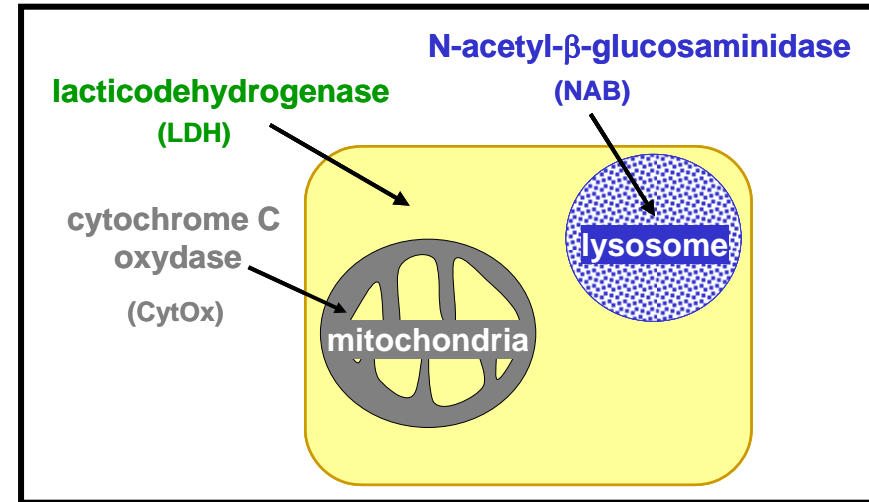
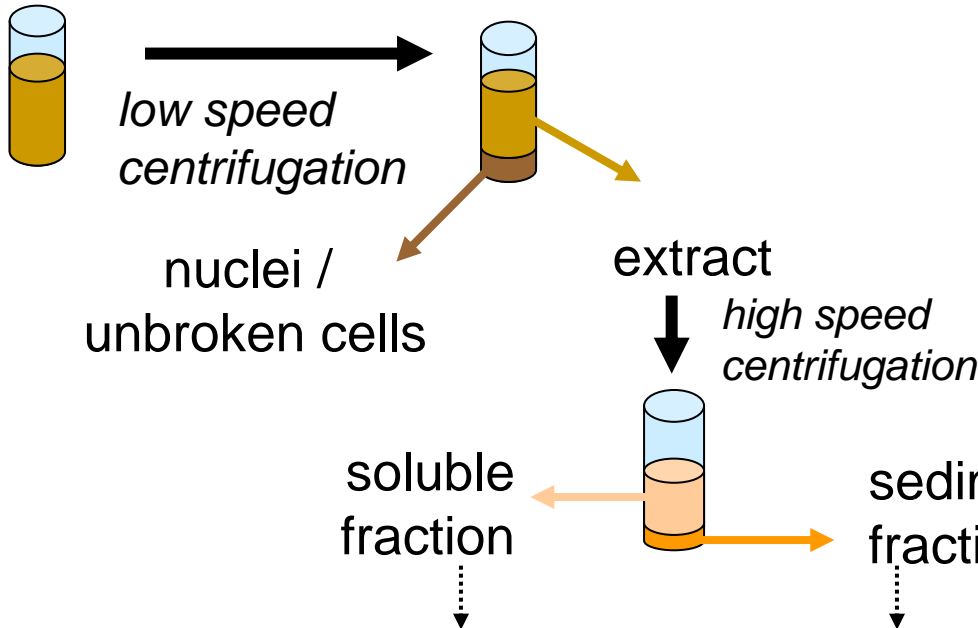
Subcellular localization of radezolid

⇒ J774 mouse macrophages



Subcellular localization of radezolid

⇒ J774 mouse macrophages

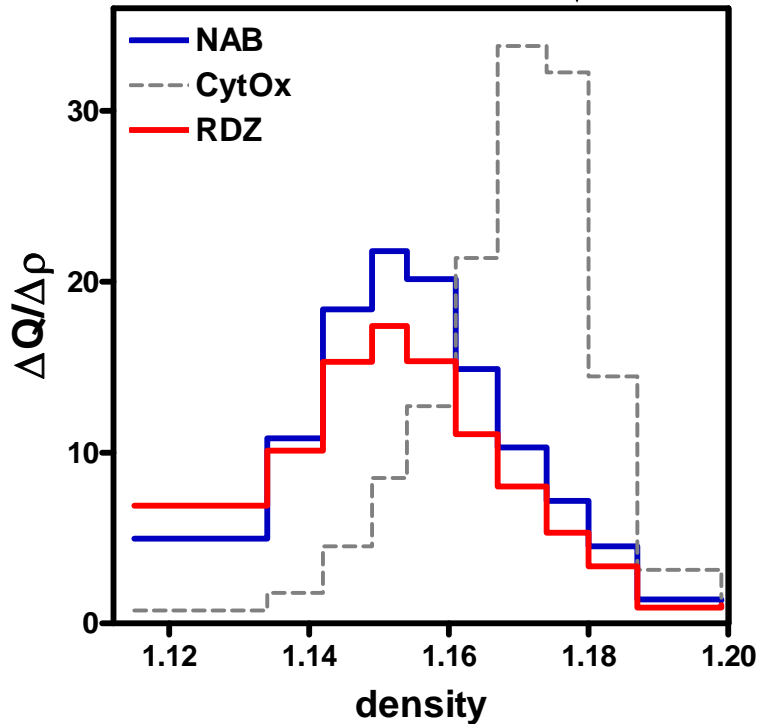
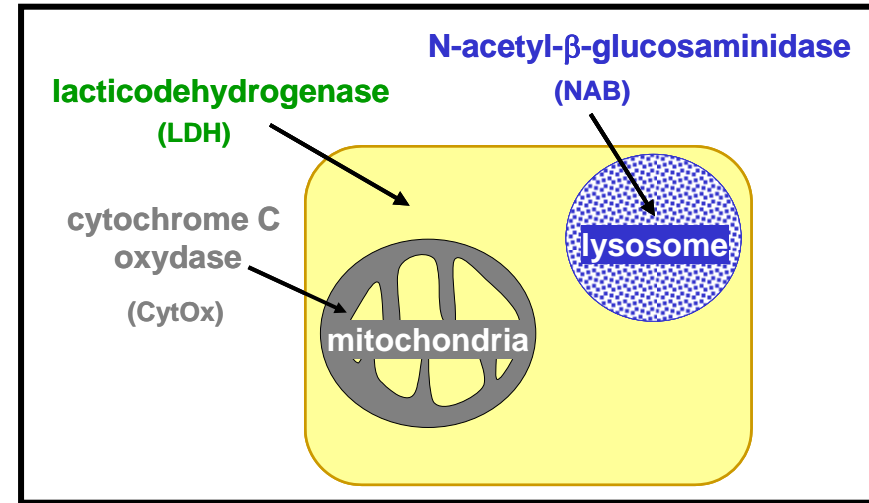
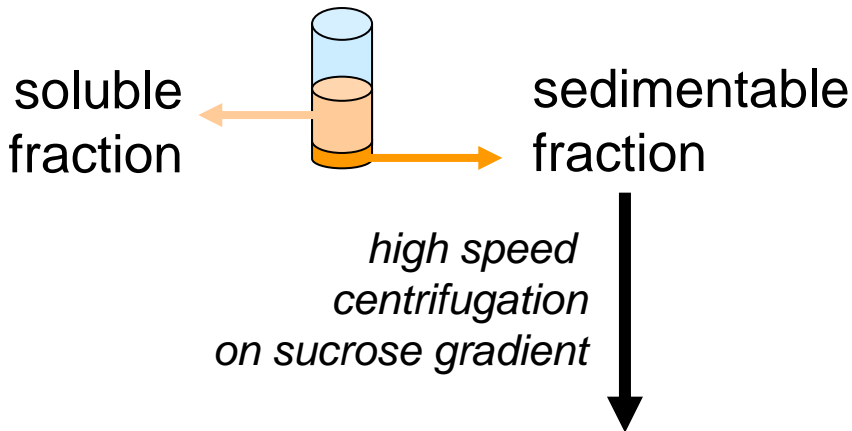


Radezolid	58.2 %	41.8 %
NAB	10.6 %	89.4 %
CytOx	1.7 %	98.3 %
LDH	97.0 %	3.0 %

dual subcellular localization

- cytosol
- organelles

Subcellular localization of radezolid



dual subcellular localization

- cytosol
- lysosomes

Aim of the study

⇒ pharmacodynamics

- to compare the intracellular activity of **radezolid** and **linezolid** against isogenic LZD-S and LZD-R strains of *Staphylococcus aureus* in THP-1 macrophages



General methodology: intracellular activity

THP-1 human macrophages

- infected by pre-opsonized *S. aureus*
- extracellular bacteria eliminated by short incubation with gentamicin
- incubation with increasing concentrations of antibiotics for 24 h
- washings

• resuspended in water

cell prot.
(Lowry)

plating and CFU counting

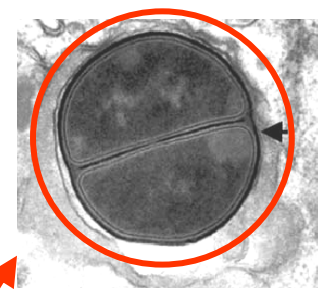
activity expressed as change from initial inoculum

Bacterial strains under study

strain	phenotype	MIC pH 7.4 (mg/L)	
		linezolid	radezolid
SA 238	LZD-S	2	1
SA 238L	LZD-R	16	2

- at neutral pH, radezolid is ~equipotent against the LZD-R strain

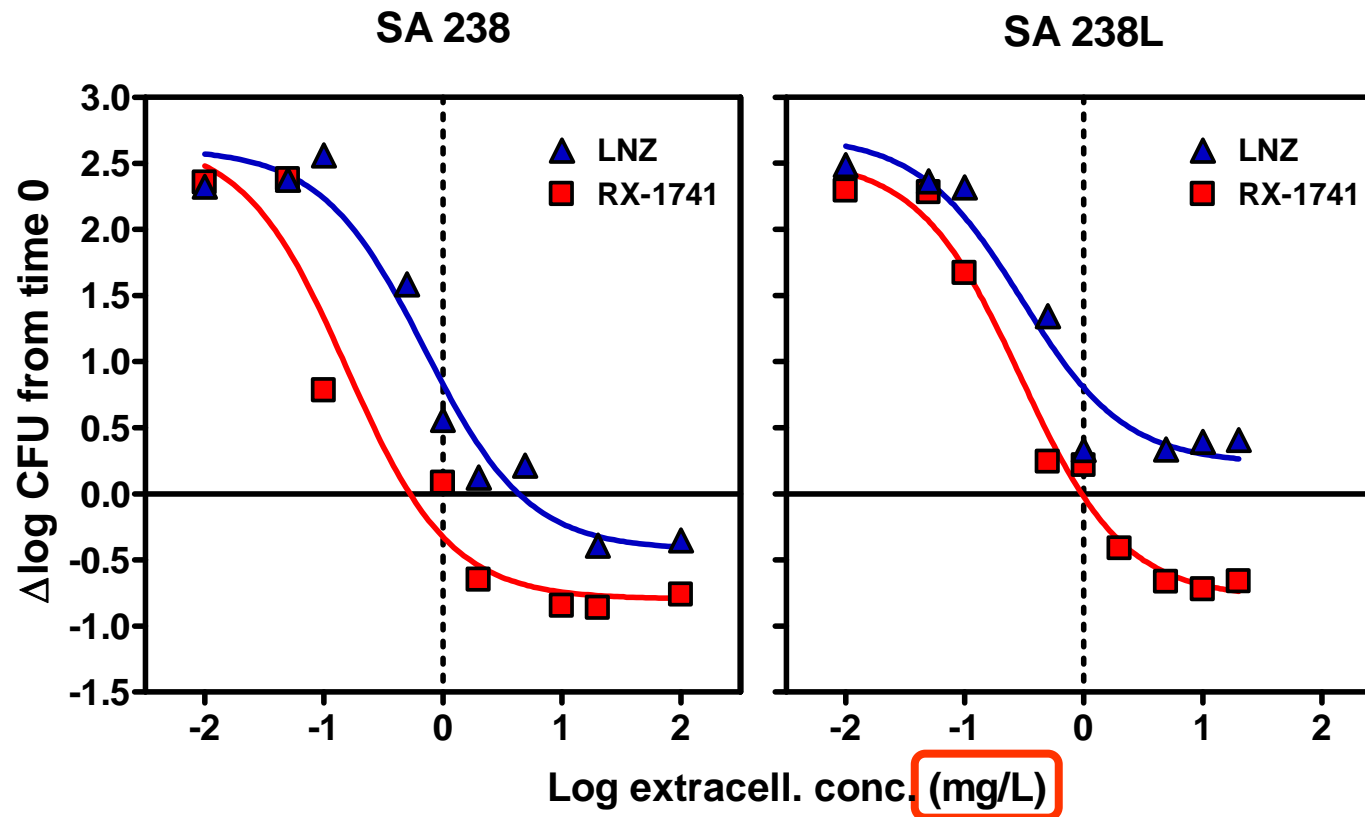
Bacterial strains under study



strain	phenotype	MIC pH 7.4 (mg/L)		MIC pH 5.5 (mg/L)	
		linezolid	radezolid	linezolid	radezolid
SA 238	LZD-S	2	1	4	16
SA 238L	LZD-R	16	2	8	16

- at neutral pH, radezolid is ~equipotent against the LZD-R strain
- at acidic pH, radezolid activity is reduced

Intracellular activity (clinically-relevant comparison)

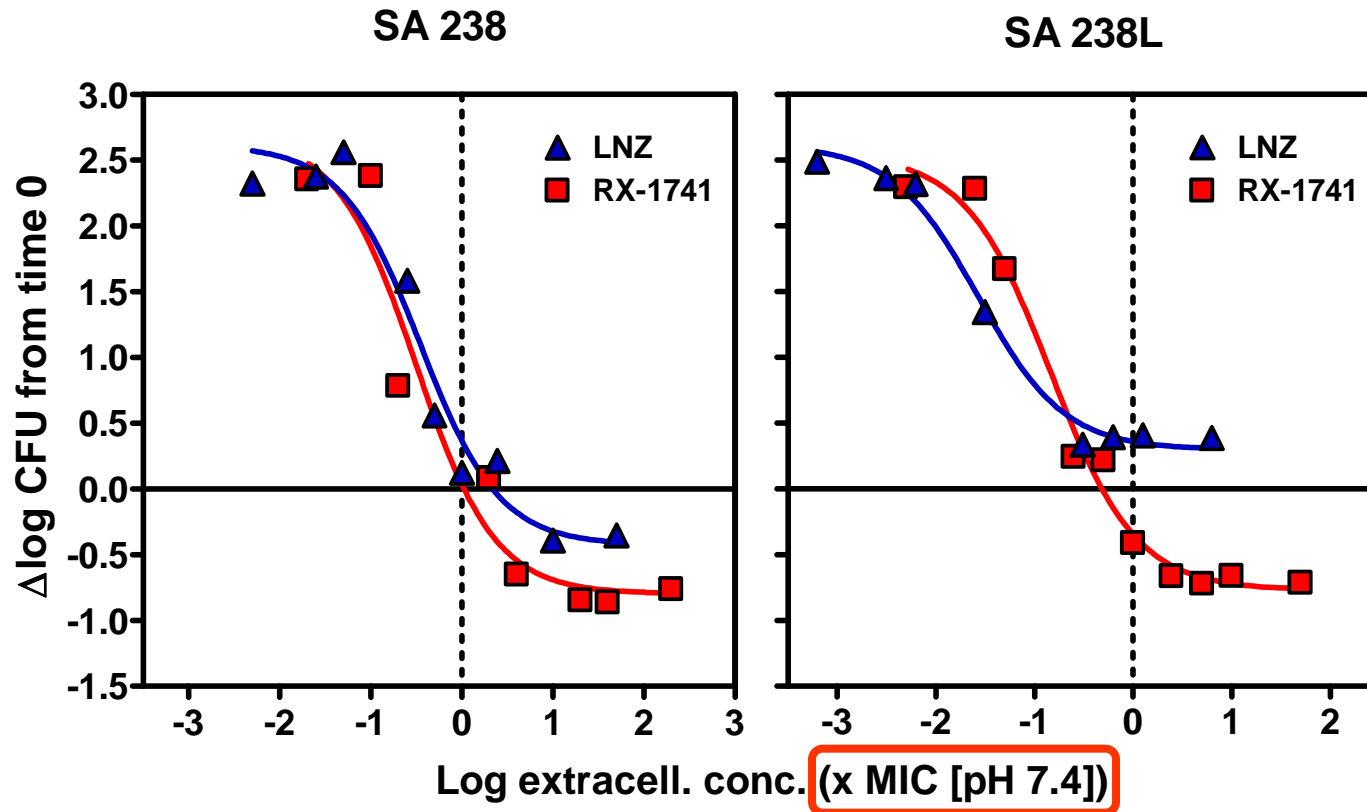


drug	SA 238		SA 238L	
	C_{static}	E_{max}	C_{static}	E_{max}
linezolid	5.8	- 0.3	> 100	0.2
radezolid	0.5	- 0.6	0.9	-0.8

Radezolid is

- more potent and effective than LZD
- as potent and effective against both strains

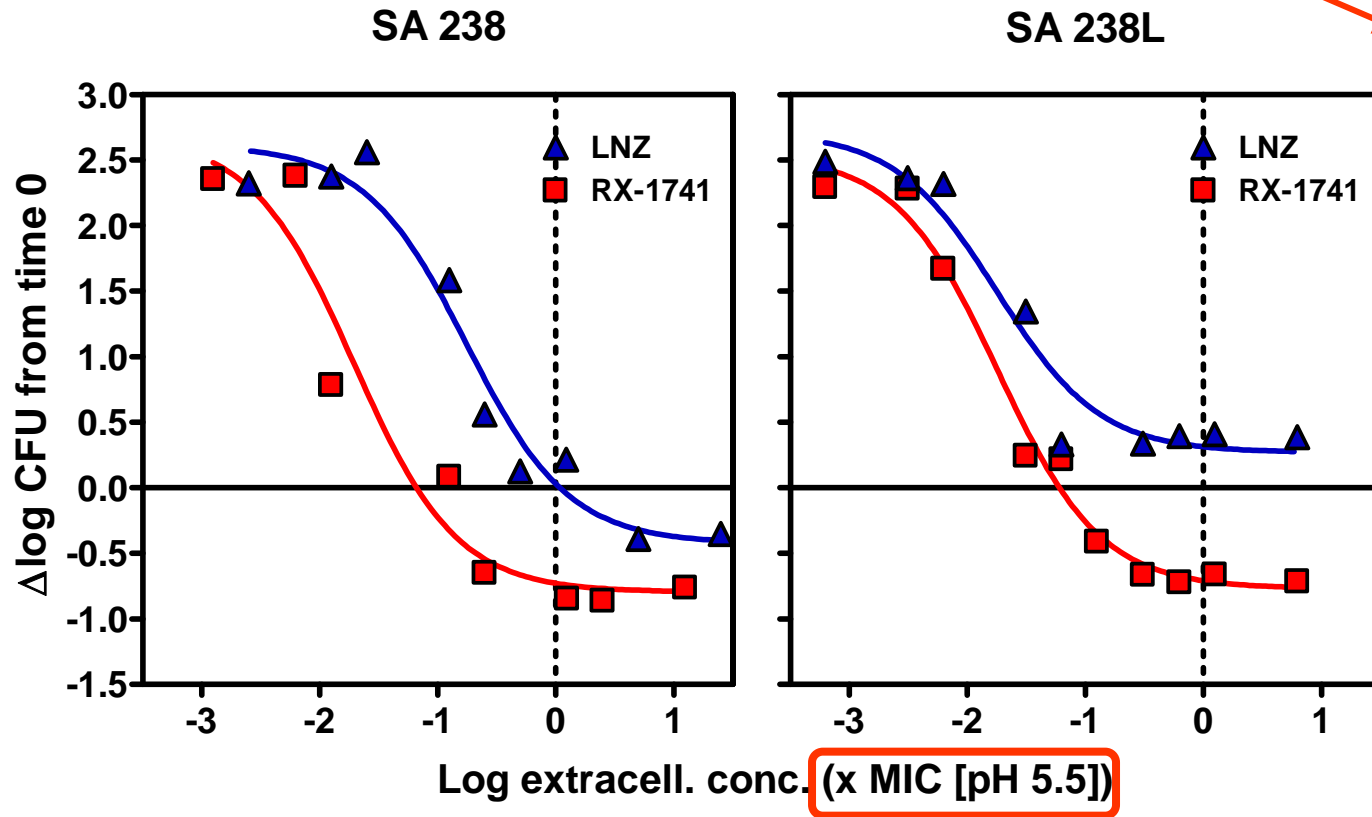
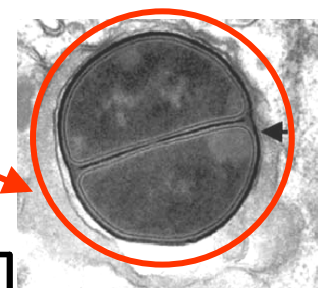
Intracellular activity (equipotent concentrations)



drug	SA 238	SA 238L
	C_{static}	C_{static}
linezolid	2.1	> 10
radezolid	1.1	0.5

- Radezolid and LZD ~ equipotent against the LZD-S strain
- Radezolid ~ equipotent against both strains

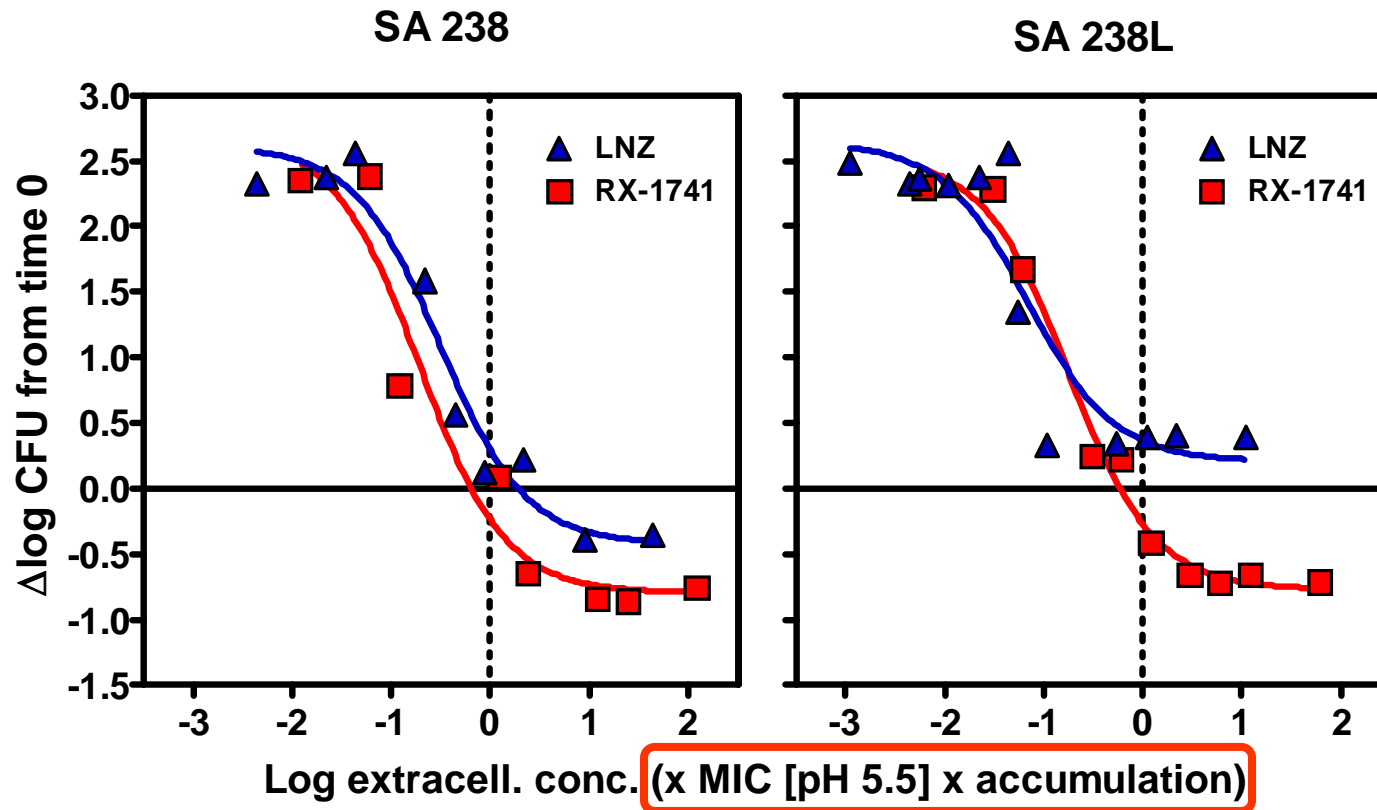
Intracellular activity (x MIC pH 5.5)



drug	SA 238	SA 238L
	C_{static}	C_{static}
linezolid	1.1	> 10
radezolid	0.1	0.1

• Radezolid is active at lower multiples of the assumed MIC in the infected compartment

Intracellular activity (pharmacological comparison)



drug	SA 238	SA 238L
	C_{static}	C_{static}
linezolid	1.9	> 10
radezolid	0.7	0.6

• cellular accumulation of Radezolid compensates for the effect of acid pH on activity

Summary

⇒ pharmacokinetics

- **radezolid** accumulates
 - ~ 10-fold in human macrophages and PMNs
 - quickly, reversibly
 - independently of extracellular concentration
 - in both cytosolic and lysosomal compartments

⇒ pharmacodynamics

- as compared to **linezolid**, **radezolid** shows
 - a higher efficacy (improved E_{\max})
 - a higher potency (lower C_{static})
- **radezolid** proves equipotent against LZD-S and LZD-R strains