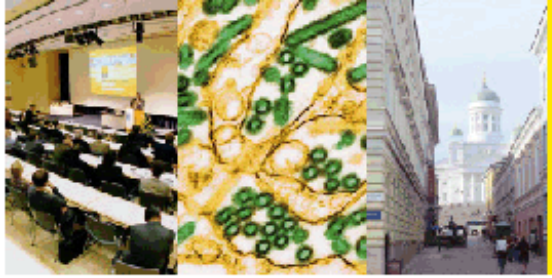

19th ECCMID

The novel oxazolidinone Radezolid (RX-1741) accumulates in THP-1 macrophages: comparative studies with linezolid and azithromycin

Sandrine Lemaire, Paul M. Tulkens, Françoise Van Bambeke

Unité de Pharmacologie cellulaire et moléculaire
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Université catholique de Louvain
<www.facm.ucl.ac.be>

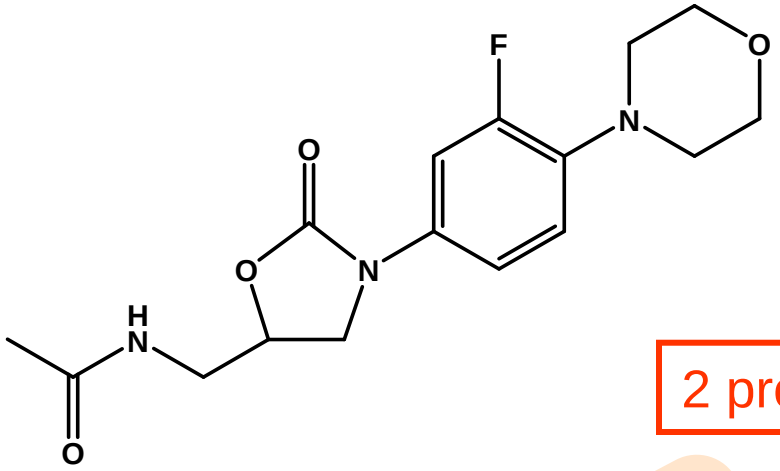
UCL Université catholique de Louvain

From Linezolid to Radezolid

designed and developed by

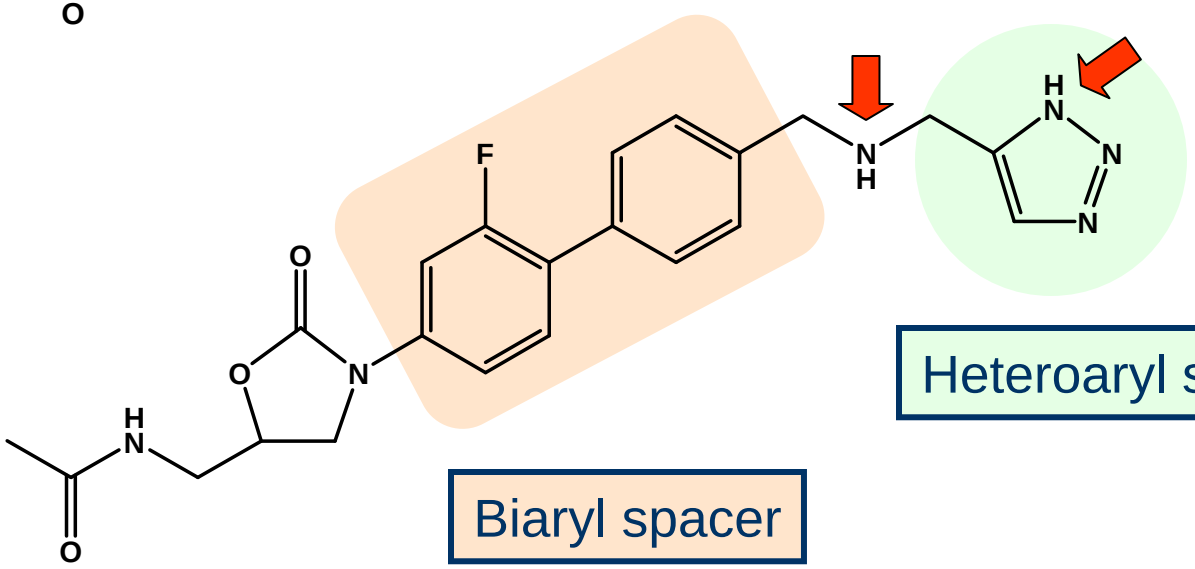
Rib-X PHARMACEUTICALS

linezolid



2 protonable aminated functions

radezolid

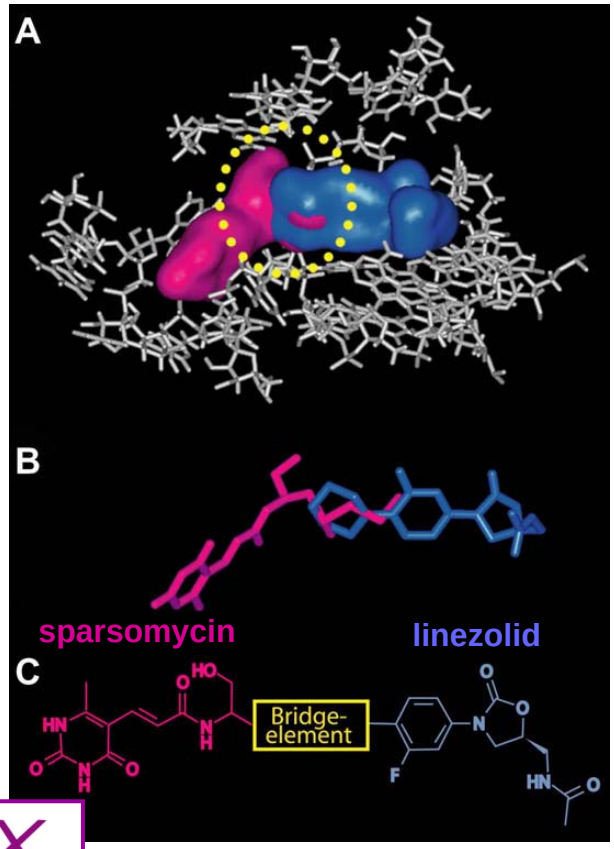


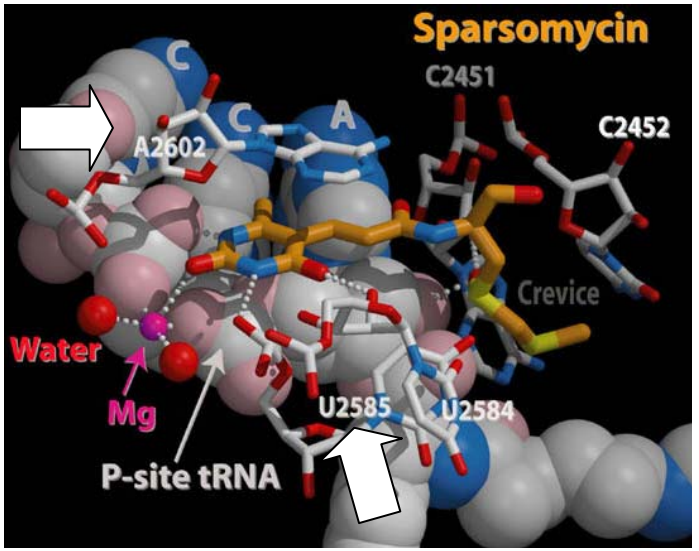
Heteroaryl substituant

Biaryl spacer

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



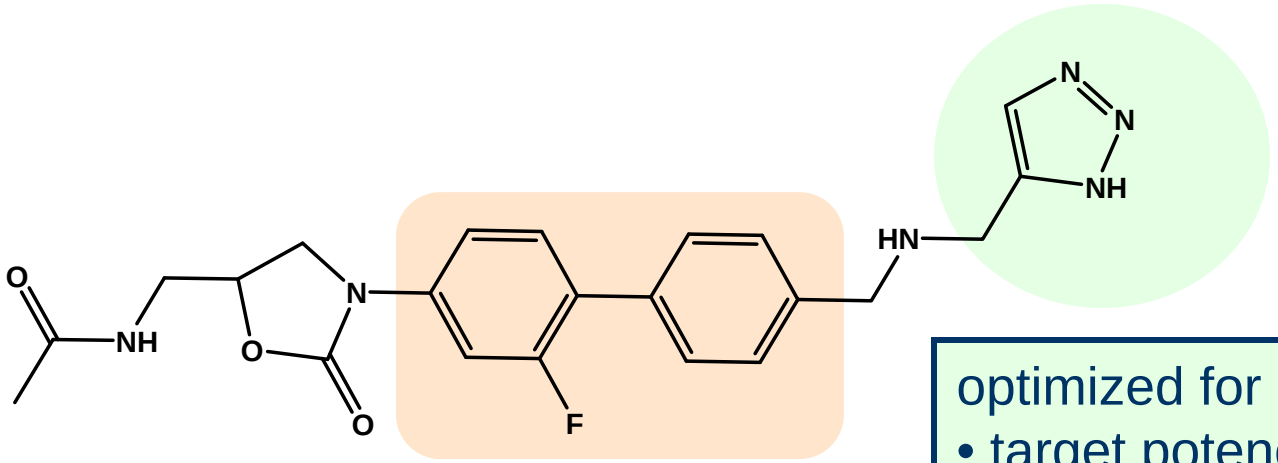


additional interaction
with A2602 and U2585
of the 50S ribosomal binding site

Zhou et al., J. Bioorg. Med. Chem. Lett. (2008) 18:6179-83
Skripkin et al. Antimicrob. Ag. Chemother. (2008) 52:3350-57

Rib-X PHARMACEUTICALS

Structure-based design of radezolid



aromatic spacer (biaryl)

⇓

π stacking with ribosome

optimized for

- target potency & selectivity
- antibacterial spectrum
- **ADME**
- **cell penetration**

Zhou et al., J. Bioorg. Med. Chem. Lett. (2008) 18:6179-83

Skripkin et al. Antimicrob. Ag. Chemother. (2008) 52:3350-57

Aim of the study

- **to investigate the cellular pharmacokinetics of radezolid**
- **to decipher the mechanisms of its cellular accumulation**
  - in a model of THP-1 human macrophages
  - in comparison with linezolid and azithromycin

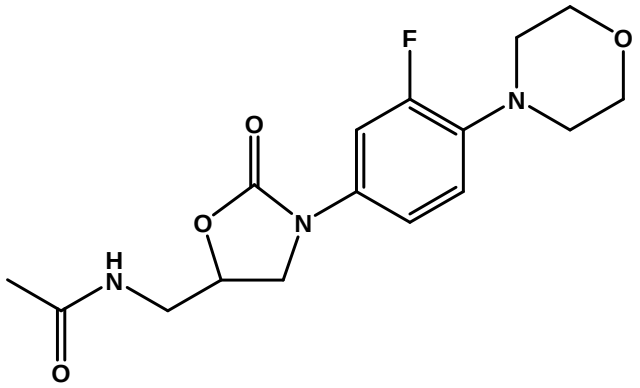
Oxazolidinone
- low accumulation
- cellular conc. ~ extracell. conc.

Macrolide
- dicationic amphiphile
- high accumulation by diffusion-segregation in acidic compartments

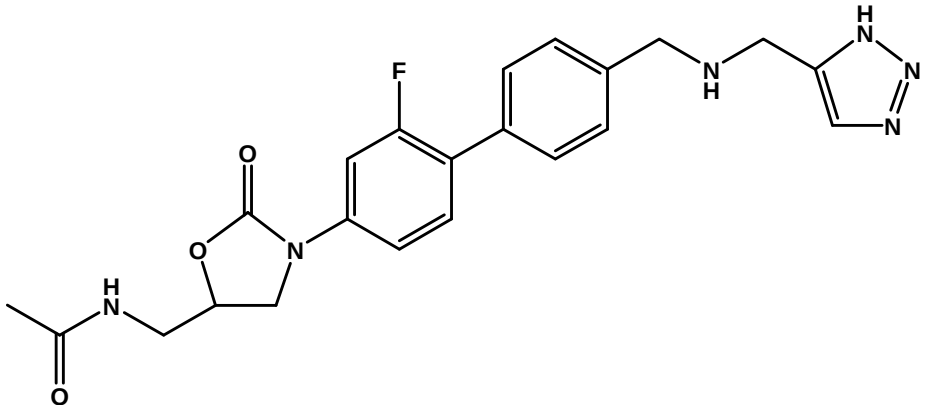
Physico-chemical properties

drug	logP (Qikprop)	pKa_1	pKa_2
Linezolid	0.47		
Radezolid	0.7	6.8	9.4
Azithromycin	2.98	8.1	8.6

Similar lipophilicity



linezolid



radezolid

Physico-chemical properties

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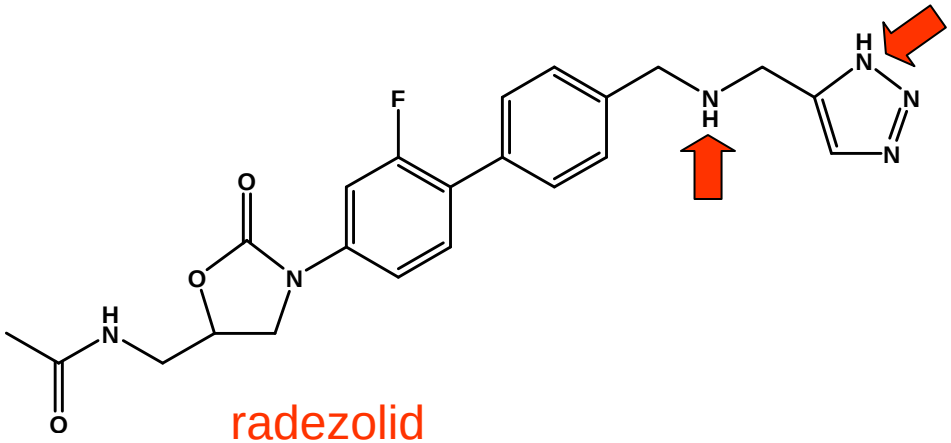
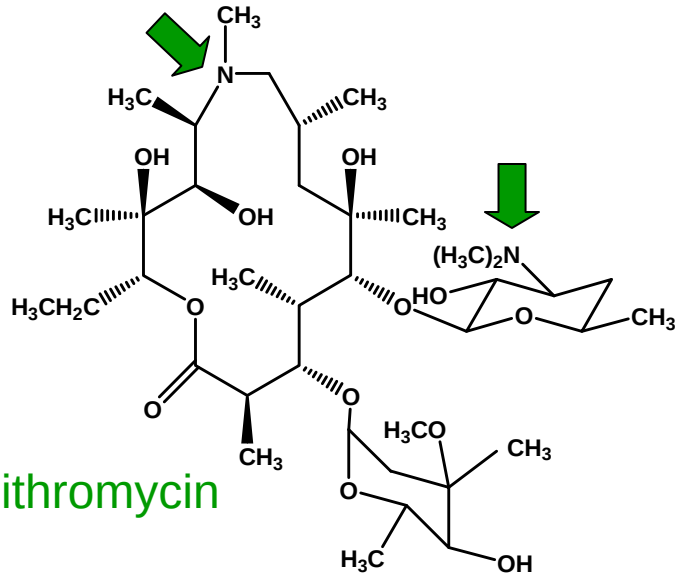
weak dibasic character

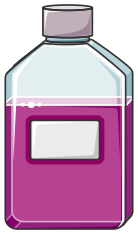


radezolid

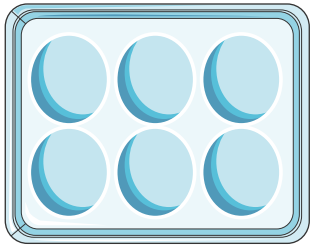


azithromycin

General methodology



THP-1 cells
growing in suspension



10^6 cells incubated with
- linezolid (250 mg/L),
- radezolid (50 mg/L, with a trace amount of ^{14}C-drug),
- azithromycin (10 mg/L)

- washed 3 X in ice-cold PBS
- collected by low-speed centrifugation

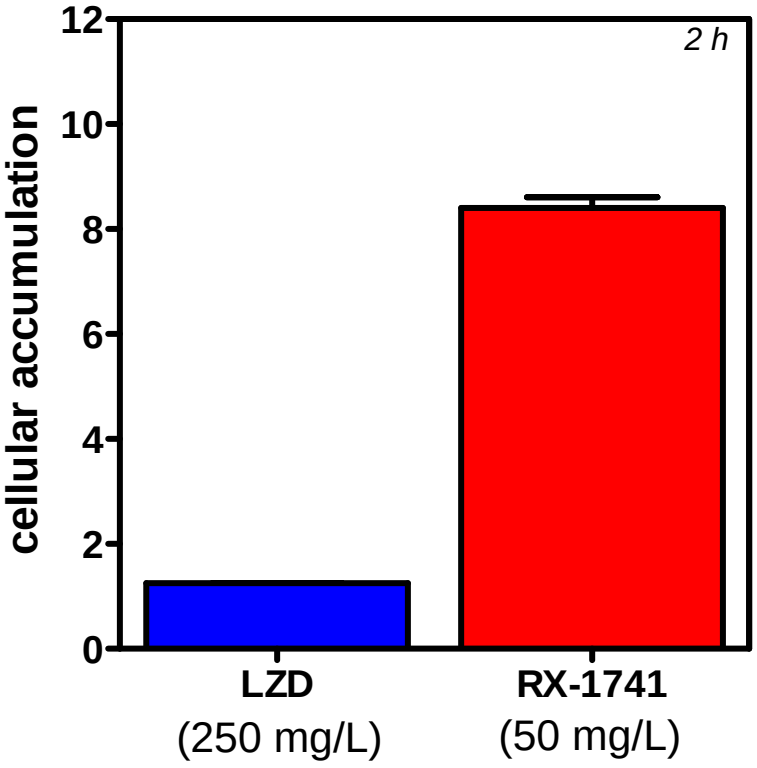
- resuspended in water

cell prot. (Lowry)

drug
- scintillation counting
- microbiological assay (*B. subtilis*)

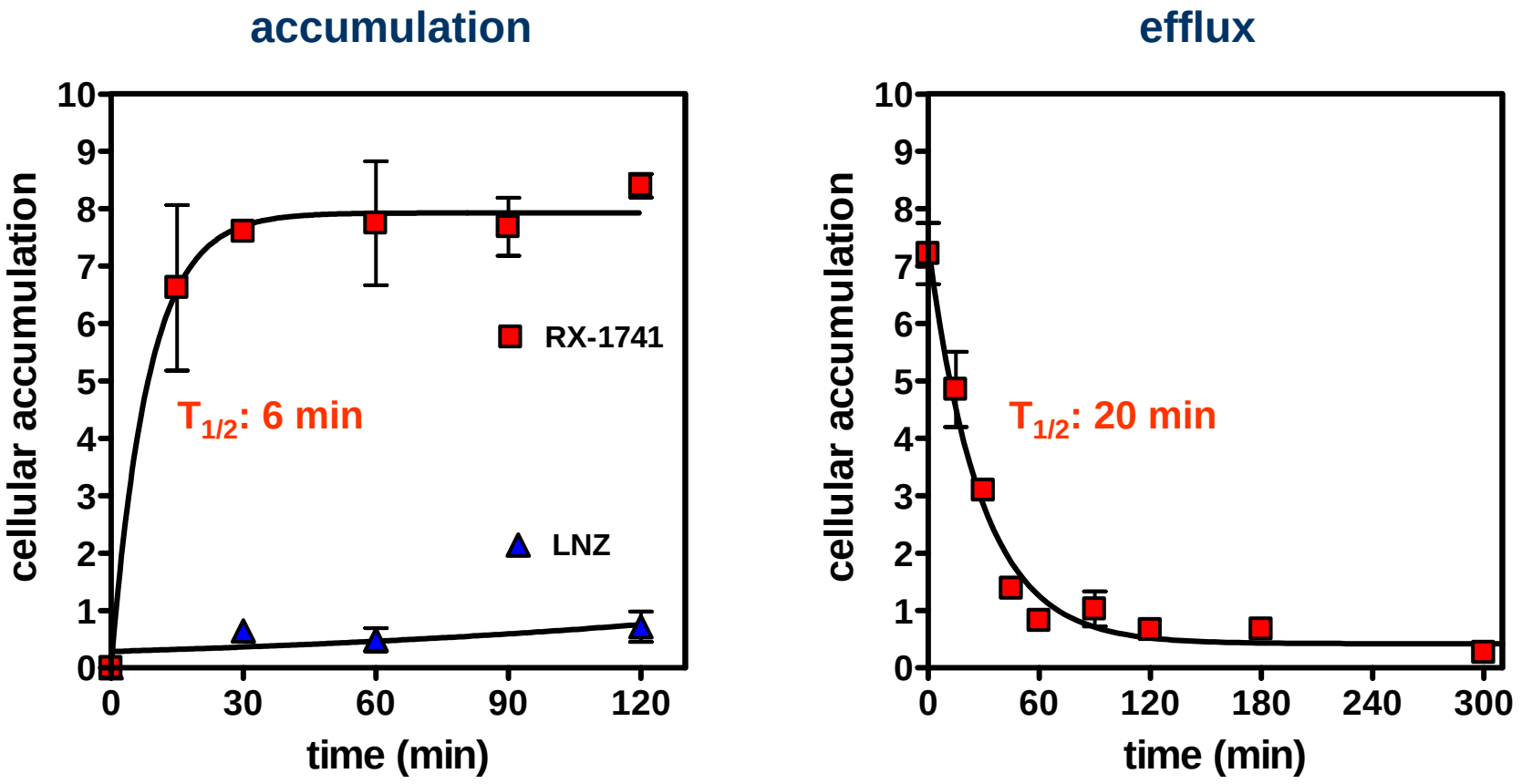
accumulation calculated considering a cell volume of 5 µl/mg prot.

Comparative accumulation level at equilibrium



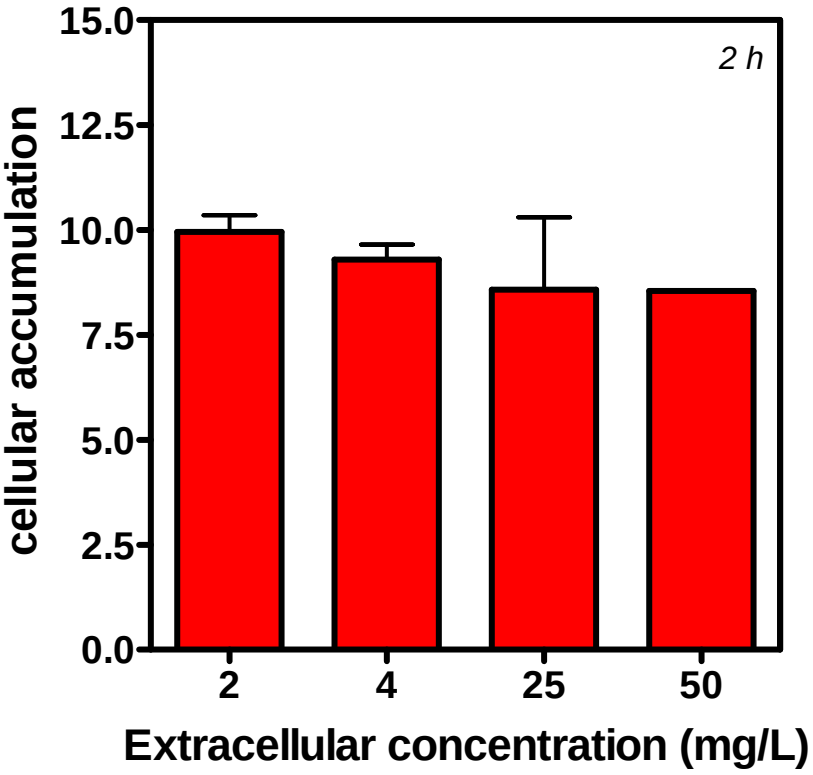
in contrast to linezolid, radezolid accumulates in eucaryotic cells !

Kinetics of accumulation and efflux



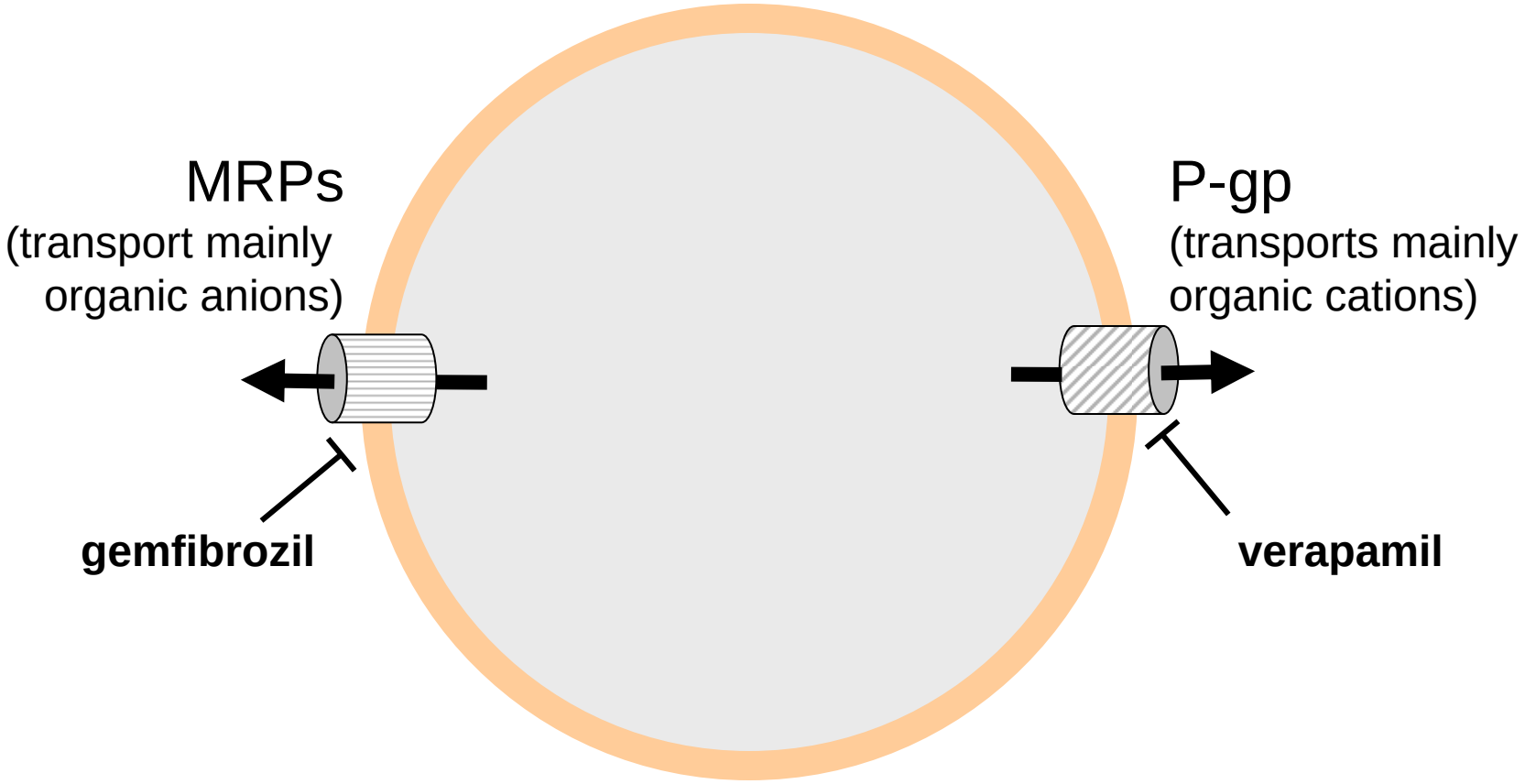
rapid accumulation; slower efflux

Concentration - effect

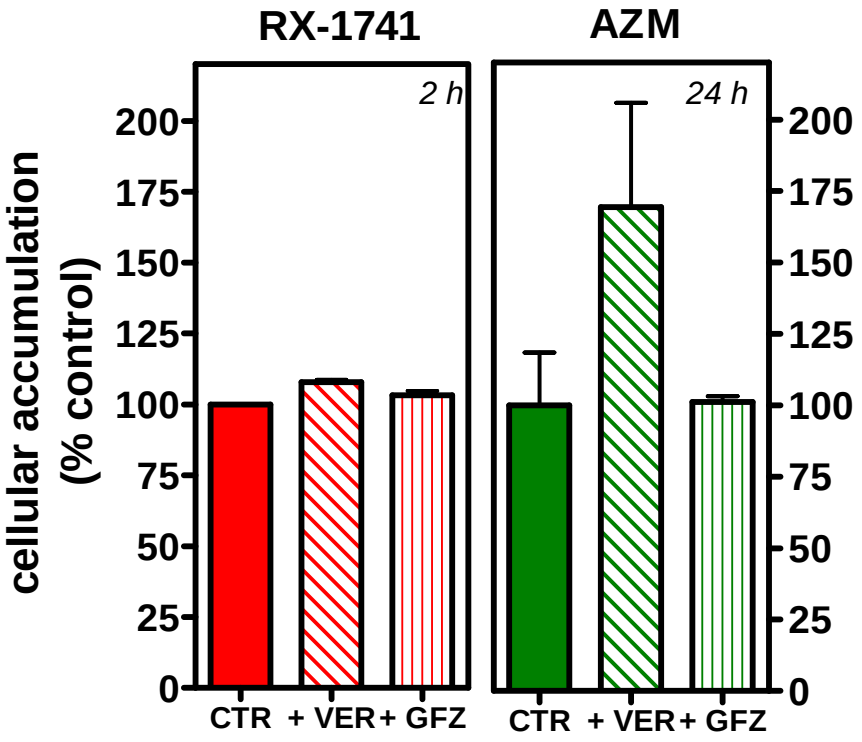


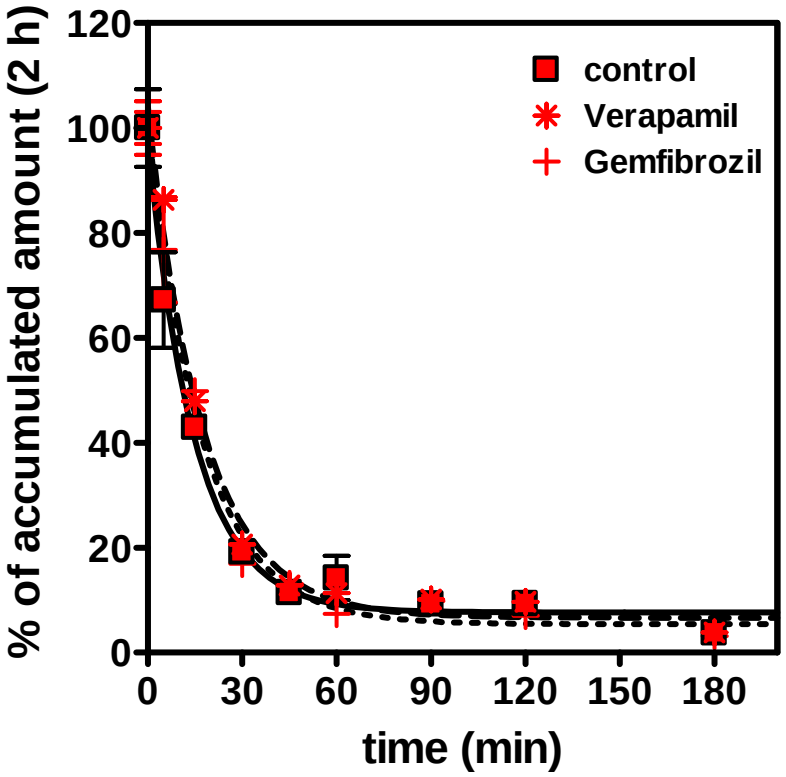
non-saturable accumulation

Role of multidrug transporters



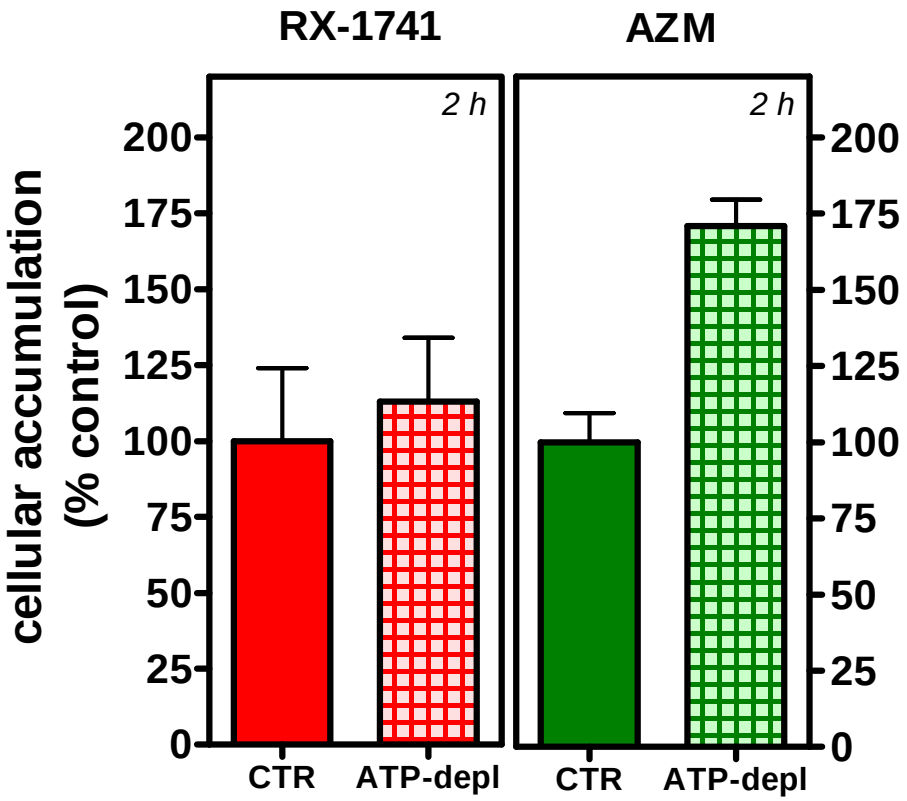
Influence of efflux pumps inhibitors





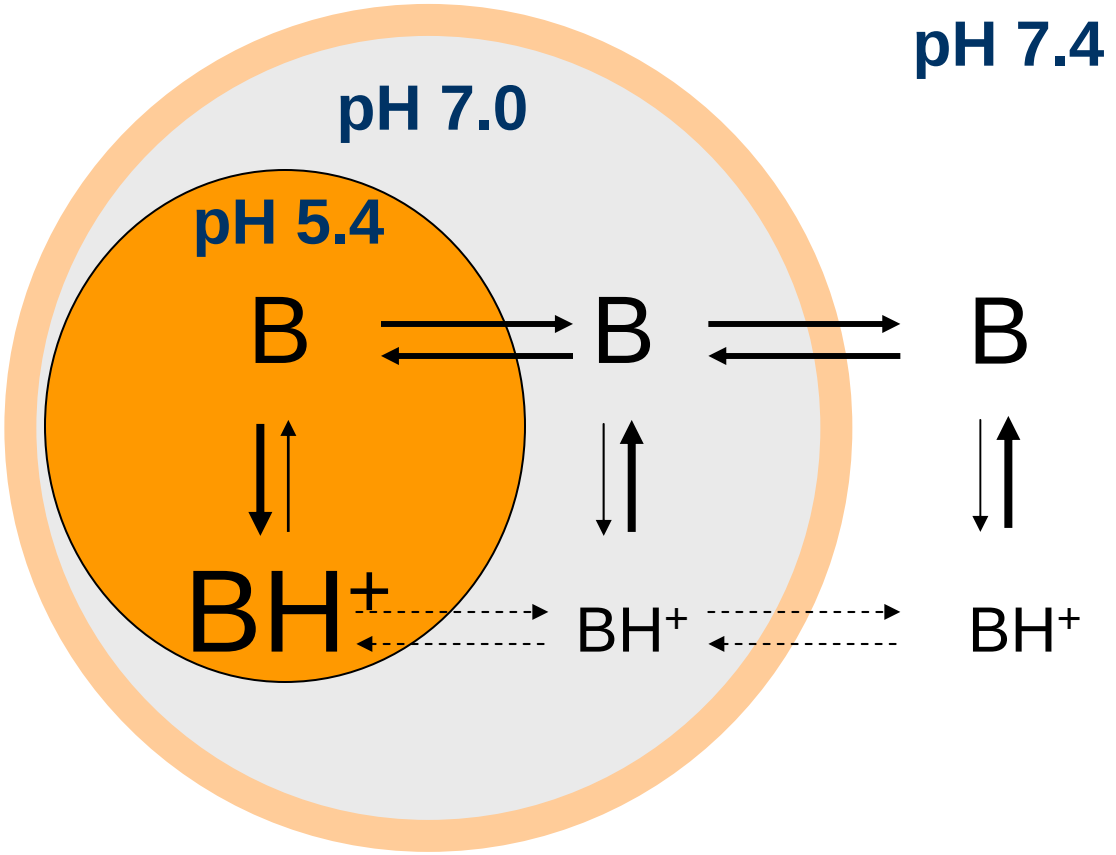
no influence of efflux pump inhibitors
on radezolid accumulation/efflux

Influence of ATP-depletion



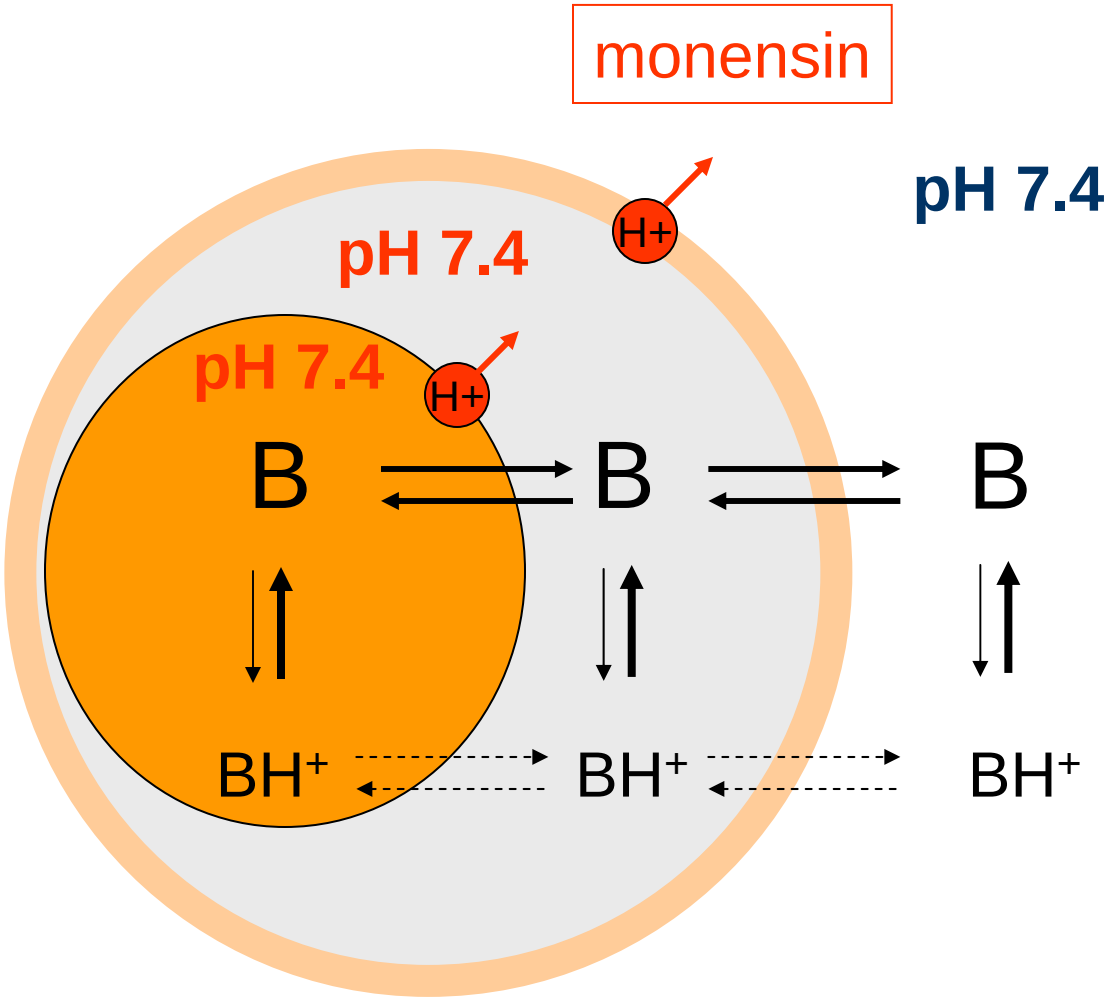
no implication of energy-dependent processes in the accumulation of radezolid

Importance of pH gradients

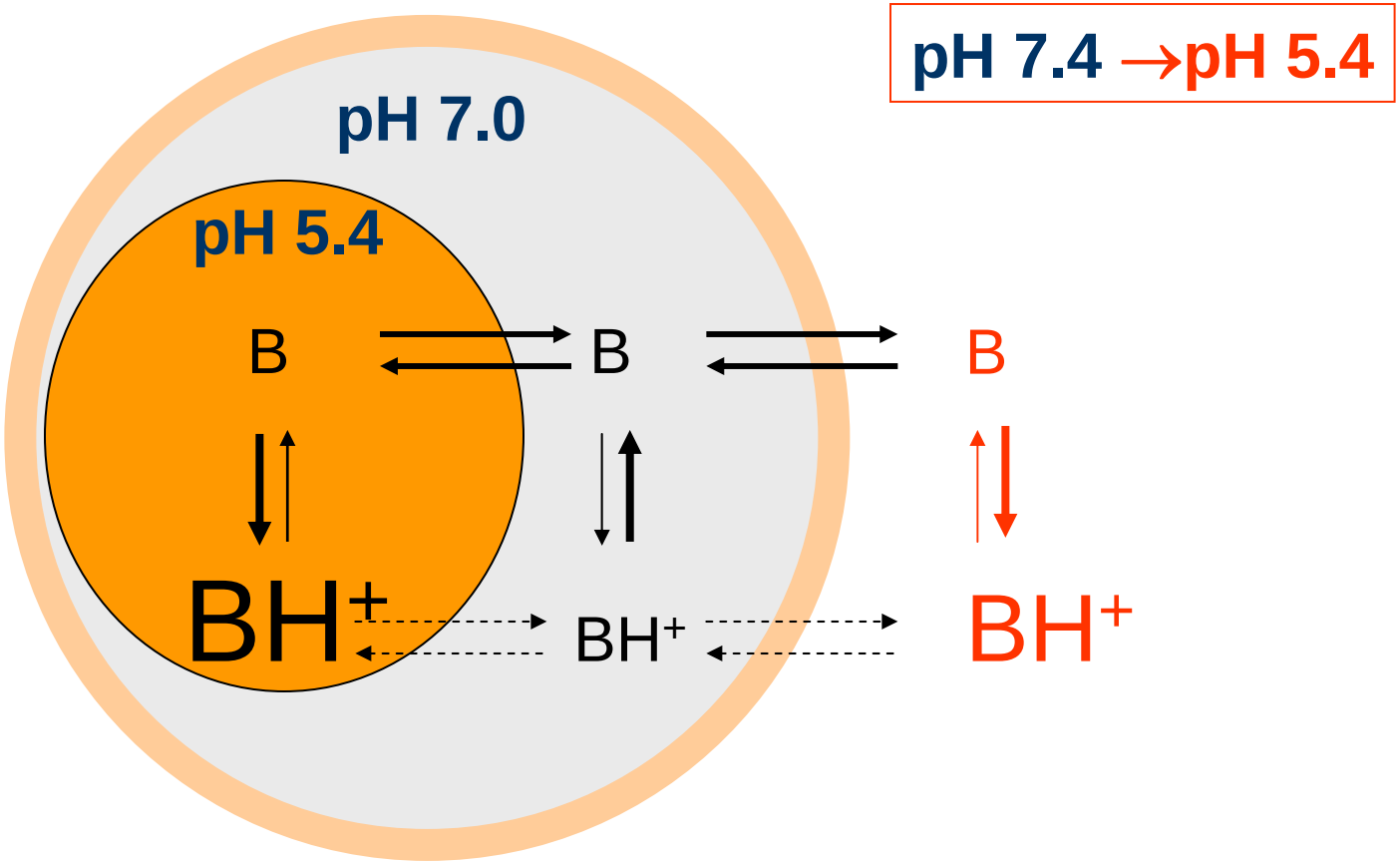


De Duve. Biochem Pharmacol. (1974) 23:2495-531

How to collapse pH gradients ?

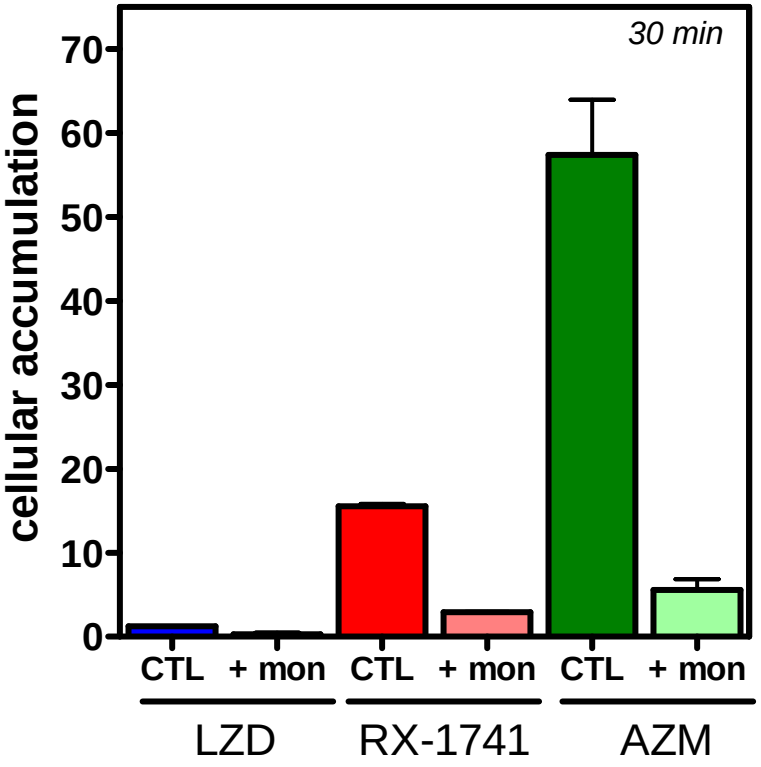


How to collapse pH gradients ?

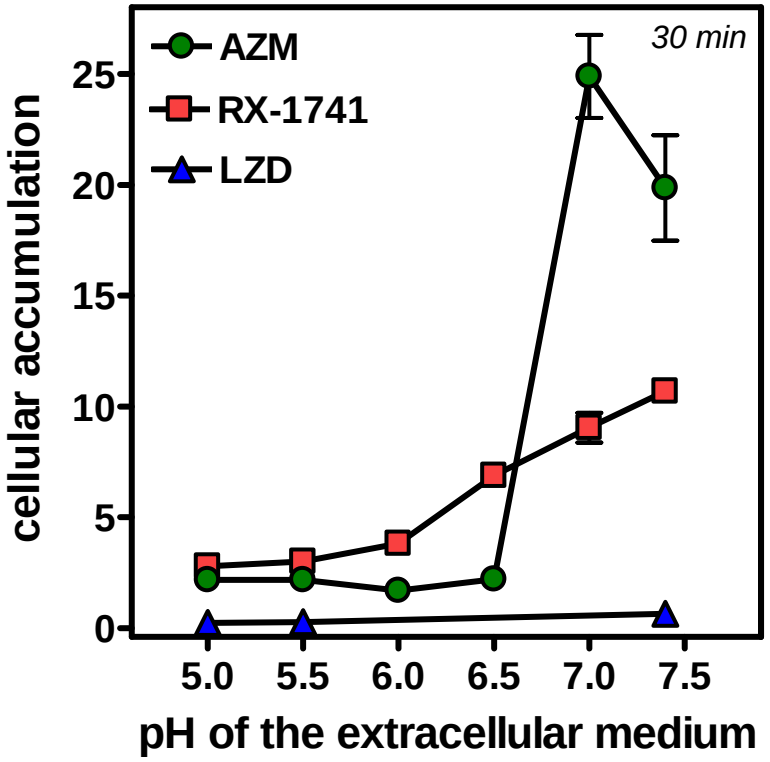


Influence of pH gradients

influence of monensin on the accumulation of LZD, RX-1741, AZM

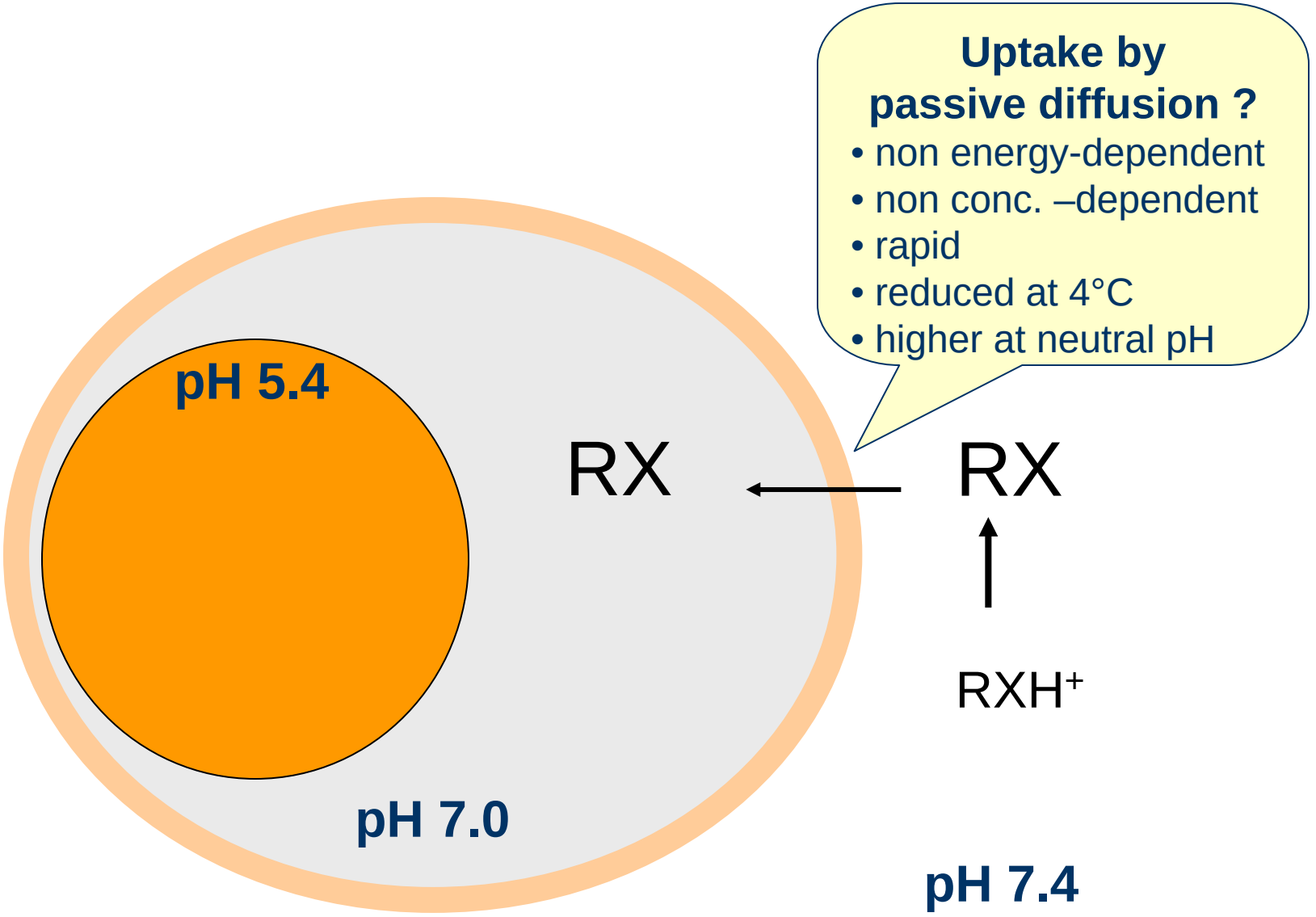


accumulation of RX-1741 in culture media at different pH



radezolid accumulation reduced by 60-80 % when collapsing pH gradients

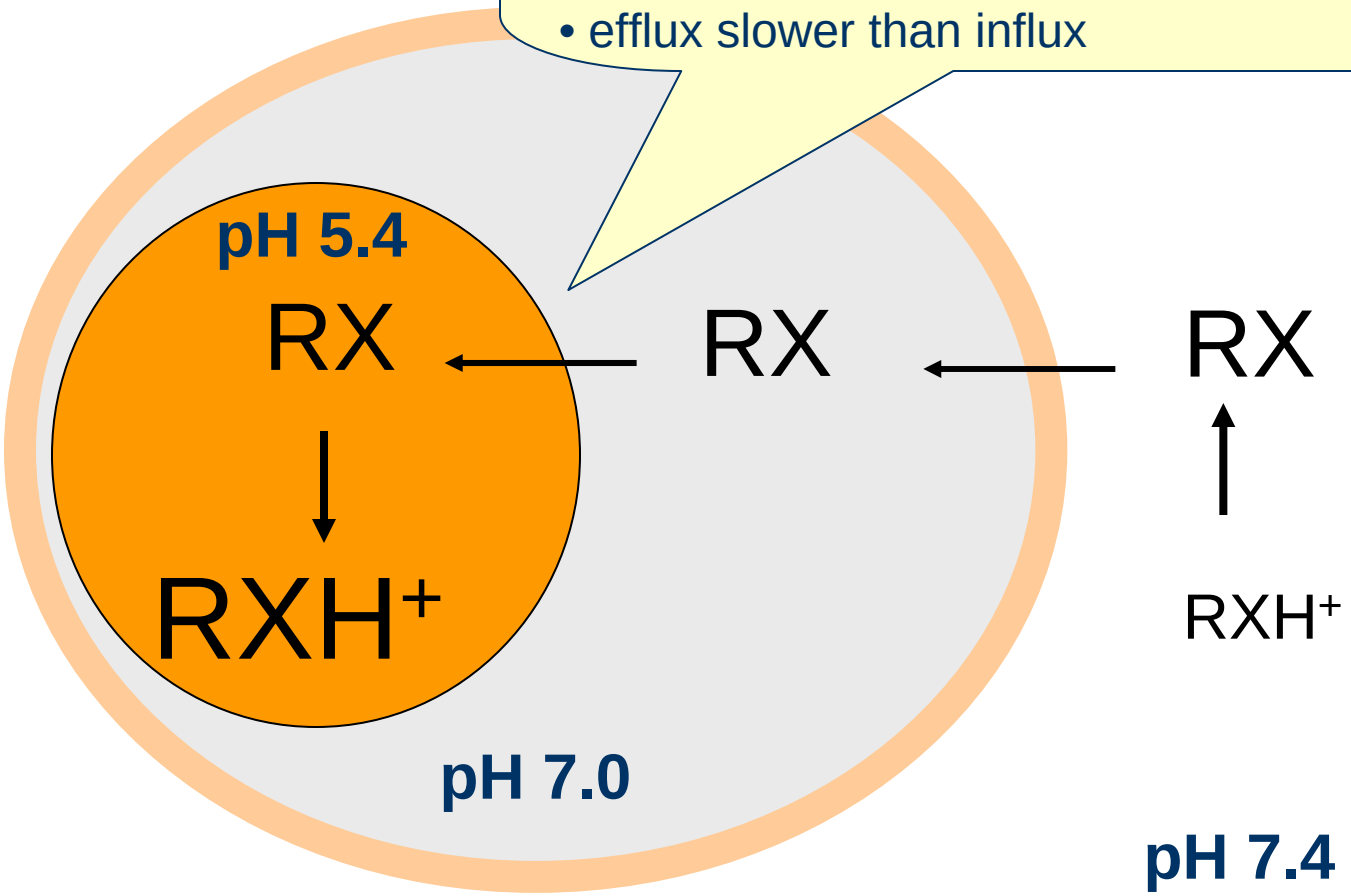
Current model for cellular accumulation of radezolid



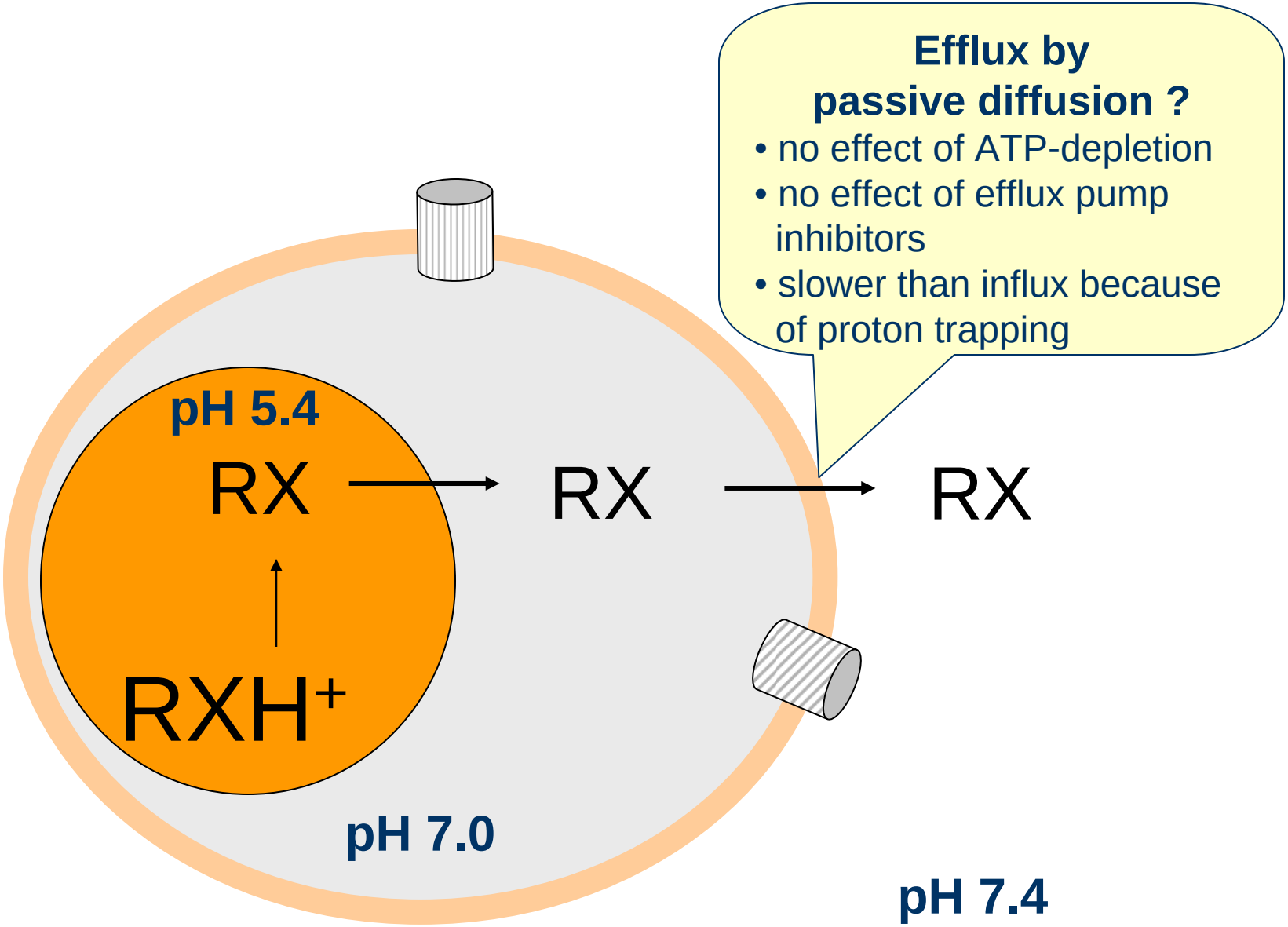
Current model for cellular accumulation of radezolid

Accumulation by segregation - proton trapping in acidic compartments ?

- inhibited by monensin
- reduced in acidic medium
- efflux slower than influx



Current model for cellular accumulation of radezolid



Potential interest of cell accumulation

- **Pharmacokinetics ?**
  - ⇒ larger volume of distribution and tissue penetration
  - ⇒ drug concentration at the site of infection

drug	Vd (mL/kg)		
	mouse	rat	dog
Linezolid	450	720	630
Radezolid	824	1239	975

RibX, data on file

- **Pharmacodynamics ?**
  - ⇒ activity on intracellular bacteria

Sibelius monument, Helsinki