

Competition between **Ciprofloxacin** and Antiviral Agents (**Adefovir**, **Saquinavir**, **Ritonavir**) for Efflux Transporters in J774 Macrophages

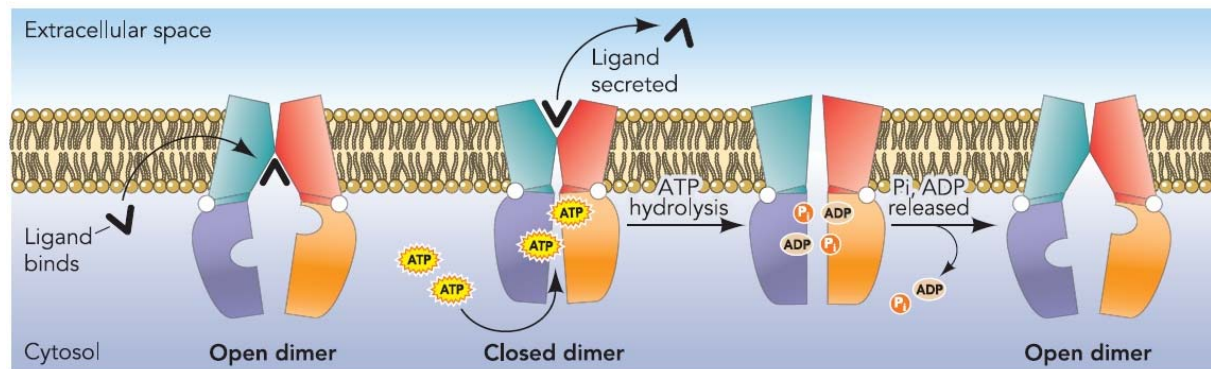
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<www.facm.ucl.ac.be>



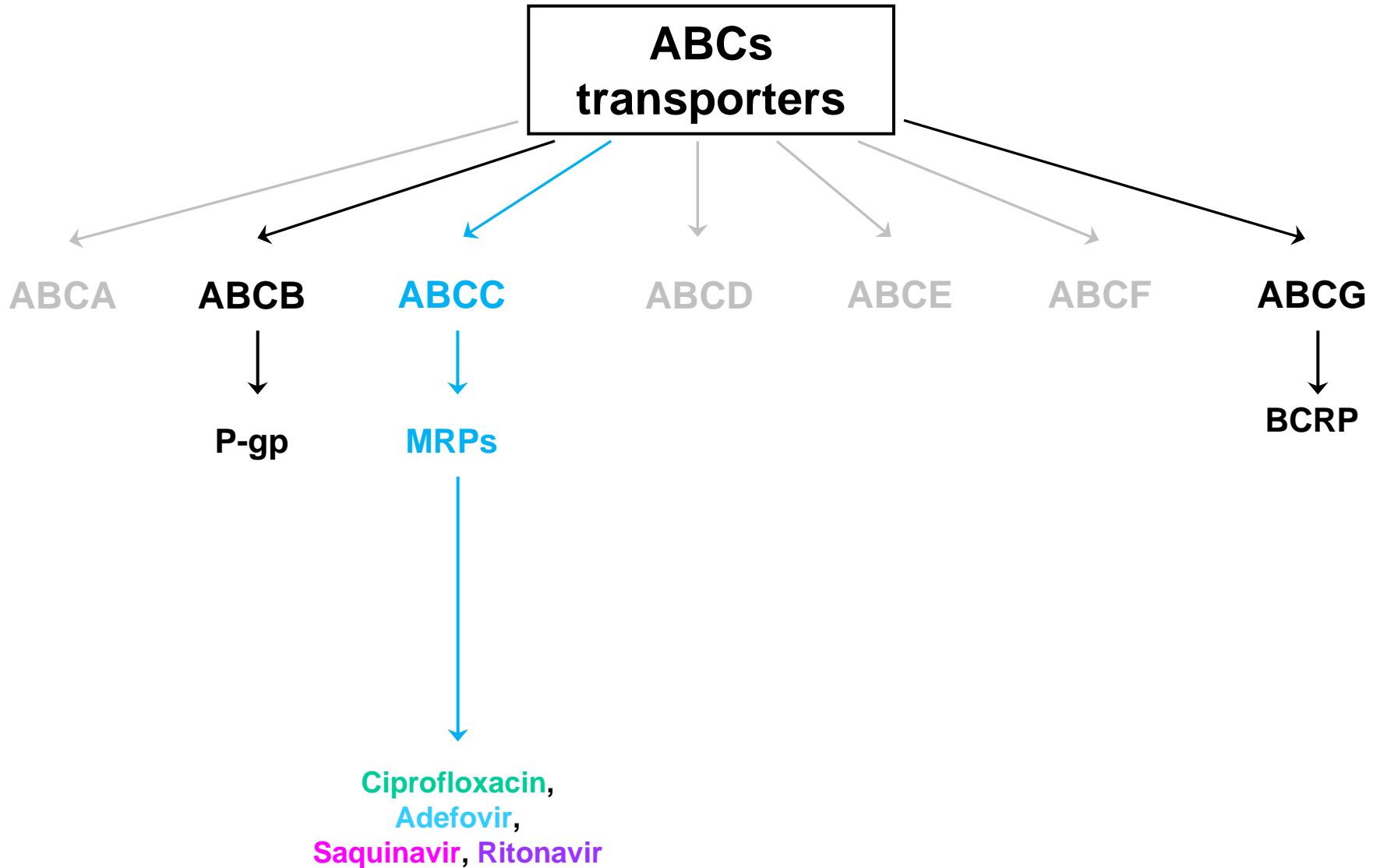
Active Efflux

Active efflux is an ubiquitous process which protects cells against foreign substances

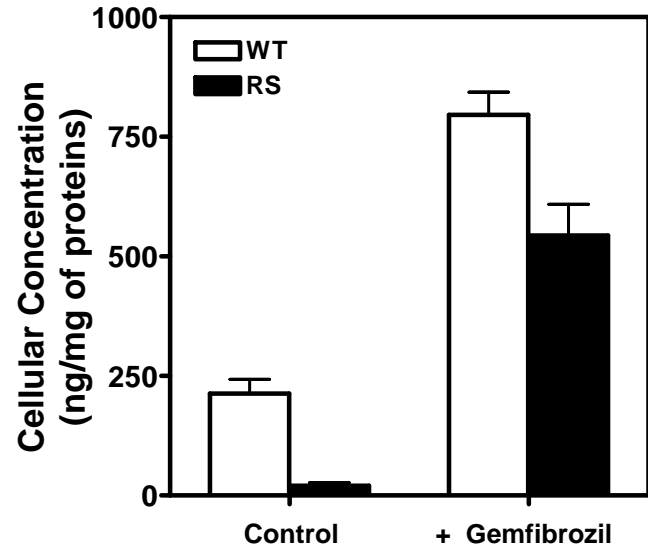
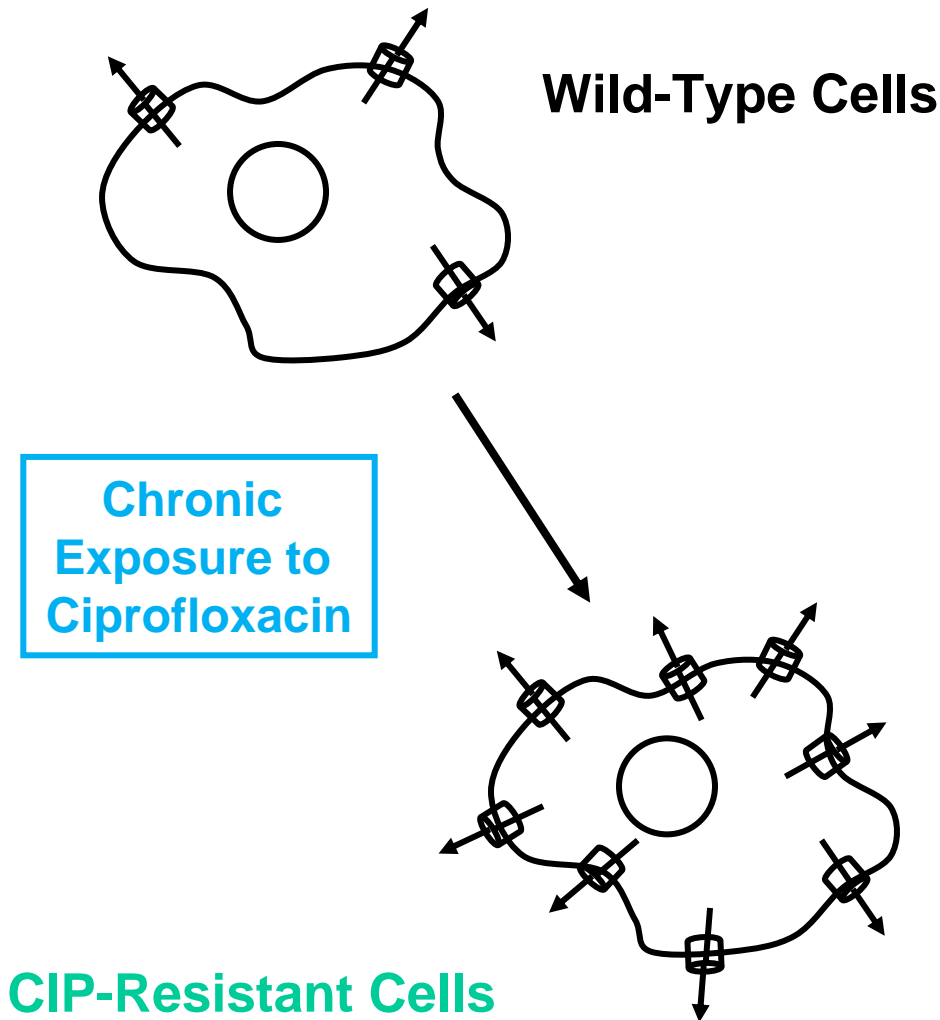


↘ Cellular Concentration
↘ Pharmacological Activity

ATP-Binding Cassette transporters



Identification of **Ciprofloxacin** transporter



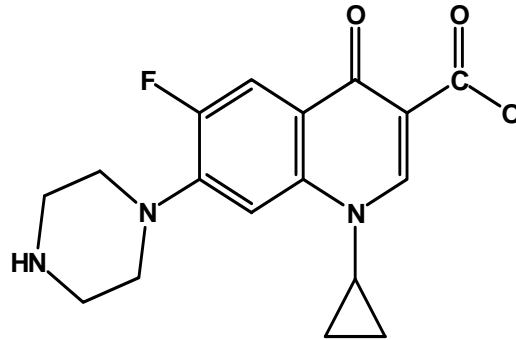
The CIPRO-resistant cells overexpress Mrp2 and Mrp4, but Mrp4 predominates

Michot et al. AAC (2006) 50:1689-1695
Marquez et al. AAC (2009) [Epub ahead of print]

Aim of our study

The aim of our work is to examine, in wild-type and Ciprofloxacin-resistant macrophages, the potential competition between Ciprofloxacin and preferential substrates of Mrp2 and Mrp4 in order to better identify and further characterize the Fluoroquinolone transporter.

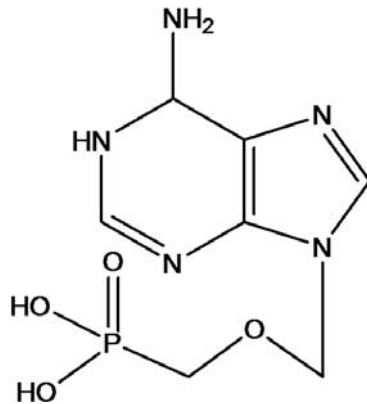
CIPROFLOXACIN



Adefovir and MRP4

PMEA

9-(2-PhosphonylMethoxyEthyl)Adenine



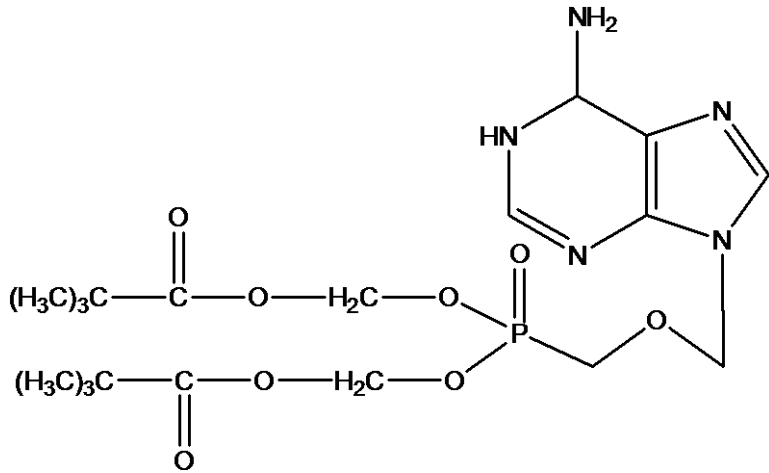
Reid et al. *Mol Pharmacol.* 2003 63:1094-103

Imaoka et al. *Mol Pharmacol.* 2007 71:619-27.

Saquinavir and MRP2

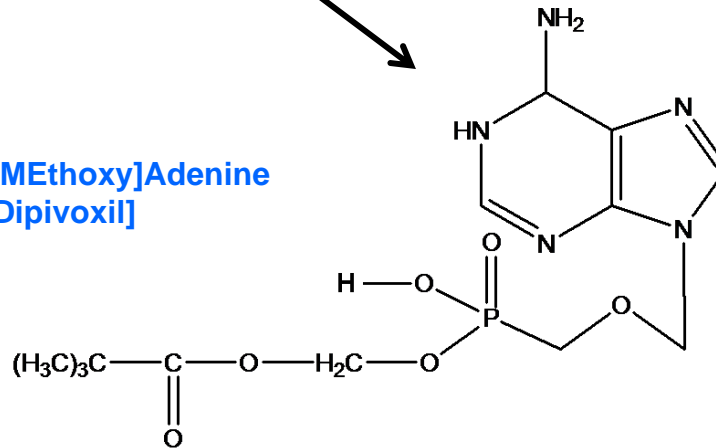
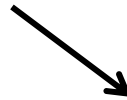
Ritonavir and MRP2

Hydrolysis of Bis(POM)PMEA

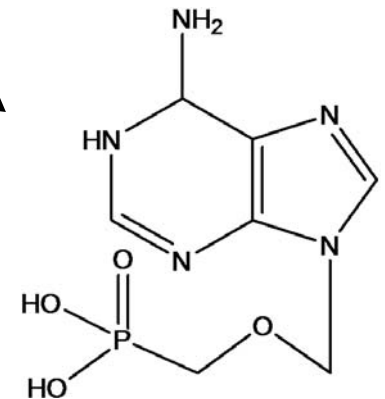


Bis(POM)PMEA

9-[2-(BisPivaloyloxyMethyl)PhosphoMethoxy]Adenine
[Bis(POM)PMEA or Adefovir Dipivoxil]



Mono(POM)PMEA

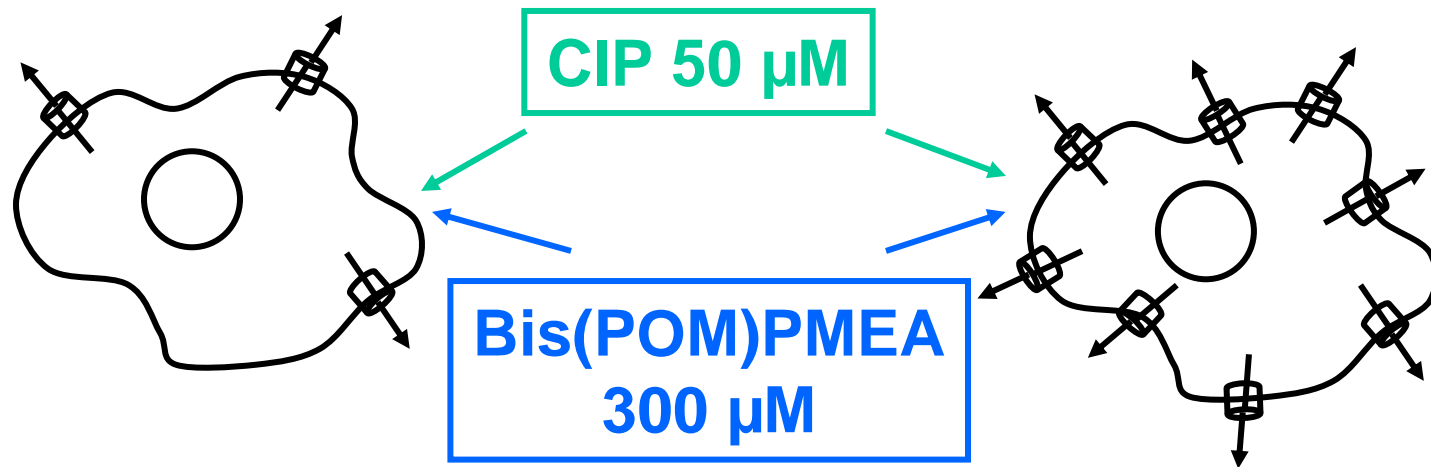


PMEa

Competition between CIP and Bis(POM)PMEA

Wild-Type Cells

CIP-Resistant Cells



Measure of cellular content in CIP

Fluorimetry: λ excitation = 275 nm; λ emission = 450 nm

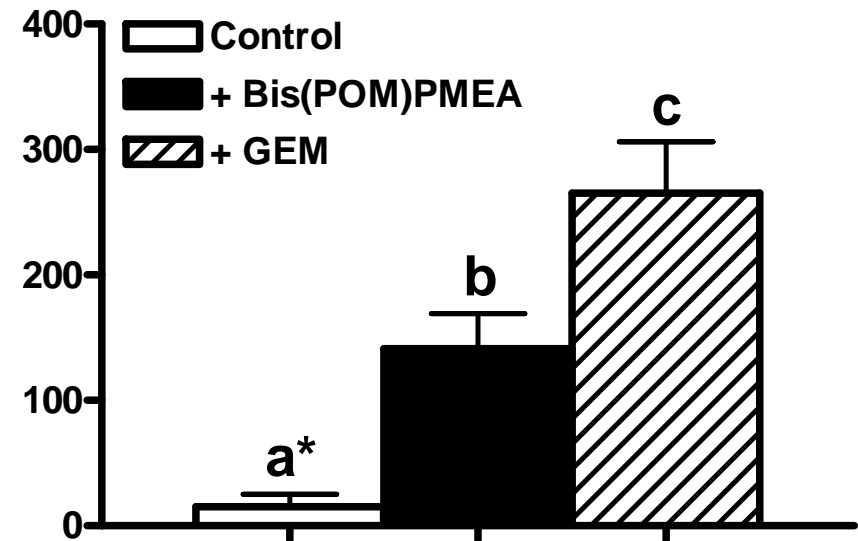
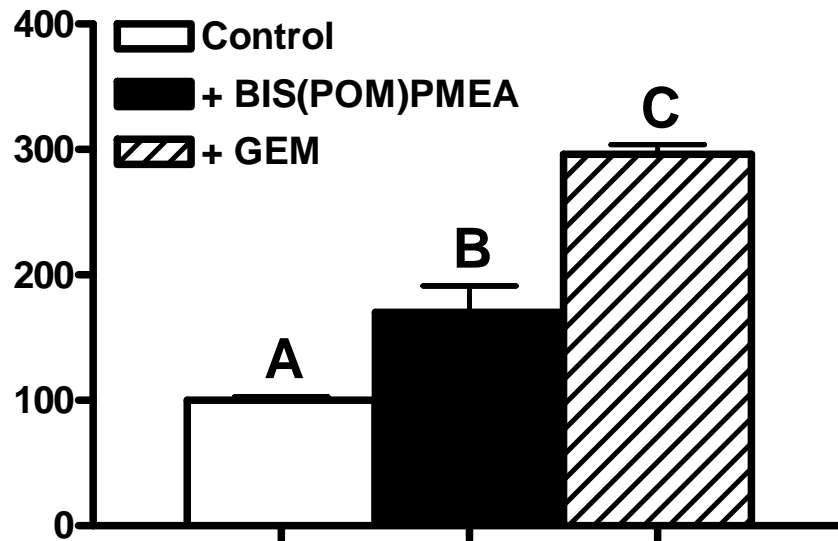
Competition between CIP and Bis(POM)PMEA

Accumulation of Ciprofloxacin

Wild-Type Cells

CIP-Resistant Cells

Extracellular Concentration of CIPRO
(% of the control in WT)

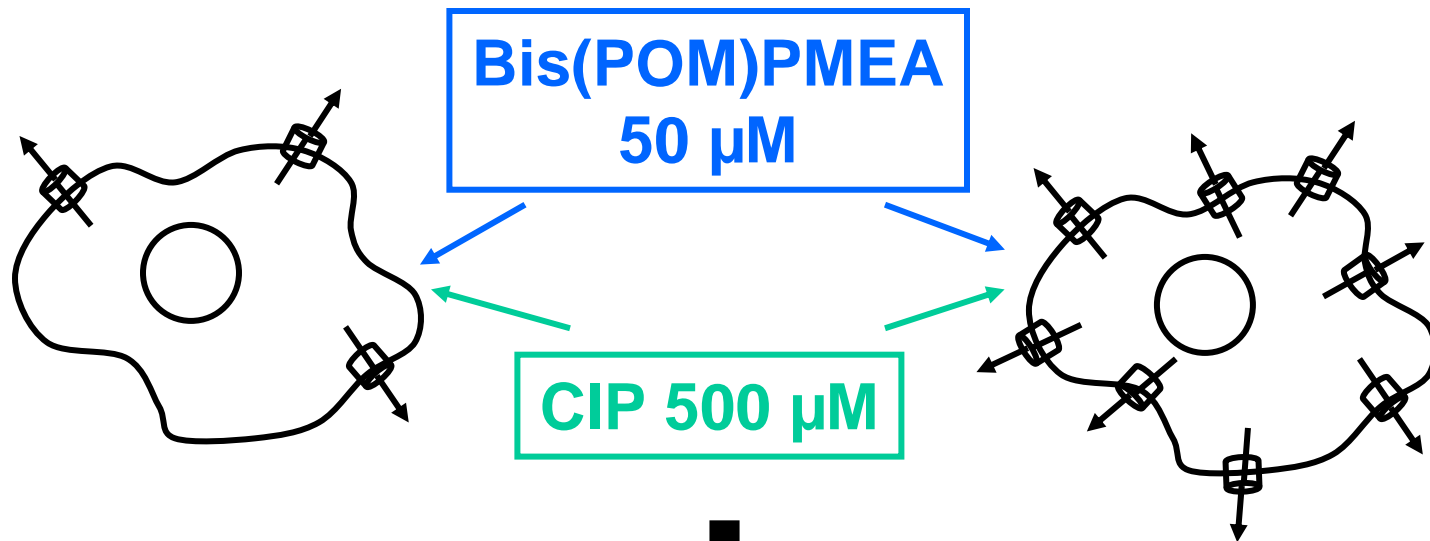


The accumulation of CIP is increased in the presence of Gemfibrozil or Bis(POM)PMEA in both cell types

Competition between Bis(POM)PMEA and CIP

Wild-Type Cells

CIP-Resistant Cells



Measure of cellular content in **PMEA**

HPLC: 4°C, flow rate 1 mL/min, volume injected 25 μL.

Stationary phase: column Agilent C8 (4,6x250mm)

Mobile phase A : Acetonitrile

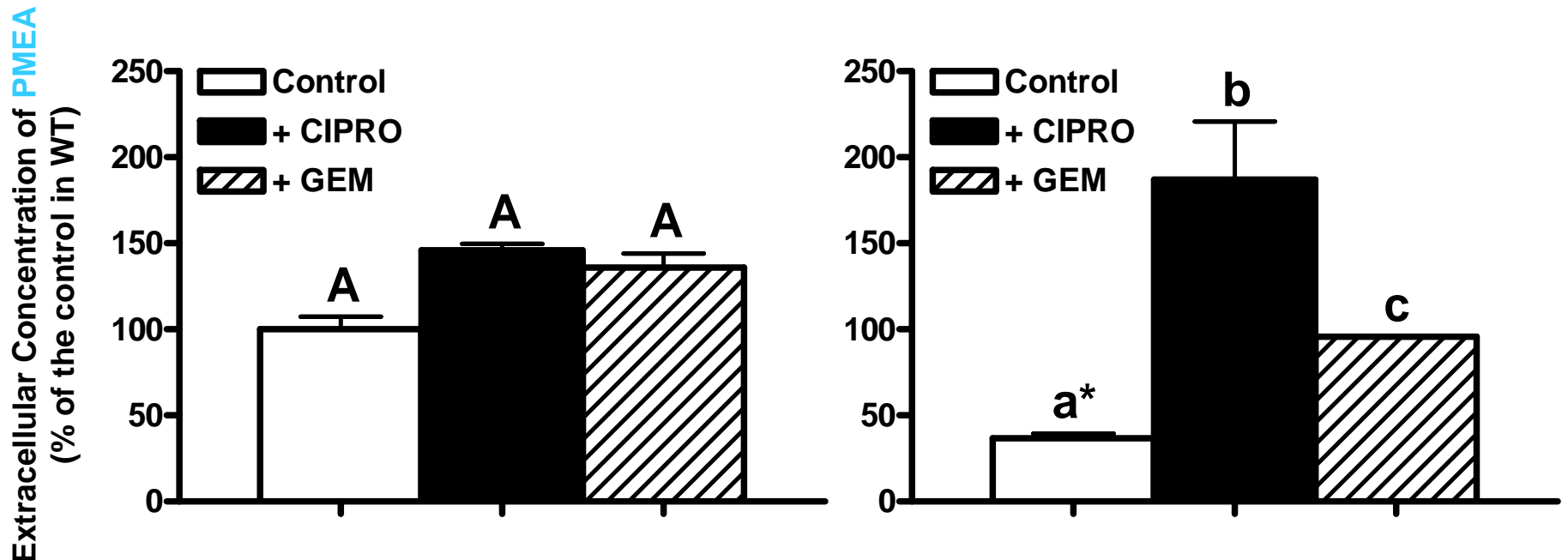
Mobile phase B : a mixture of 900 mL of buffer [10 mM KH_2PO_4 et 2 mM $(\text{But})_4\text{N}^+ \text{HSO}_4^-$] and 50 mL Acetonitrile.

Competition between Bis(POM)PMEA and CIP

Accumulation of PMEA

Wild-Type Cells

CIP-Resistant Cells



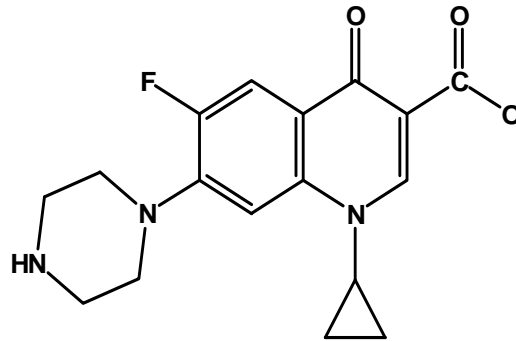
The accumulation of PMEA is increased in the presence of Gemfibrozil or CIP, especially in resistant cells

Conclusions

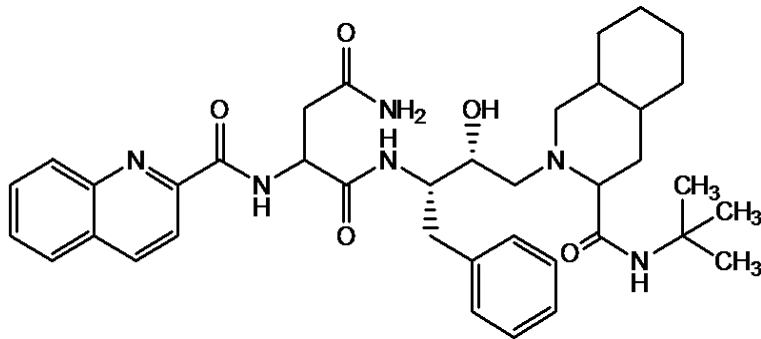
- x The accumulation of **CIP**
 - ↘ in CIPRO-resistant cells
 - ↗ in the presence of **Bis(POM)PMEA**
- x The accumulation of **PMEA**
 - ↘ in CIPRO-resistant cells
 - ↗ in the presence of **CIP**

➔ **PMEA** is substrate and inhibitor of the **CIP** transporter

CIPROFLOXACIN

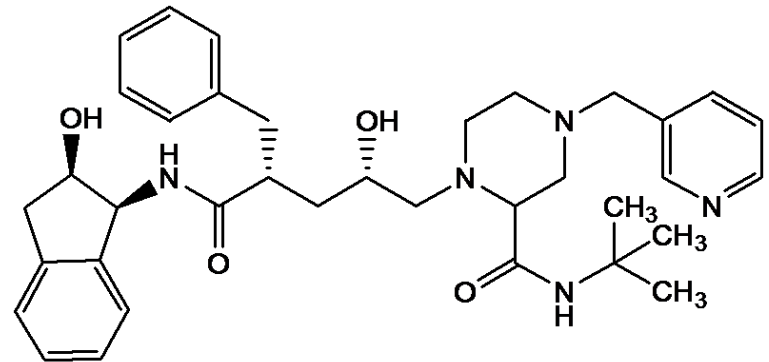


Saquinavir and MRP2



Huisman et al. *AIDS*. 2002 16:2295-301.
Williams et al. *AAC*. 2002 46:3456-62.

Ritonavir and MRP2

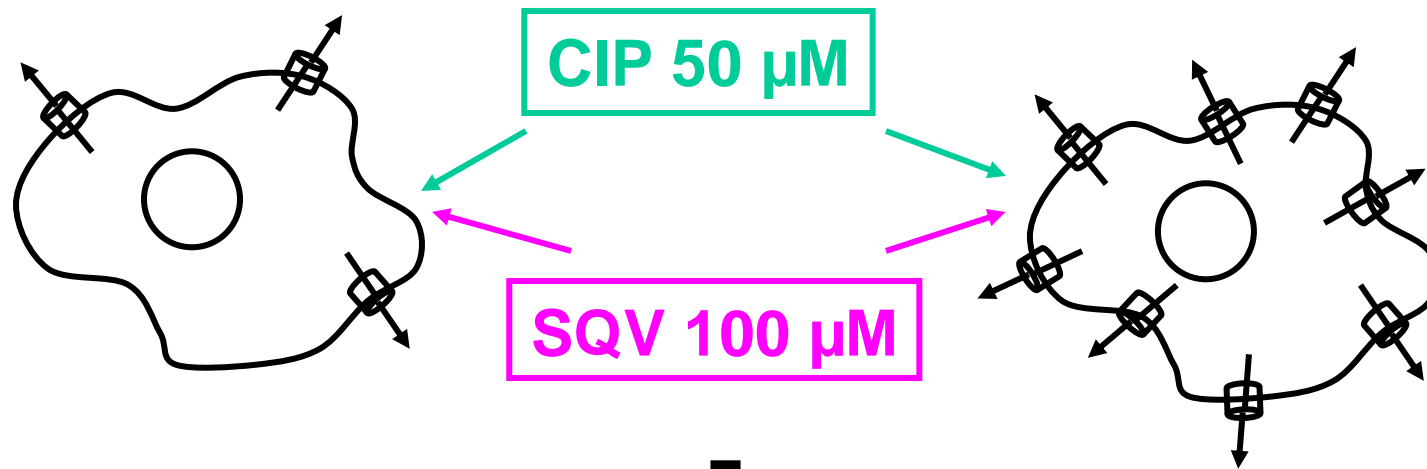


Huisman et al. *AIDS*. 2002 16:2295-301.
Williams et al. *AAC*. 2002 46:3456-62.

Competition between CIP and SQV

Wild-Type Cells

CIP-Resistant Cells



Measure of cellular content in CIP

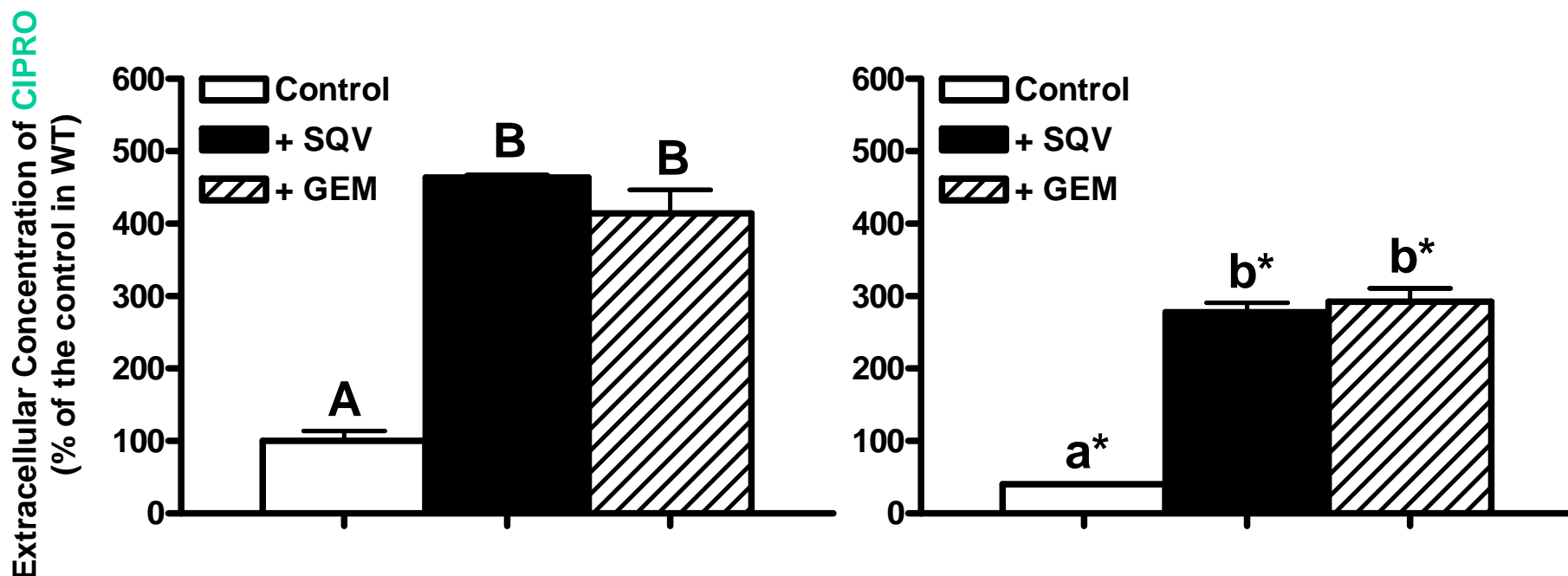
Fluorimetry: λ excitation = 275 nm; λ emission = 450 nm

Competition between CIP and SQV

Accumulation of Ciprofloxacin

Wild-Type Cells

CIP-Resistant Cells

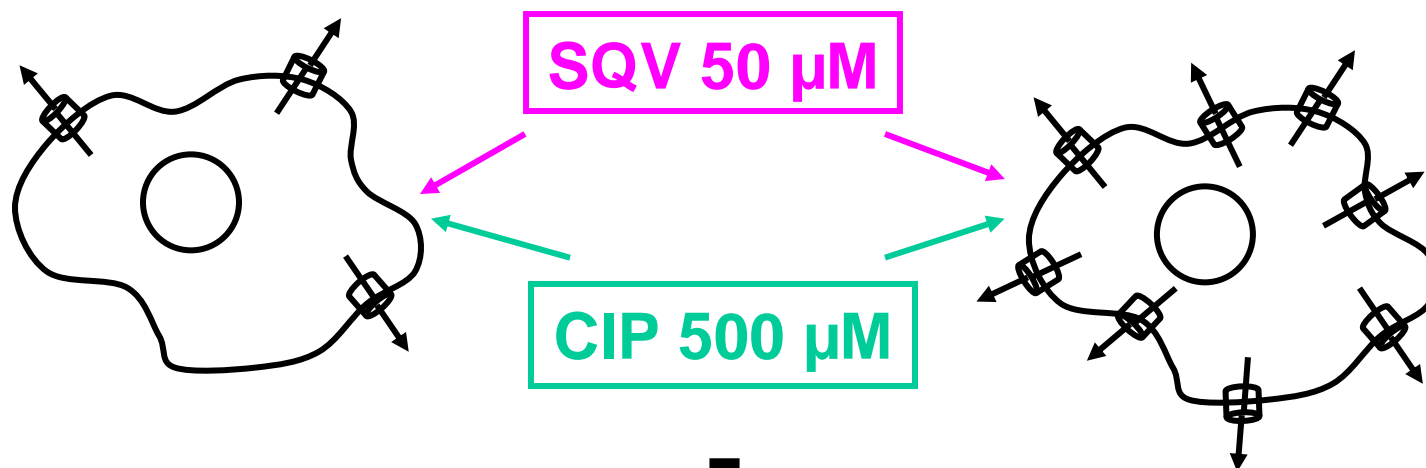


The accumulation of CIP is increased in the presence of Gemfibrozil or SQV in both cell types

Competition between SQV and CIP

Wild-Type Cells

CIP-Resistant Cells



Measure of cellular content in SQV

HPLC: 25°C, gradient isocratique, flow rate 1 mL/min.

Stationary phase : column Agilent C8 (4,6x250mm)

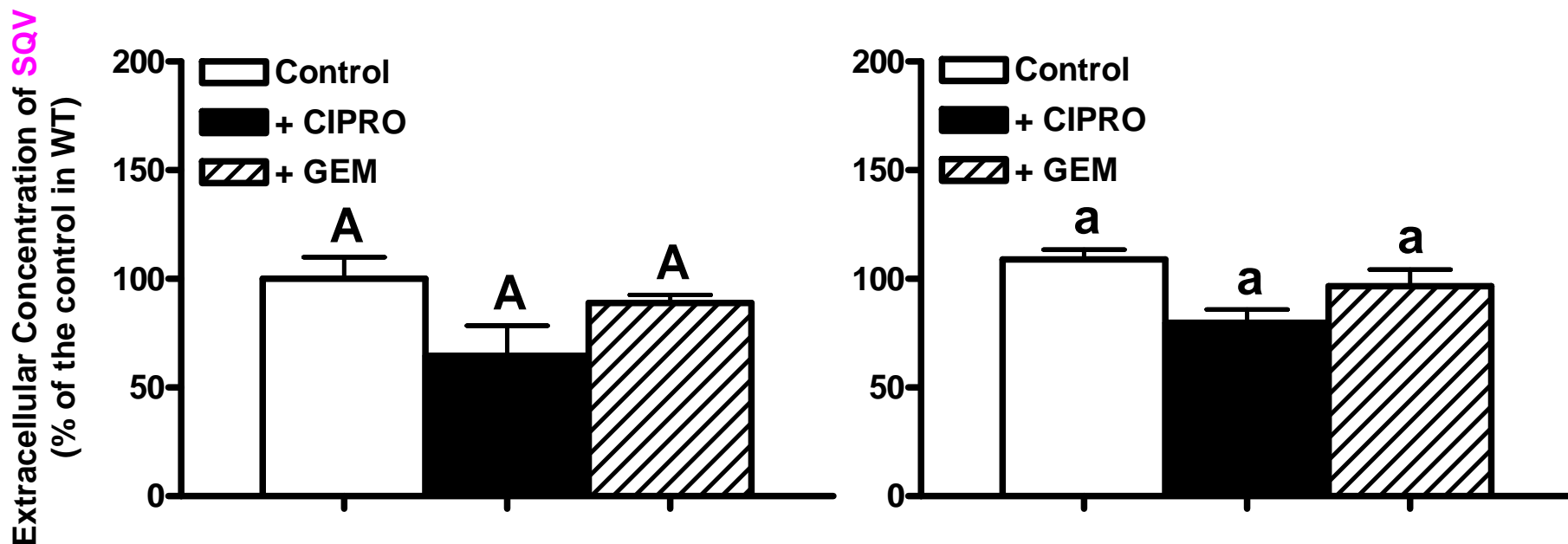
Mobile phase : 45% Acetonitrile / 55% of buffer [3,042 g KH_2PO_4 and 0,6 mL Triethylamine /L, adjusted to pH 3,1]

Competition between SQV and CIP

Accumulation of Saquinavir

Wild-Type Cells

CIP-Resistant Cells



The accumulation of SQV is not affected by CIP or by Gemfibrozil in the wild-type and resistant cells

Conclusions

- × The accumulation of **CIP**
 - ↘ in CIPRO-resistant cells
 - ↗ in the presence of **SQV**
- × The accumulation of **SQV** is unchanged in CIPRO-resistant cells or in the presence of **CIP**

➔ **SQV** is a potent inhibitor (but NOT substrate) of the **CIP** transporter

- × NO effect of **RTV** on the **CIP** accumulation
- × NO effect of **CIP** on the **RTV** accumulation

➔ **RTV** is neither an inhibitor nor a substrate of the **CIP** transporter

General Conclusions

➔ **PMEA** is substrate and inhibitor of the **CIP** transporter

➔ **SQV** is a potent inhibitor (but NOT substrate) of the **CIP** transporter

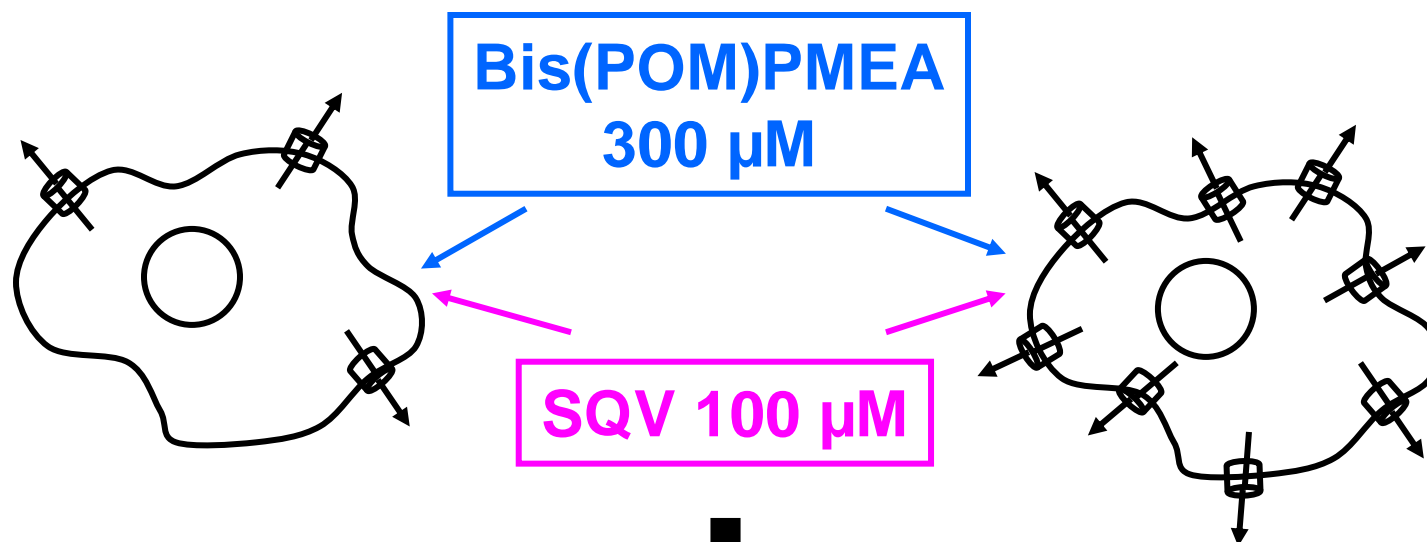
➔ Do **PMEA** and **SQV** act upon the same transporter?

➔ Is there a competition between **PMEA** and **SQV**, as inhibitor?

Competition between Bis(POM)PMEA and SQV

Wild-Type Cells

CIP-Resistant Cells



Measure of cellular content in **PMEA**

HPLC: 4°C, flow rate 1 mL/min, volume injected 25 μL.

Stationary phase: column Agilent C8 (4,6x250mm)

Mobile phase A : Acetonitrile

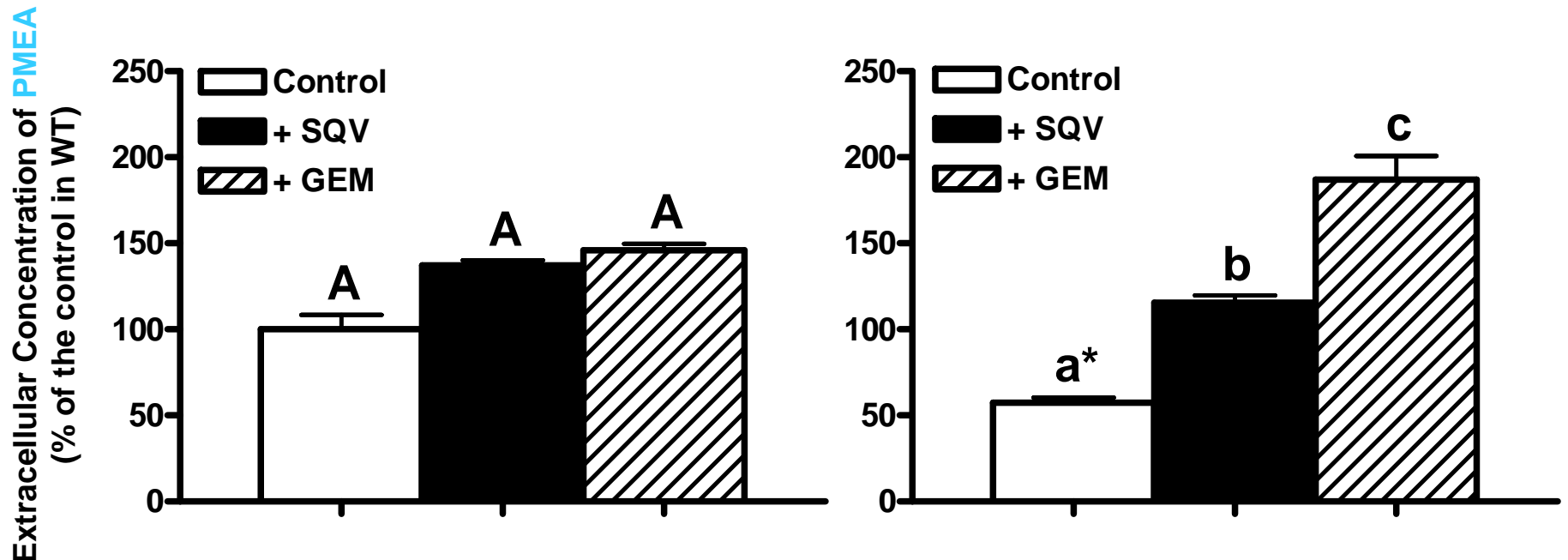
Mobile phase B : a mixture of 900 mL of buffer [10 mM KH_2PO_4 et 2 mM $(\text{But})_4\text{N}^+ \text{HSO}_4^-$] and 50 mL Acetonitrile.

Competition between Bis(POM)PMEA and SQV

Accumulation of PMEAs

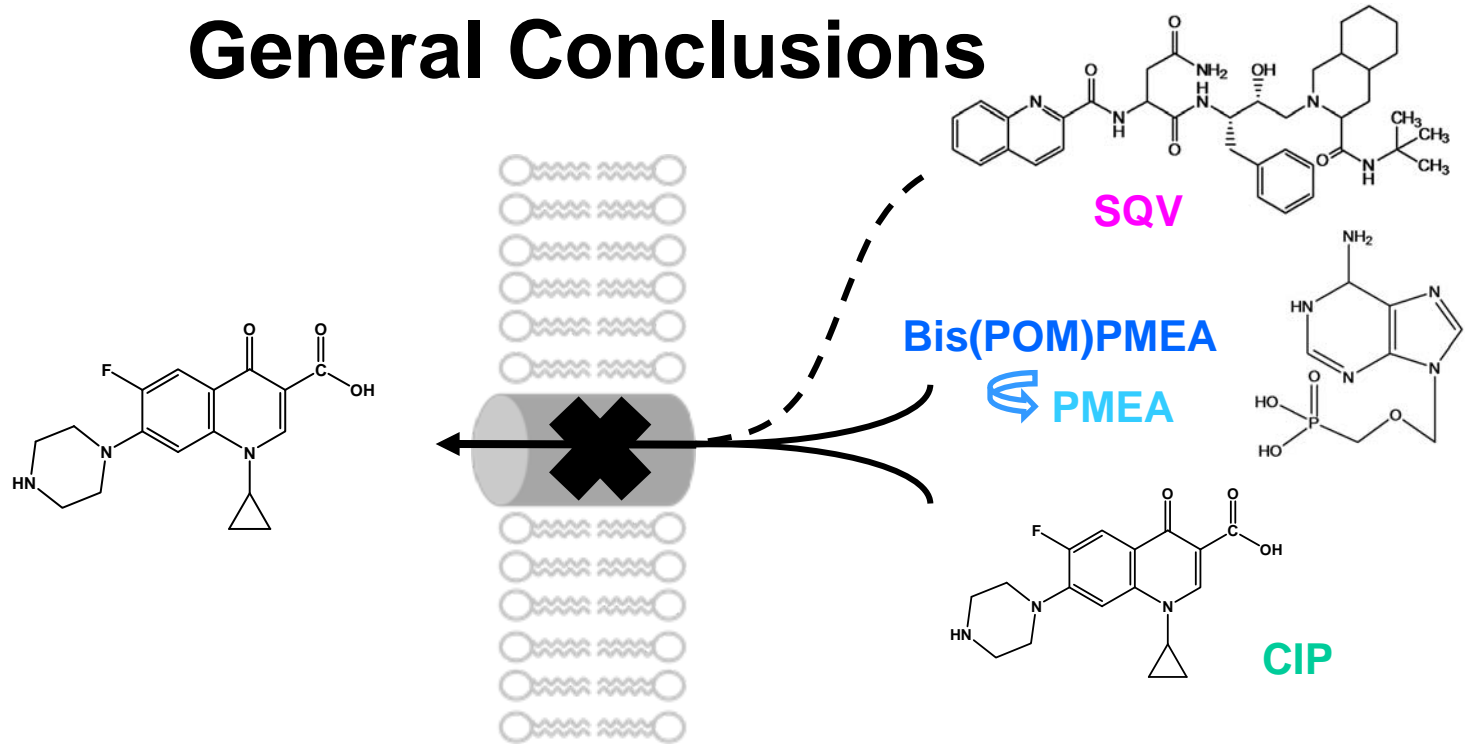
Wild-Type Cells

CIP-Resistant Cells



The accumulation of PMEAs is increased in the presence of SQV, especially in resistant cells

General Conclusions



- ➔ There is a competition between **Ciprofloxacin** and **Antiviral Agents**
- ➔ This competition leads to a **cross-modulation** of their cellular concentration
- ➔ This may alter their activity on intracellular bacteria or target viruses