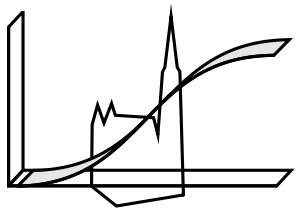
MEDICAL UNIVERSITY OF VIENNA

Department of

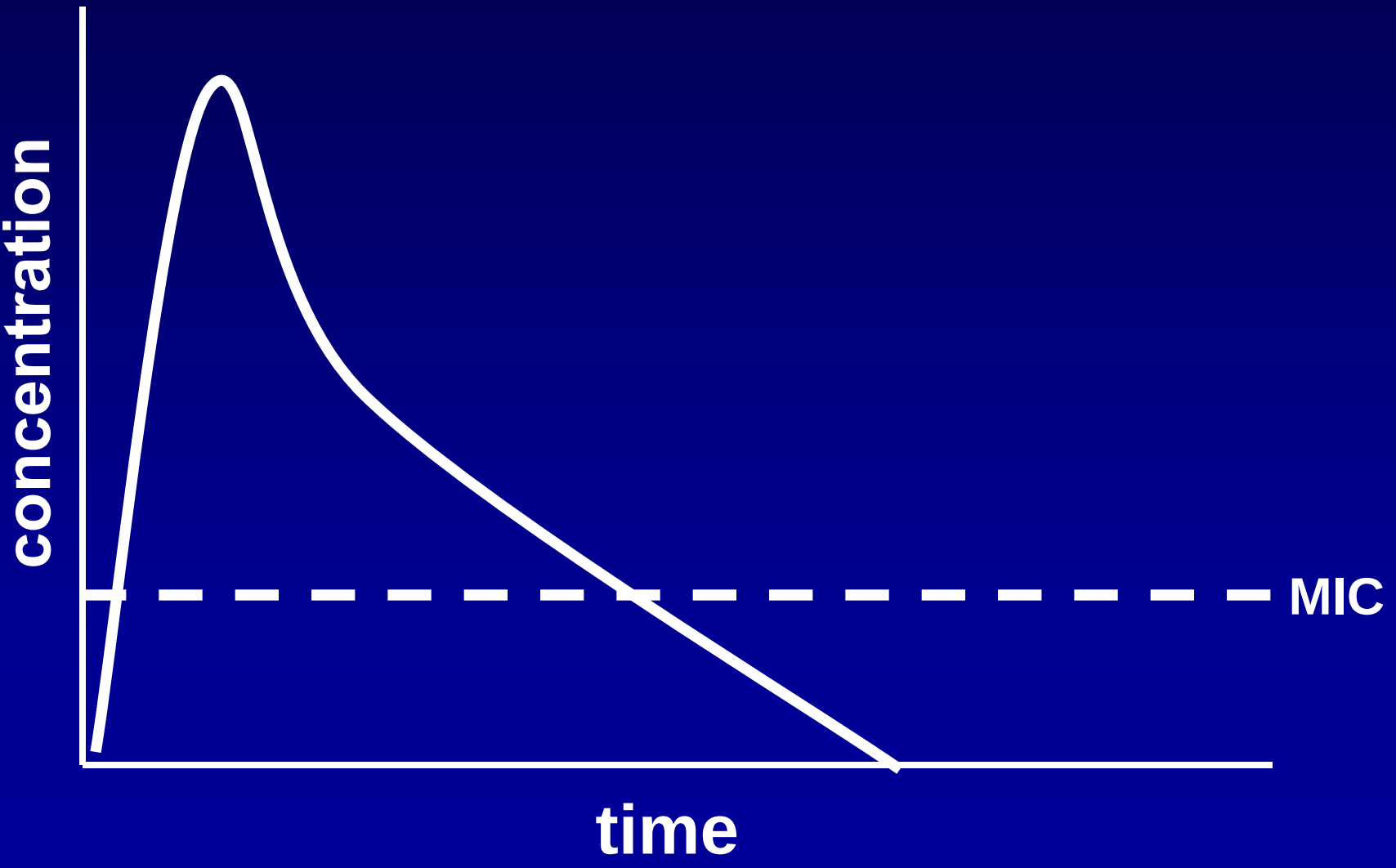
Clinical Pharmacology



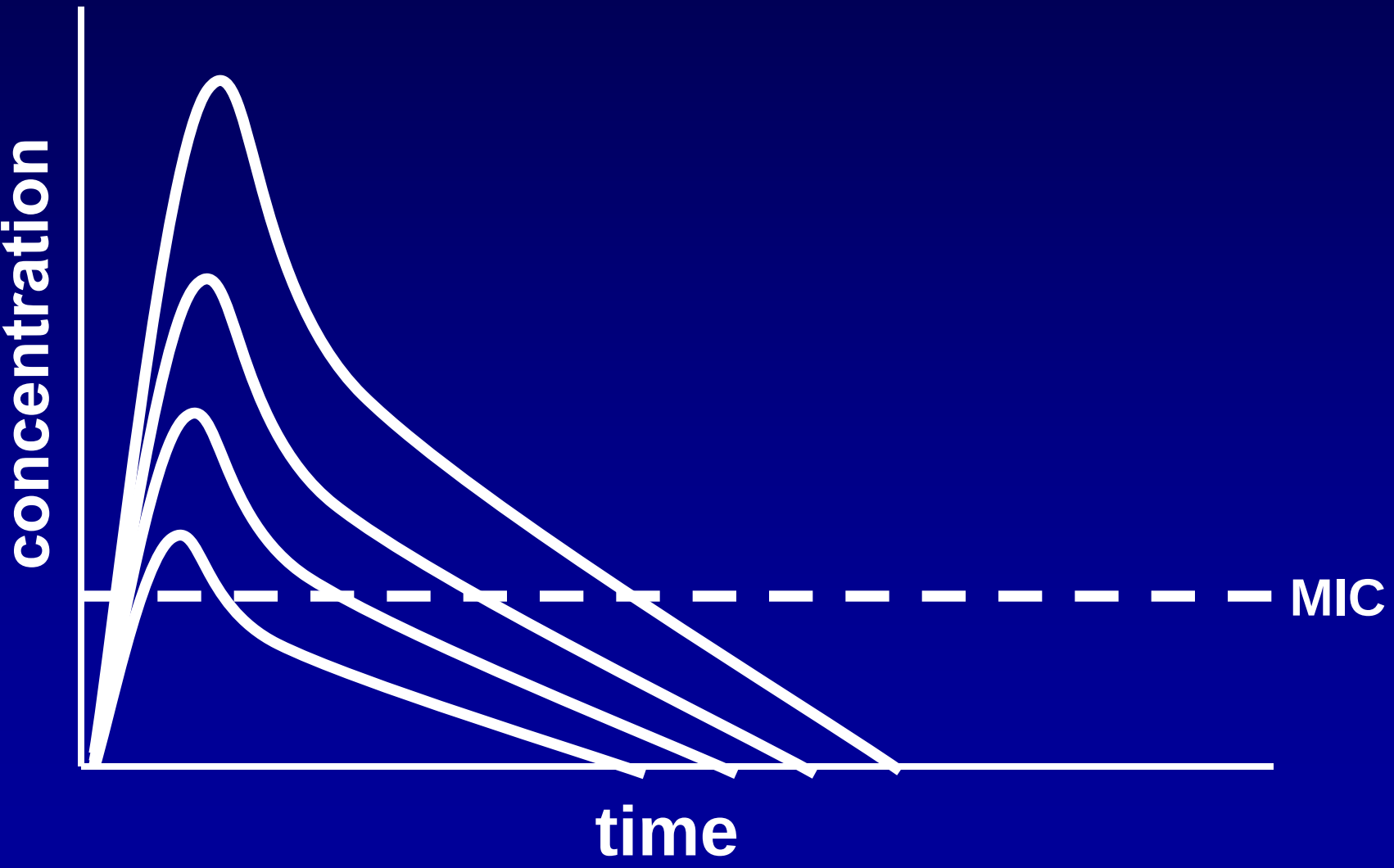
Effluxpumpeninhibitoren – alternative Therapiestrategie bei mikrobieller Multiresistenz

Markus Zeitlinger

Clinical resistancy I



Clinical resistancy II




ROBERT THOM

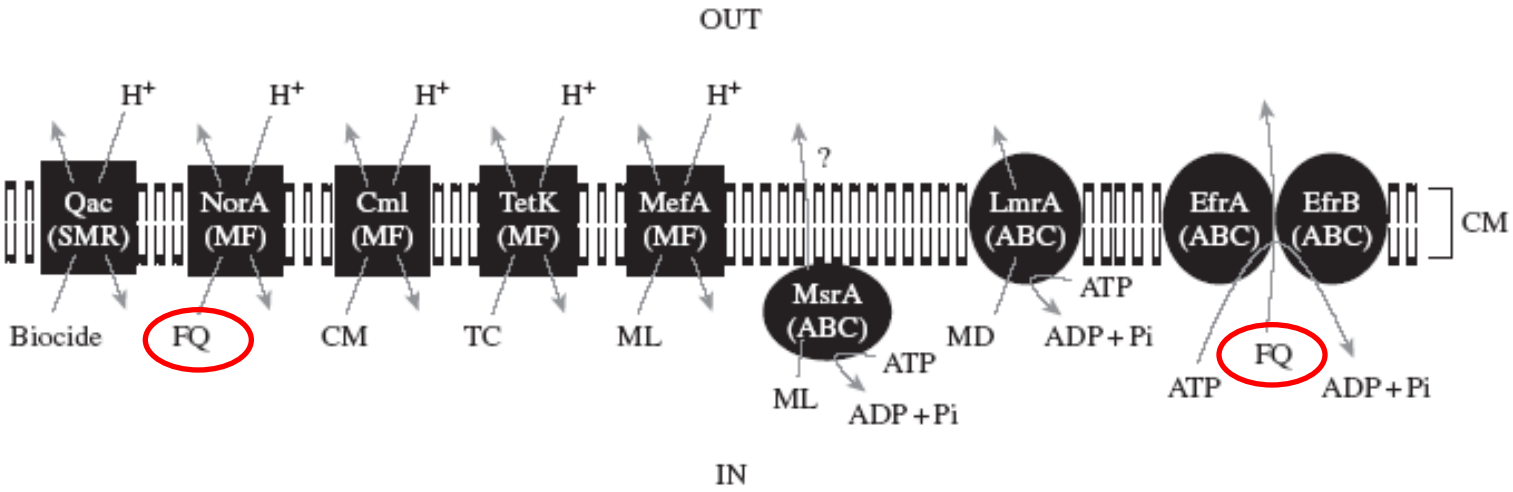
“Magic Bullet” by neutralising transporters in antimicrobial therapy

- Overcome bacterial resistance/ enhance susceptibility
- Overcome PK barriers in human

- *Improve efficacy*
- *Reduction of dose and side-effects*

Barriers in bacteria

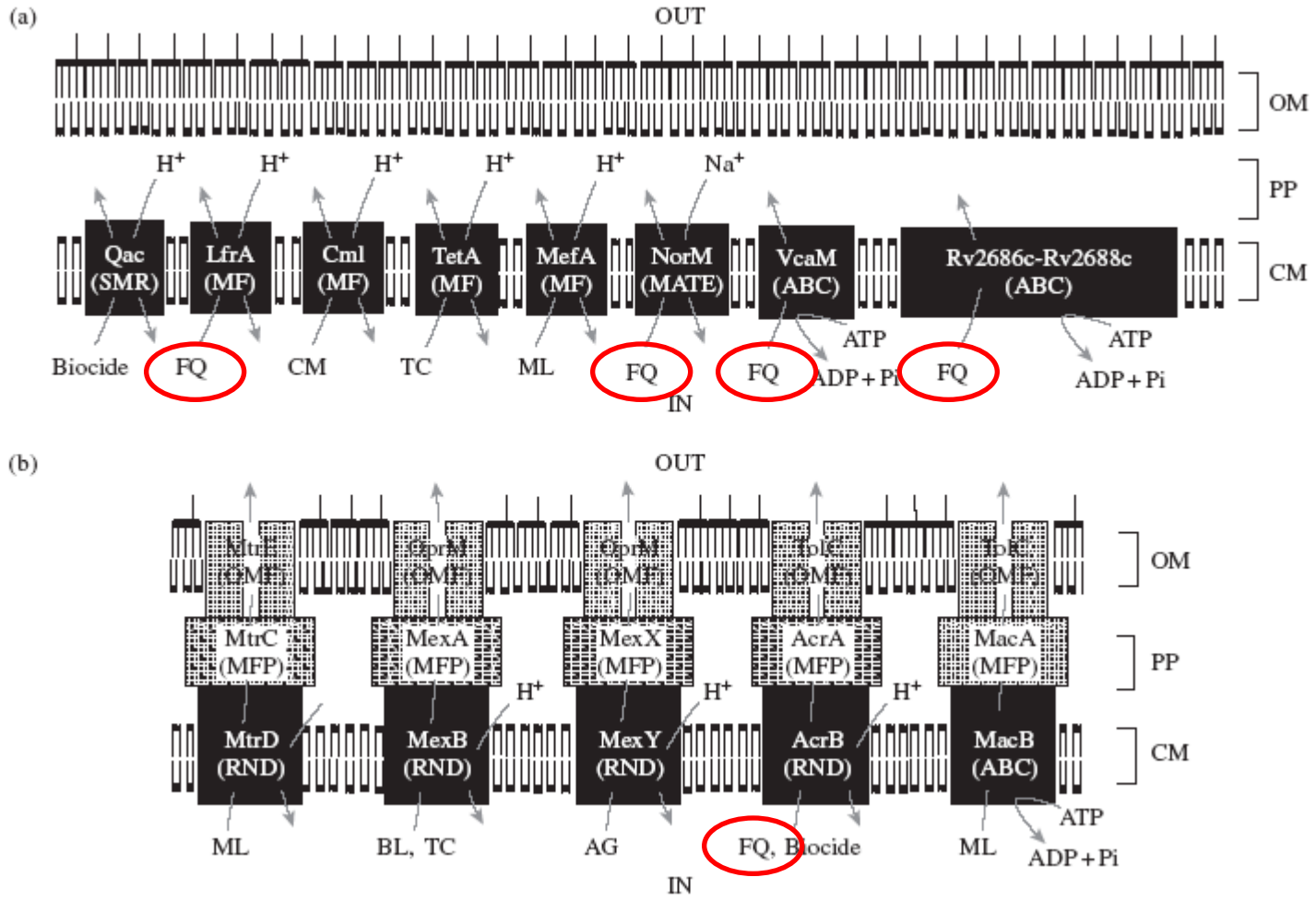
FQ - efflux pumps Gram-positive



- Major faciliator (MF) superfamily
- ATP binding casette (ABC) family

Poole K. Ann Med. 2007

FQ - efflux pumps Gram-negative



- Multidrug and toxic compound extrusion (MATE)
- Resistance nodulation division (RND)

Requisits of an ideal inhibitor

- Free of pharmacological activity
  - Determines impact on eukaryotic cells

- Feasible production and application
  - Isolation, purification, stability, solubility

- Proteolytically stable
  - Stabilty in plasma

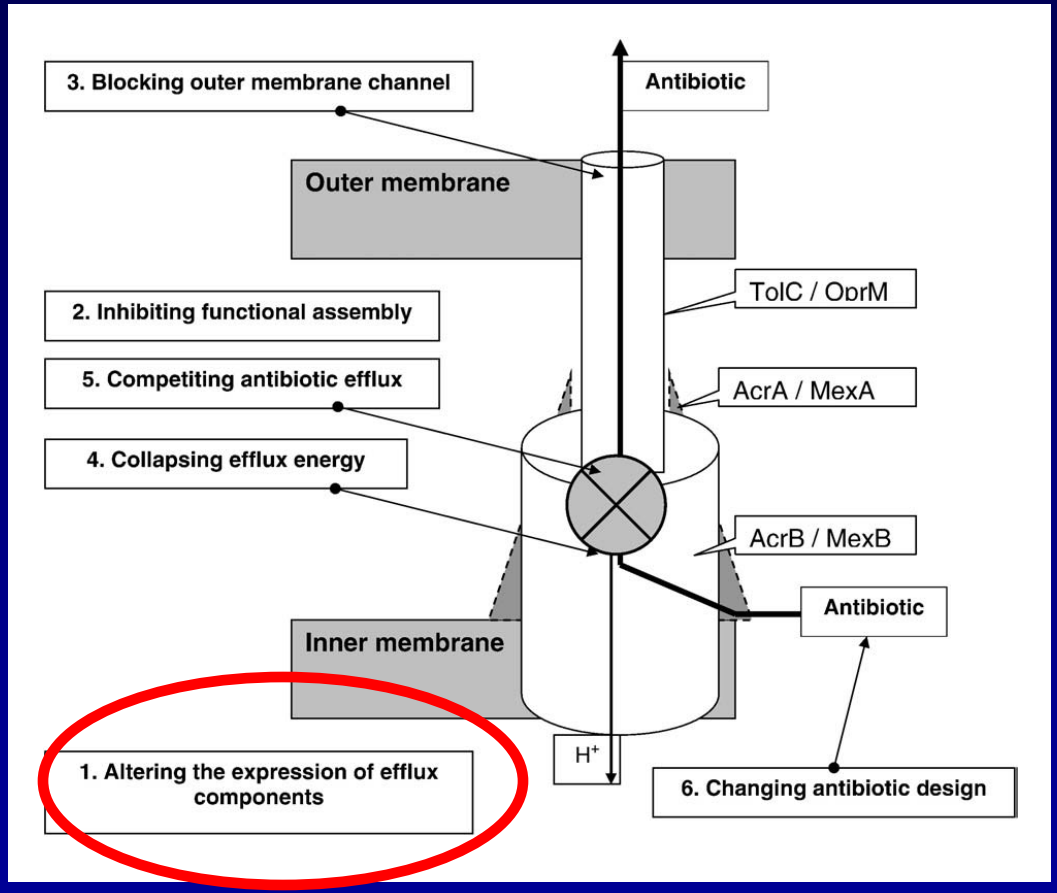
- Therapeutic index

- PK profile

- Devoid of antibiotic activity

Bhardway AK. Recent Patents Anti-Infect Drug Ther. 2012

Strategies to overcome Efflux



Altering regulatory steps for the expression of the pump

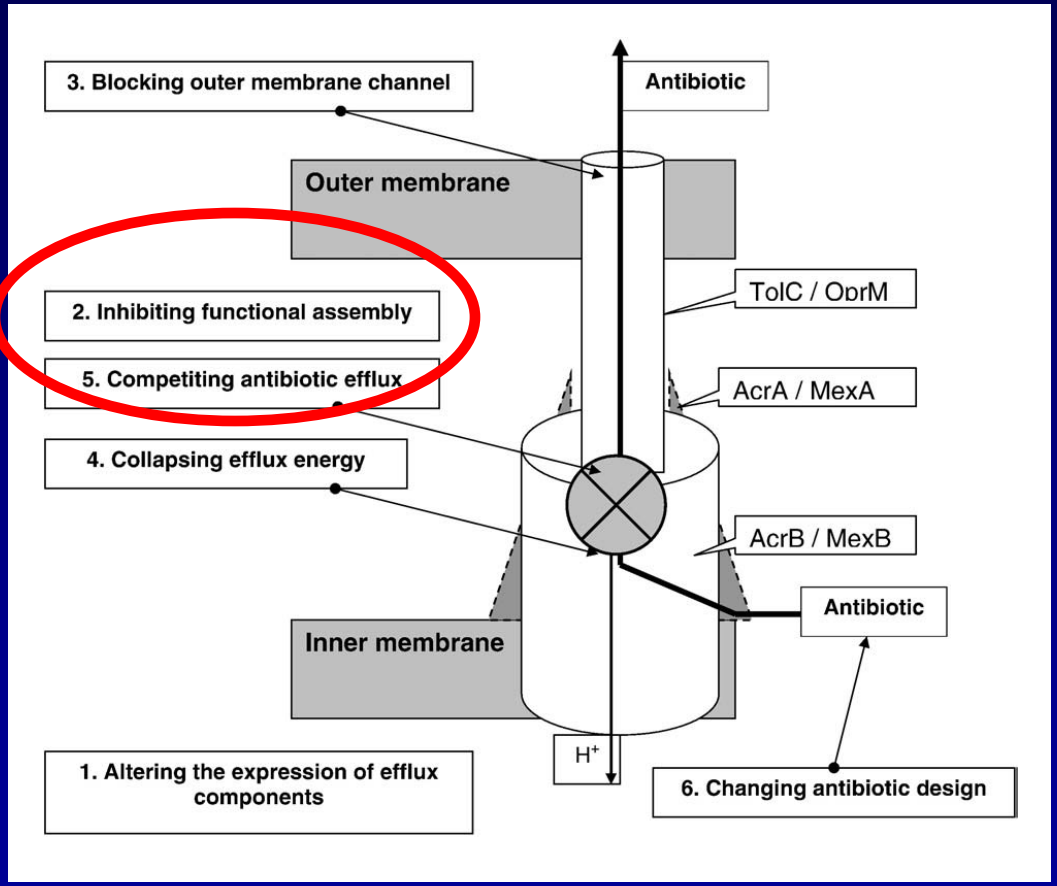
-Anitsense oligonucleotide

-Small interfering RNA -(AcrAB of *E.coli*)

-Mar A (both AcrAB and Porins)

Pages JM. Biochimica et Biophyica Acta. 2009

Strategies to overcome Efflux



Inhibiting functional assembly

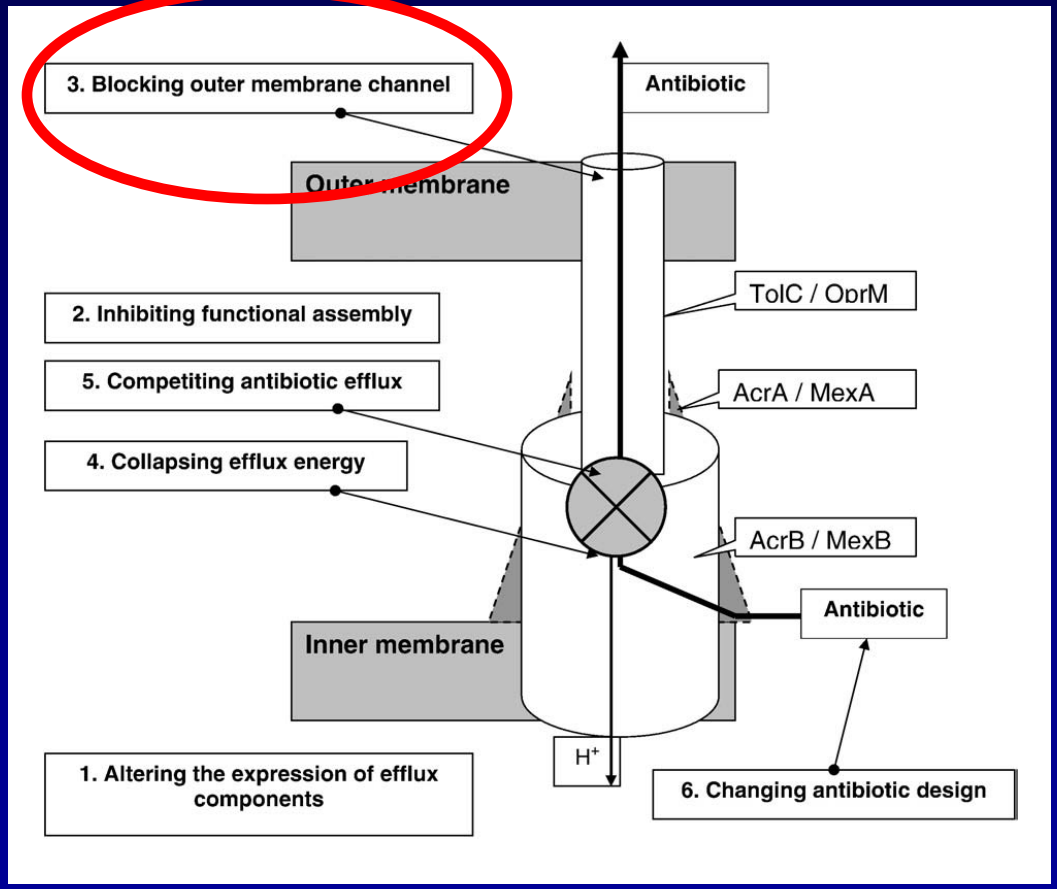
-Fatty acid metabolism

-Impacting signal peptidase II (AcrA is envelope lipoproteins)

-Tripartite efflux pumps like RND of Gram-neg.

Pages JM. Biochimica et Biophyica Acta. 2009

Strategies to overcome Efflux



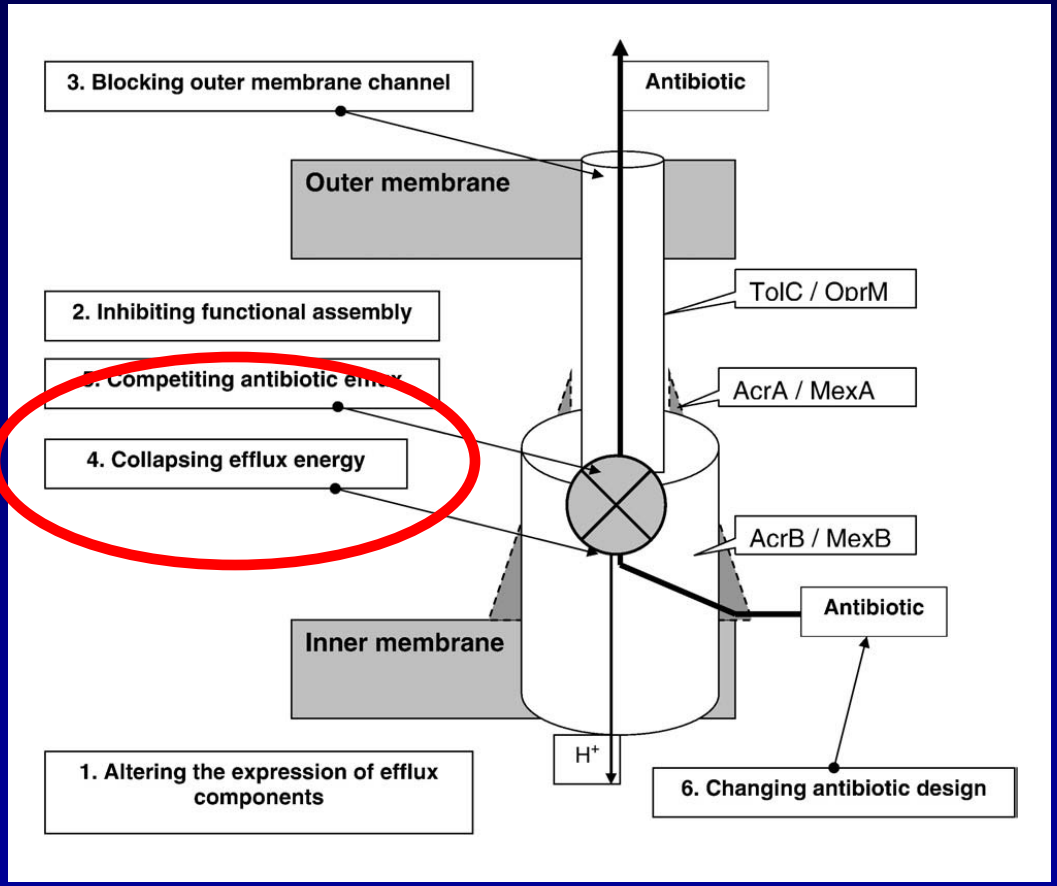
Blocking outer membrane channel (OMC)

-Nano antibodies as pore blocker

-Hypothesis at moment

Pages JM. Biochimica et Biophyica Acta. 2009

Strategies to overcome Efflux



Collapse of cell energy

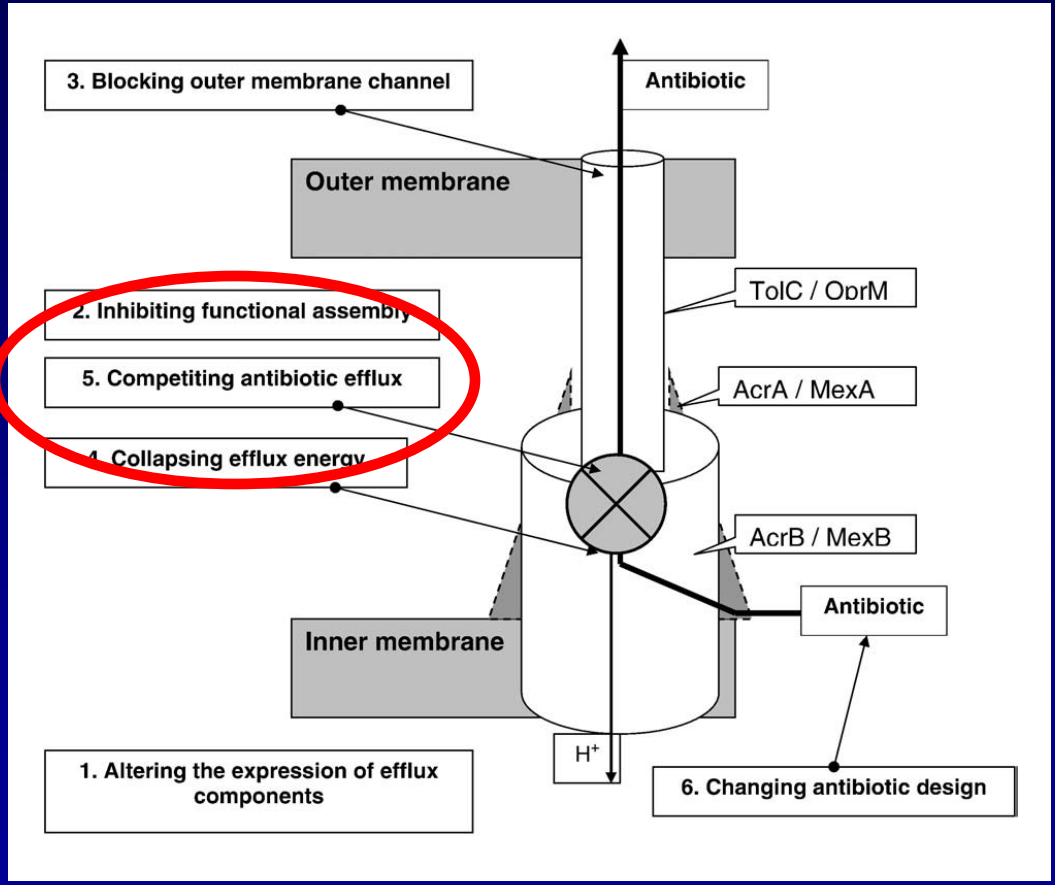
-Proton Motive Force (PMF)

-Carbonyl cyanide m-chlorophenylhydrazone (CCCP)

-?alteration of cell envelope itself

Pages JM. Biochimica et Biophyica Acta. 2009

Strategies to overcome Efflux

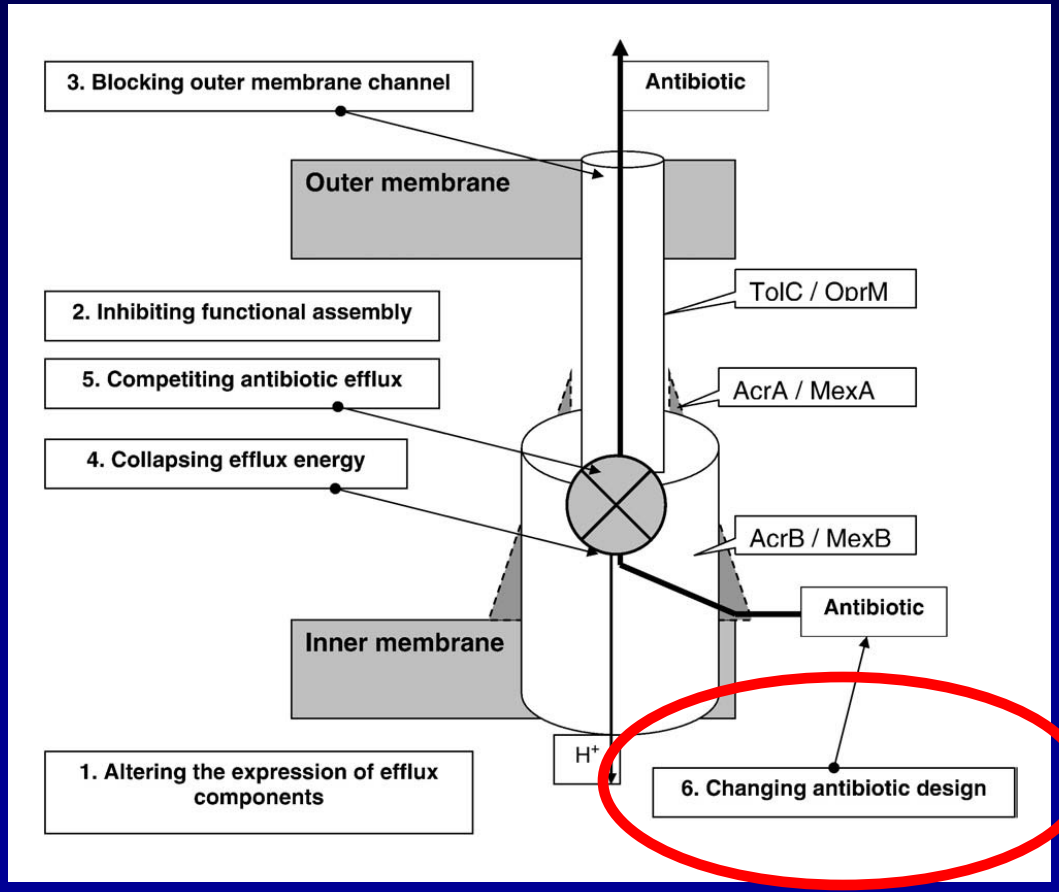


Competiting antibiotic efflux

-Classic EPI

Pages JM. Biochimica et Biophyica Acta. 2009

Strategies to overcome Efflux



Changing antibiotic design

-Glycylcyclines (Tigecycline bypasses MFS pumps)

-Ketolides (Telithromycin bypasses MefA/E and AcrAB)

-Spectinamides (TBC)

Pages JM. Biochimica et Biophyica Acta. 2009

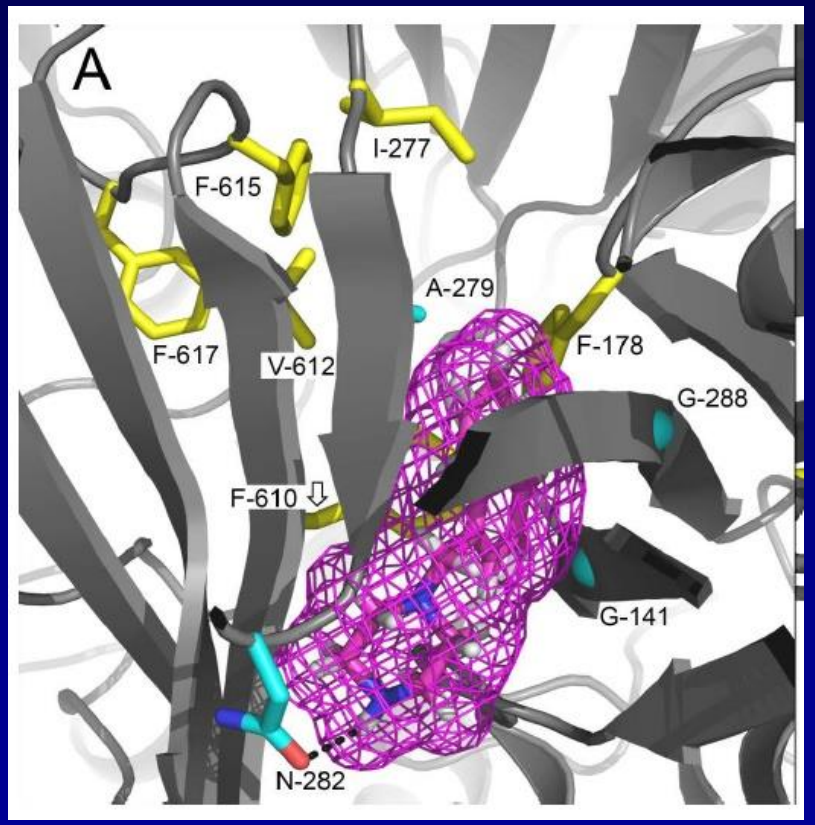
Drugs used for other purposes

- Reserpine
  - NorA of *S. aureus* (Norfloxacin)
- Verapamil
  - ABC of *S.aureus*, RND of Gram neg. (Tobramycin)
- Omeprazole
  - NorA of *S.aureus* (Norfloxacin)
- Phenothiazine (Thioridazin)
  - BpeAB-OprB, AmrAB-OprA of *Burkholderia spp.* (Aminoglycosides and macrolides)
  - RND of *E.coli* (Penicillin G)
- Paroxetine
  - NorA and MepA of *S.aureus*
  - AcrAB of *E.coli*

Bhardway AK. Recent Patents Anti-Infect Drug Ther. 2012

Specific EPIs

- Arylpiperazines
  - 1-(1-naphthylmethyl)-piperazine (NMP)
  - Blocking of AcrAB and AcrEF
  - Linezolid, tetracyclines, macrolides, fluoroquinolones
- Peptidomimetics
  - phenyl-arginine-beta-naphthylamide (PAßN)
  - Linezolid, rifampicin, macrolides, fluoroquinolones



www.pymol.org

Bhardway AK. Recent Patents Anti-Infect Drug Ther. 2012

Identification of Natural Compound Inhibitors for Multidrug Efflux Pumps of Escherichia coli

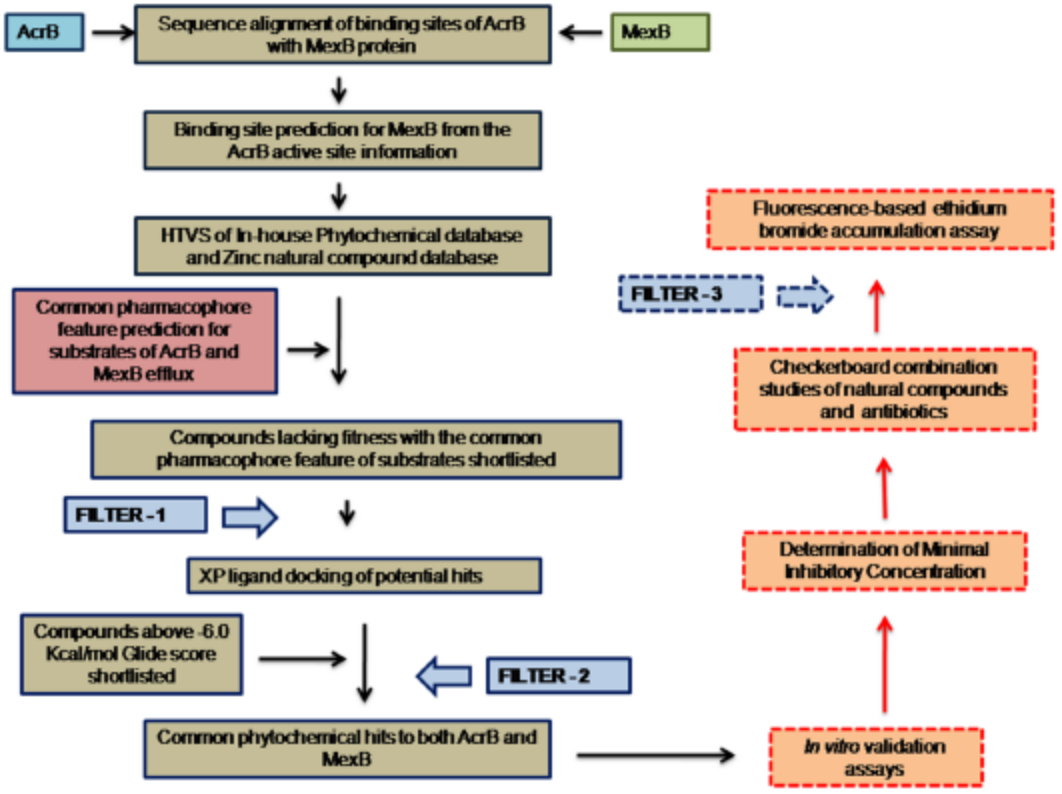
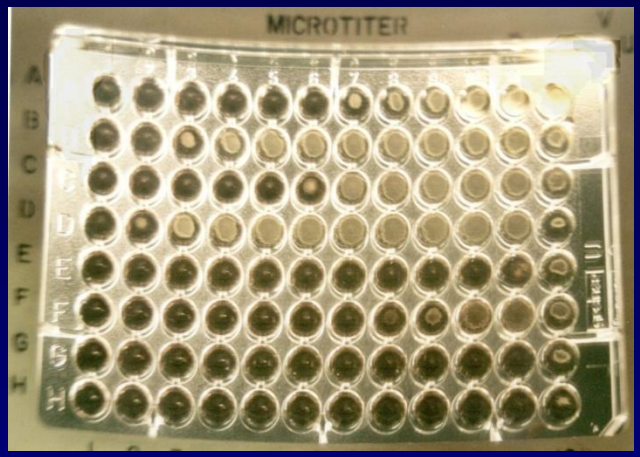


Figure 1. Flow chart of Virtual screening, pharmacophore-based filtering and experimental screening strategy for identifying efflux inhibitors.
doi:10.1371/journal.pone.0101840.g001

Apama V. Plos One. 2014

Methods Example Ciprofloxacin

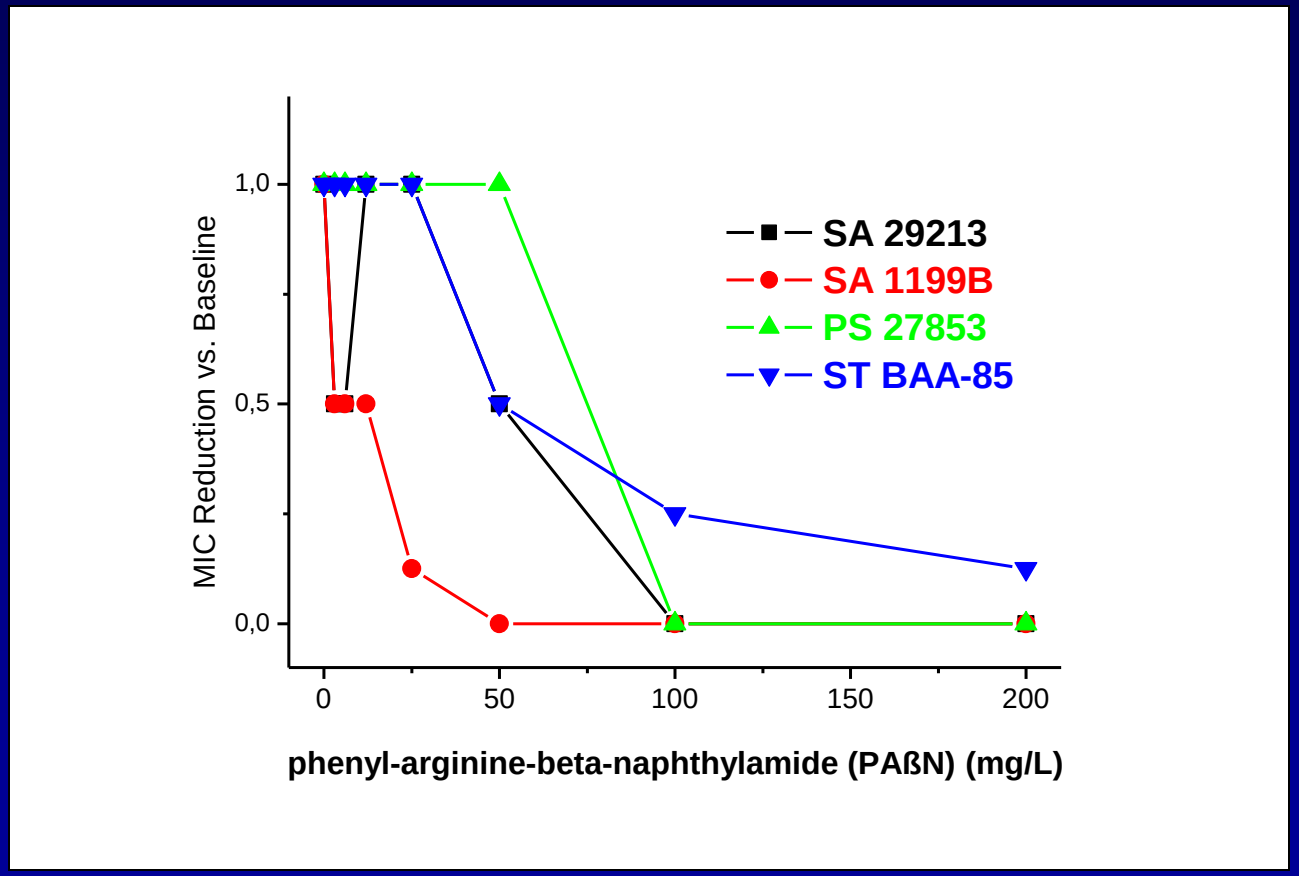
- 4 Stains
  - Staphylococcus aureus ATCC 29213 (MIC 0,5 µg/ml)
  - Staphylococcus SA-1199B (MIC 16 µg/ml)
  - Pseudomonas aeruginosa ATCC 27853 (MIC 0,5 µg/ml)
  - Stenotrophomonas maltofilia ATCC BAA-85 (MIC 16 µg/ml)



- Antimicrobial actvity

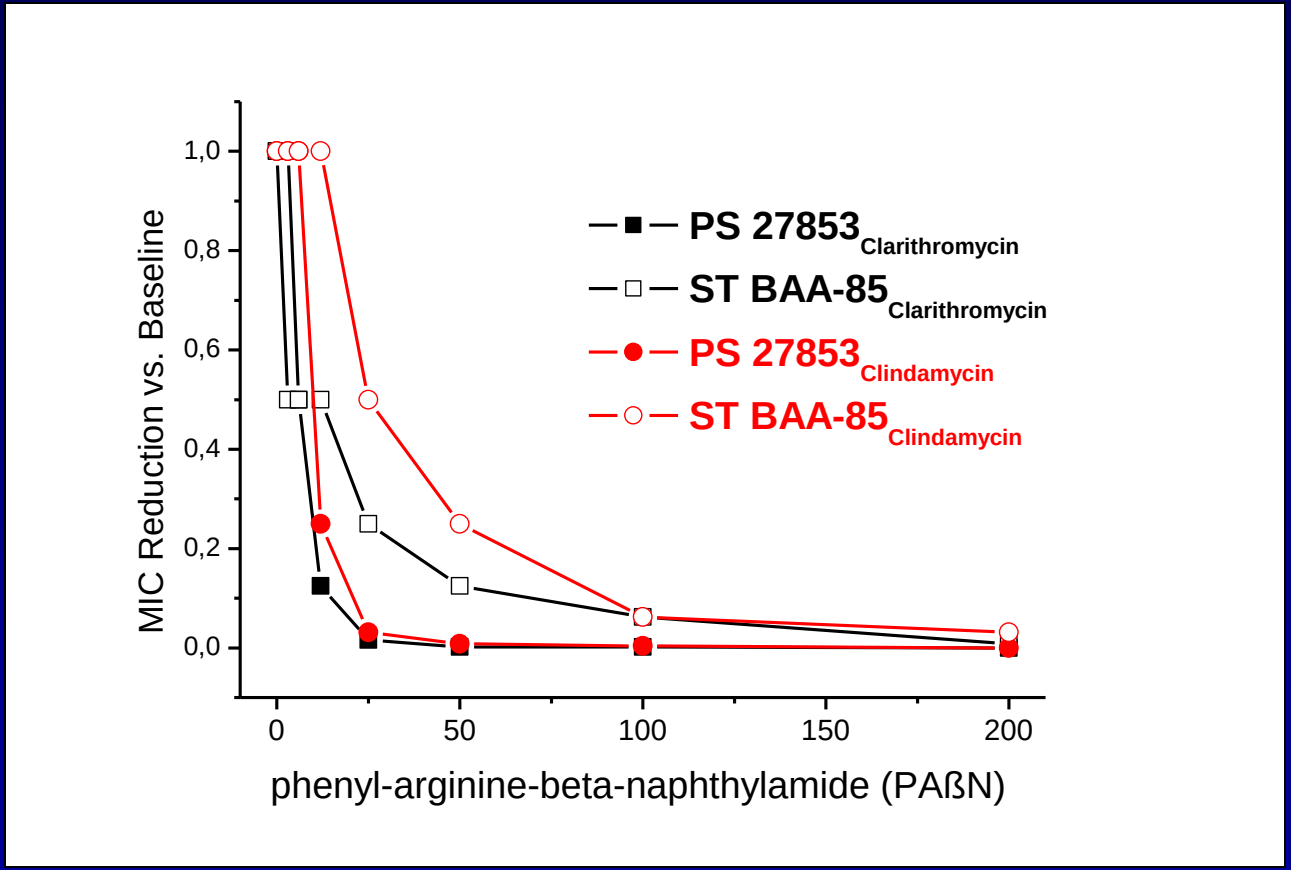
- Intracellular concentration

Ciprofloxacin and PAßN



Unpublished data

Clarithromycin and Clindamycin



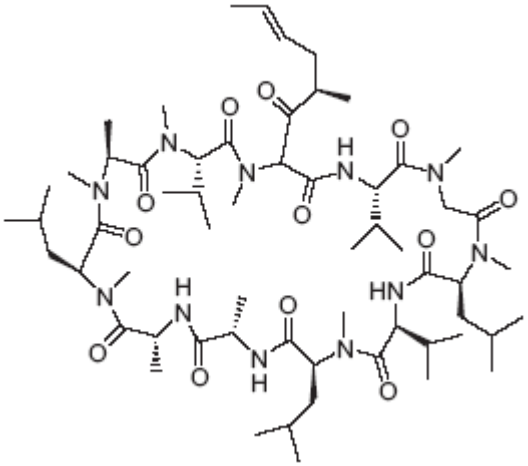
Unpublished data

MIC (mg/L) of efflux-pump-inhibitors without addition of antibiotic

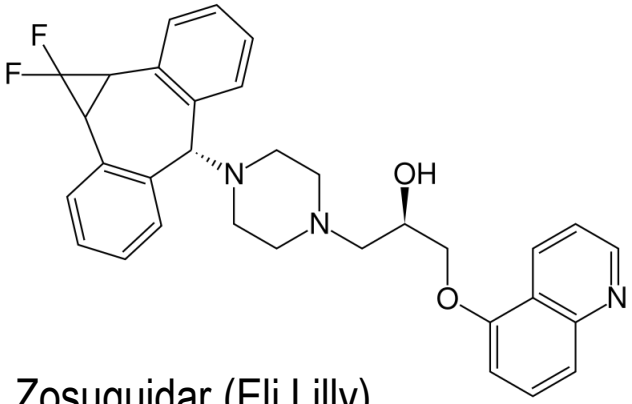
	PAßN
SA 29213	32
SA 1199B	16
PS 27853	256
ST BAA-85	512

phenyl-arginine-beta-naphthylamide (PAßN)

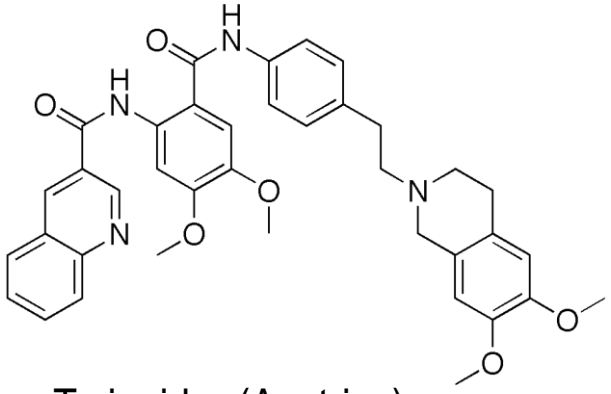
New-generation P-gp modulators



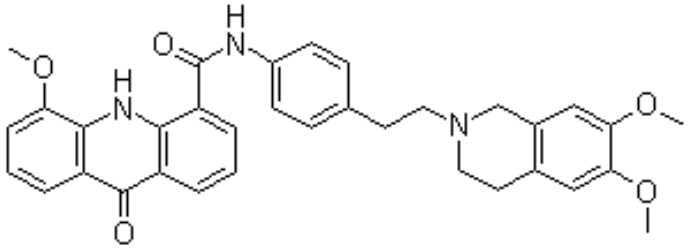
Valspodar (Novartis)



Zosuquidar (Eli Lilly)



Tariquidar (Azatrius)

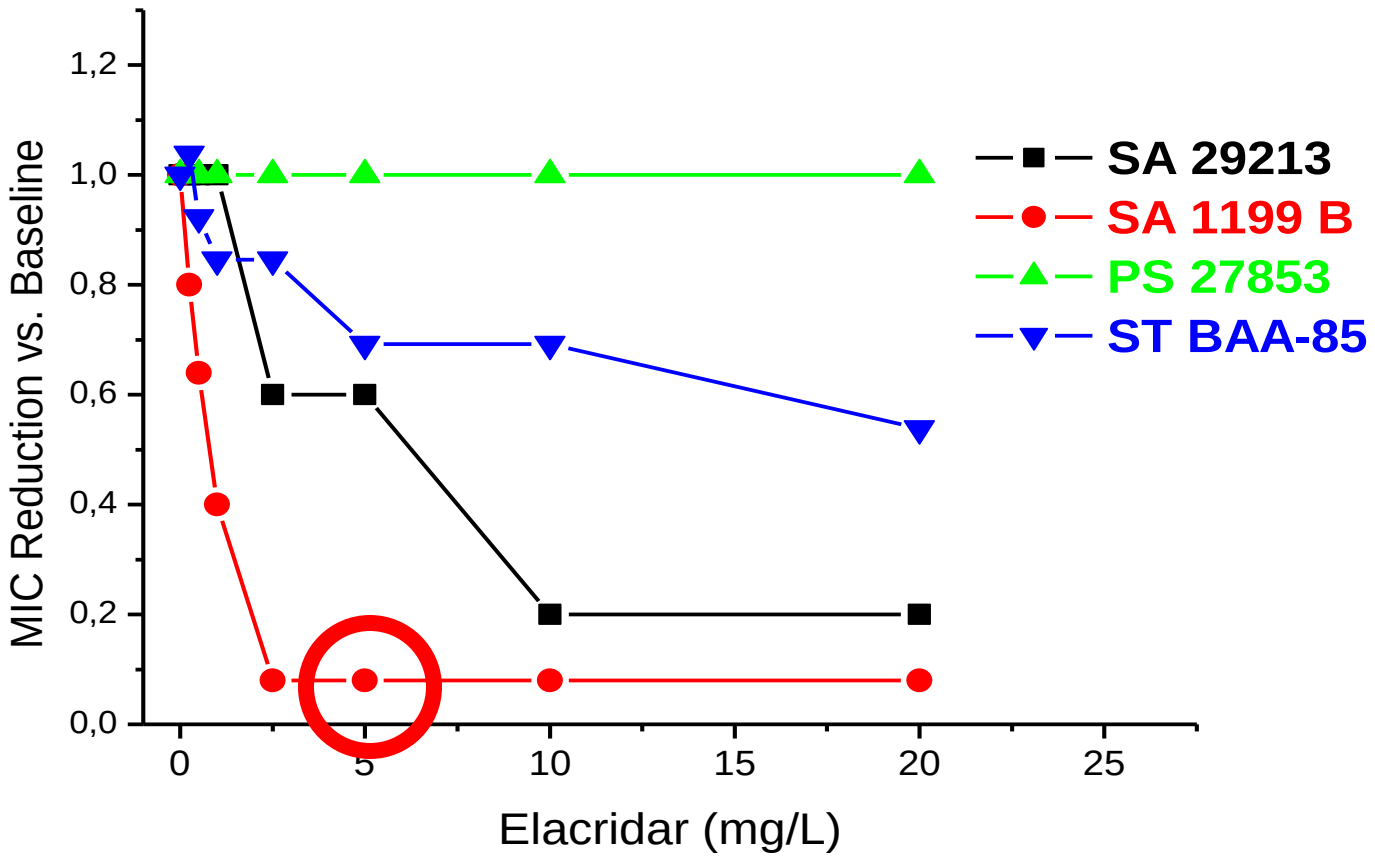


Elacridar (GSK)

MIC (mg/L) of efflux-pump-inhibitors without addition of antibiotic

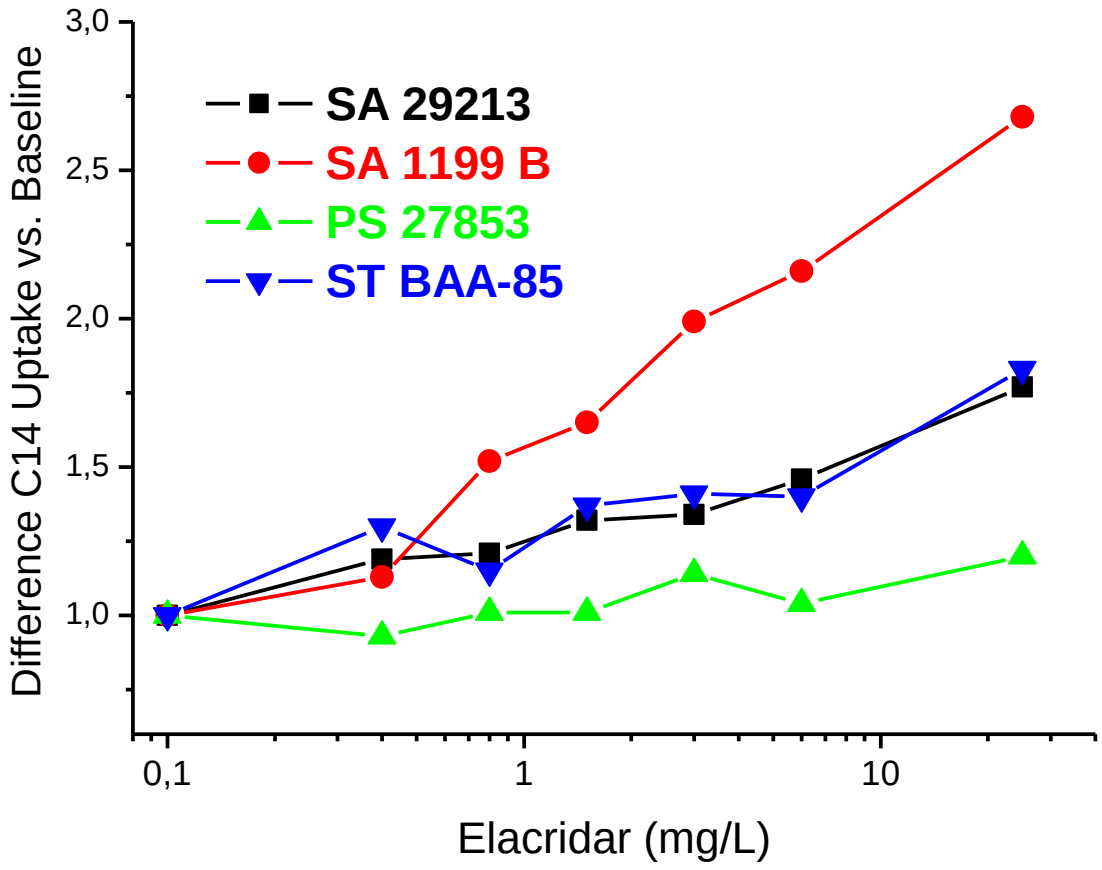
	Tariquidar	Elacridar
SA 29213	>64	>64
SA 1199B	>64	>64
PS 27853	>64	>64
ST BAA-85	>64	>64

Reduction of MIC of Ciprofloxacin for Gram positive and negative bacteria by Elacridar



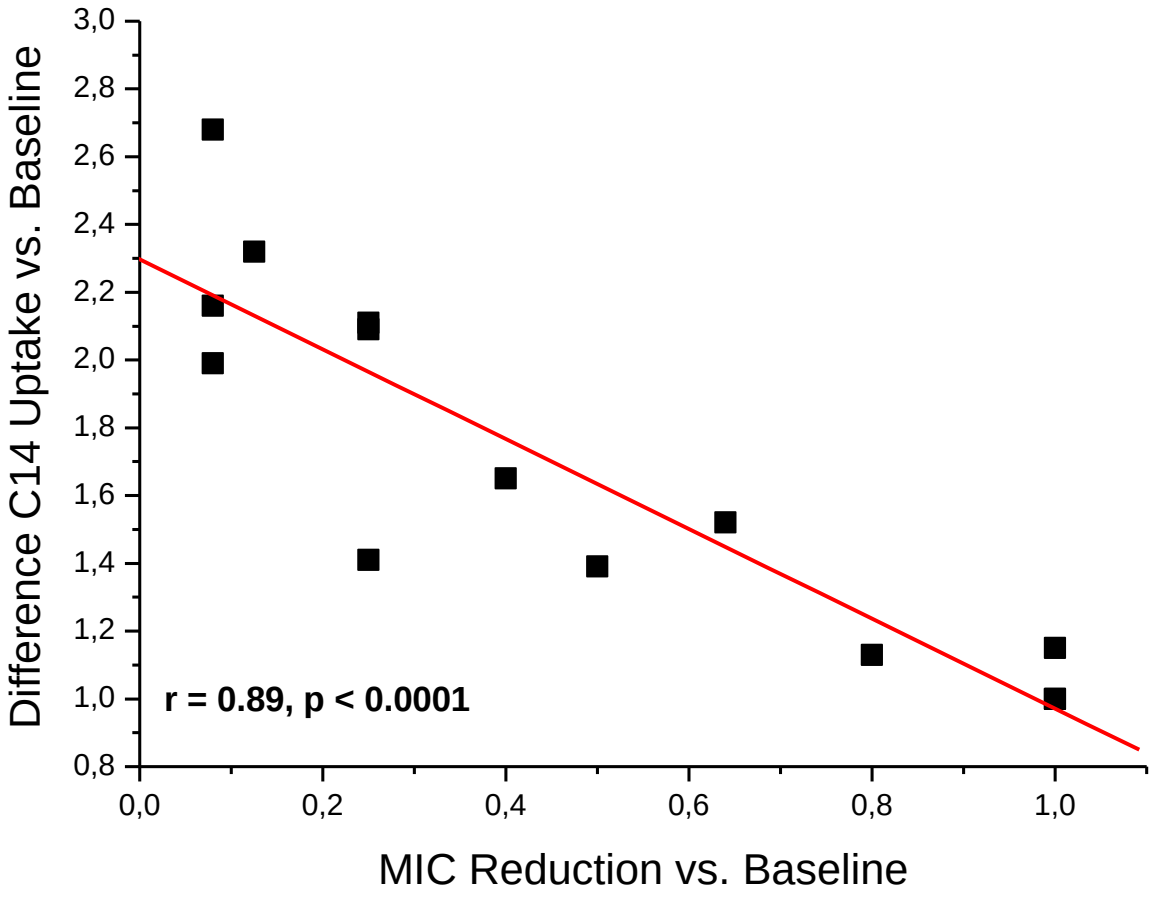
Change in MIC of SA 1199 B from 16 to 1 µg/ml

Enhancement of Uptake of [C14]ciprofloxacin by Gram positive and negative bacteria by Elacridar



Leitner I. J Antimicrob Agents 2010

Correlation between change in MIC and Uptake of [C14]ciprofloxacin for SA 1199B



Leitner I. J Antimicrob Agents 2010

Efflux Pump Induction Is a General First Step in the Evolution of Mycobacterial Drug resistance

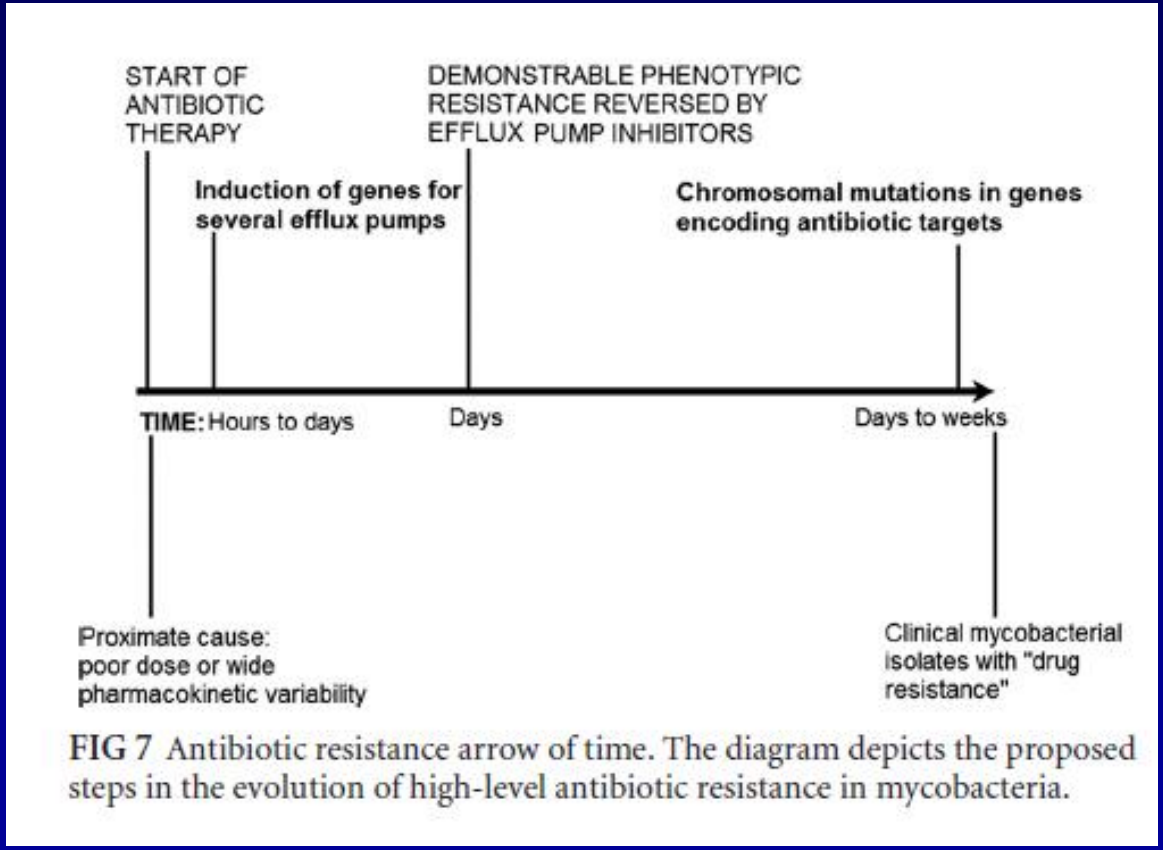


FIG 7 Antibiotic resistance arrow of time. The diagram depicts the proposed steps in the evolution of high-level antibiotic resistance in mycobacteria.

Schmalstieg AM . Antimicrob Agents Chemother. 2012

Effect of EPI on drug susceptibility of ofloxacin resistant Mycobacterium tuberculosis isolates

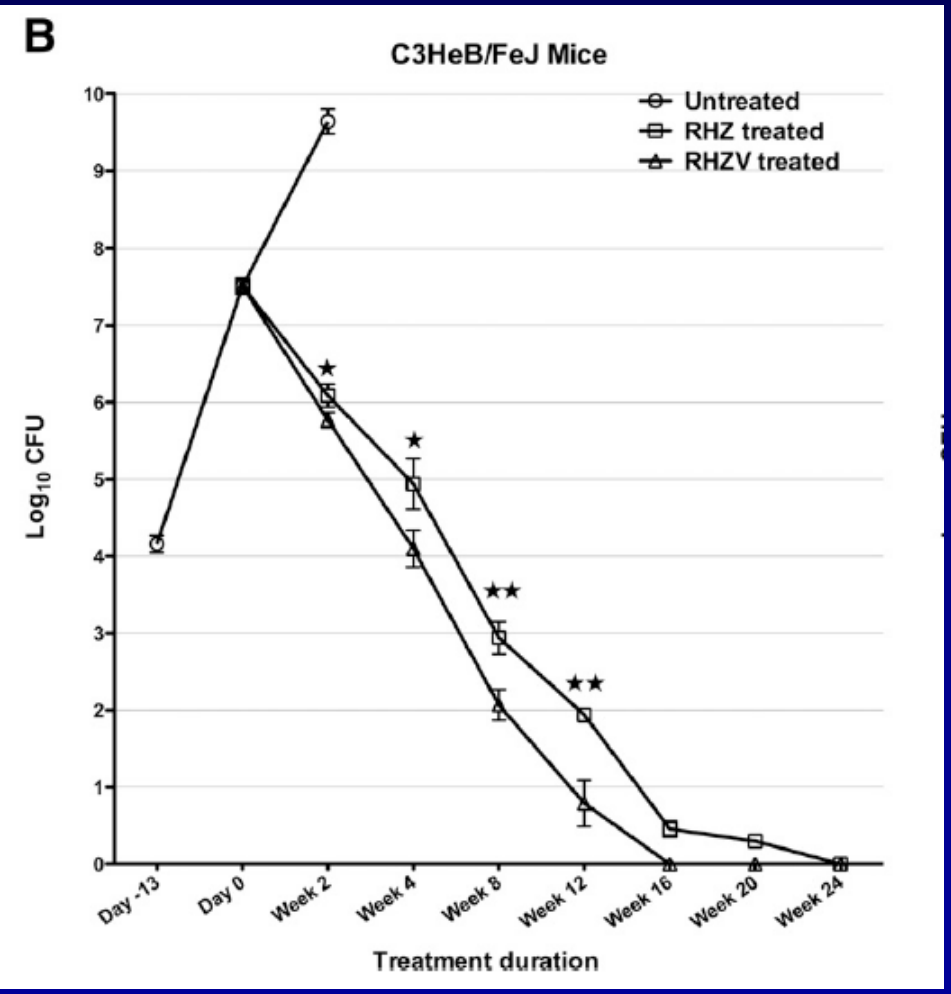
Table II. Fold changes in ofloxacin MIC of *M. tuberculosis* isolates (n=45) in presence of efflux inhibitors (CCCP, DNP and verapamil)

Efflux inhibitors (No. of isolates)	Fold changes in presence of efflux inhibitors in ofloxacin resistant isolates (%)		
	2	4	8
CCCP (n =16; 35.5%)	13 (81.3)	2 (12.5)	1 (6.3)
DNP (n =21; 46.6%)	11 (52.3)	5 (23.8)	5 (23.8)
Verapamil (n =24; 53.3%)	19 (79.2)	4 (16.6)	1 (4.2)

- CCCP
- 2,4-dinitrophenol (DNP)
- Verapamil

Singh M. Indian J Med Res. 2011

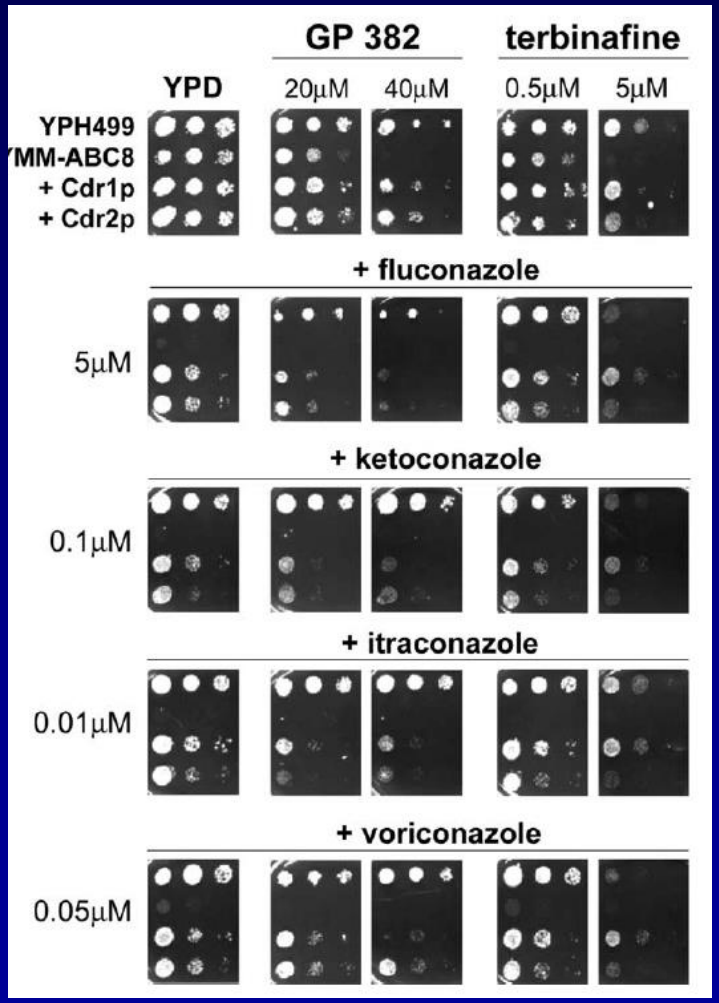
Acceleration of Tuberculosis Treatment by Adjunctive Therapy with Verapamil as an Efflux Inhibitor



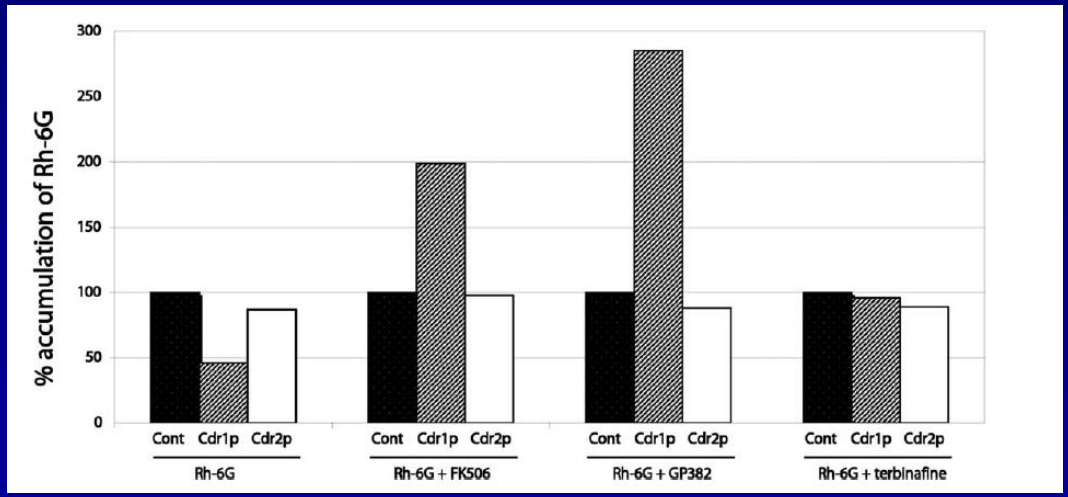
- rifampin (R; 10 mg/kg)
- isoniazid (H; 10 mg/kg)
- pyrazinamide (Z; 150 mg/kg),
- verapamil (V; 9.40 mg/kg)

Gupta S. Am J Resp Crit Care Med. 2013

Reversal of ABC-efflux pumps resistance in yeast



- Immunosuppressive
  - FK506, PSC833
- Antifungal Terbinafine



Schützer-Mühlbauer M . Int J Antimicrob Agents. 2003

Reversal of efflux mediated antifungal resistance with monoterpenes

- principal chemical components of thyme oil:
  Thymol and Carvacrol
- Fluconazol resistant (11) and susceptible (38) strains
- All partners ½ MIC

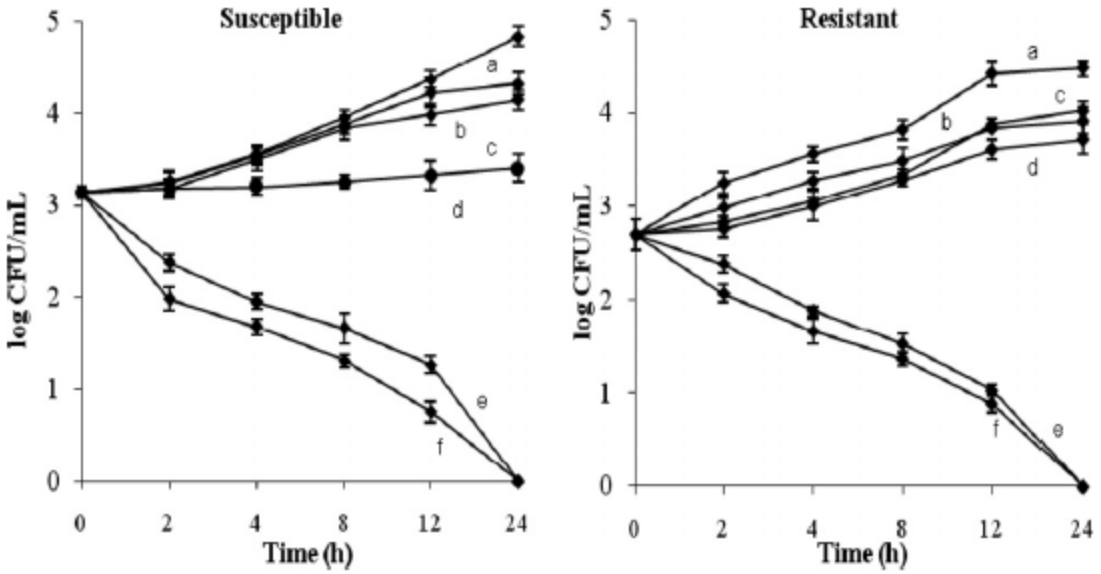
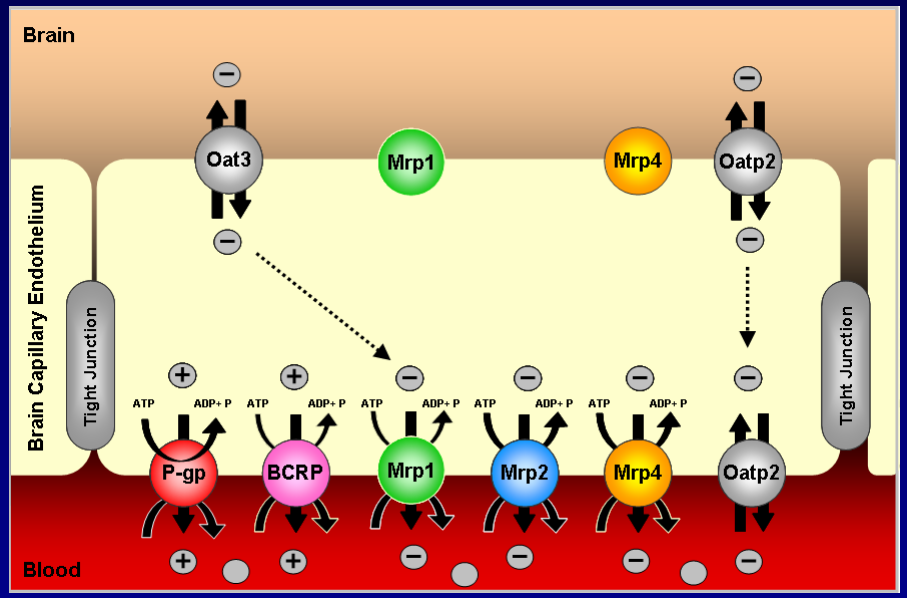


Fig. 1. Representative time-kill curves of *Candida* isolates following exposure to (a), ½ MIC of thymol (b), ½ MIC of carvacrol (c), ½ MIC of fluconazole (d), ½ MIC of fluconazole combined with ½ MIC of thymol (e) and ½ MIC of fluconazole combined with ½ MIC of carvacrol (f). (a) represents the untreated *Candida* cells (Control).

Ahmad A . Eu J Pharmaceutical Sciences. 2013

Barriers in the body

Multidrug Transporter at biological barriers



Cell

P-glycoprotein (ABCB1)
Multidrug resistance proteins
Breast cancer resistance protein
Organic anion transporting polypeptides
Organic anion transporter

Fluroquinolone Transporters

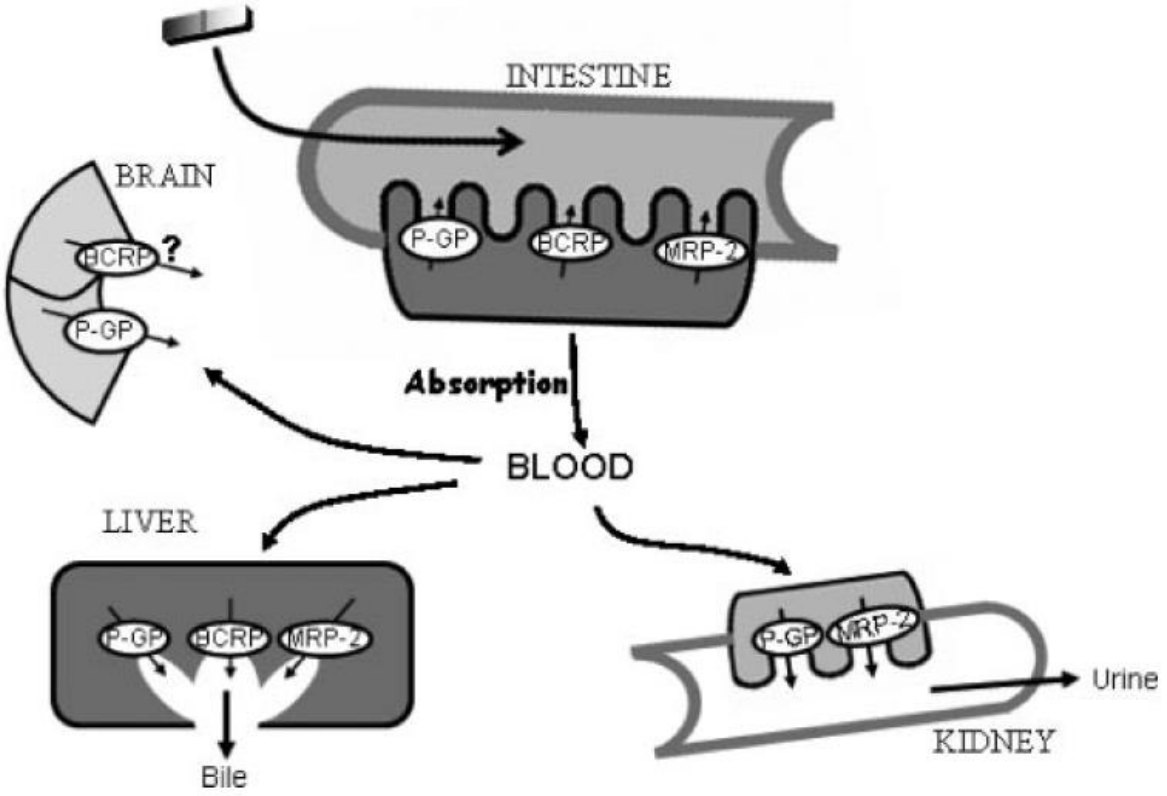
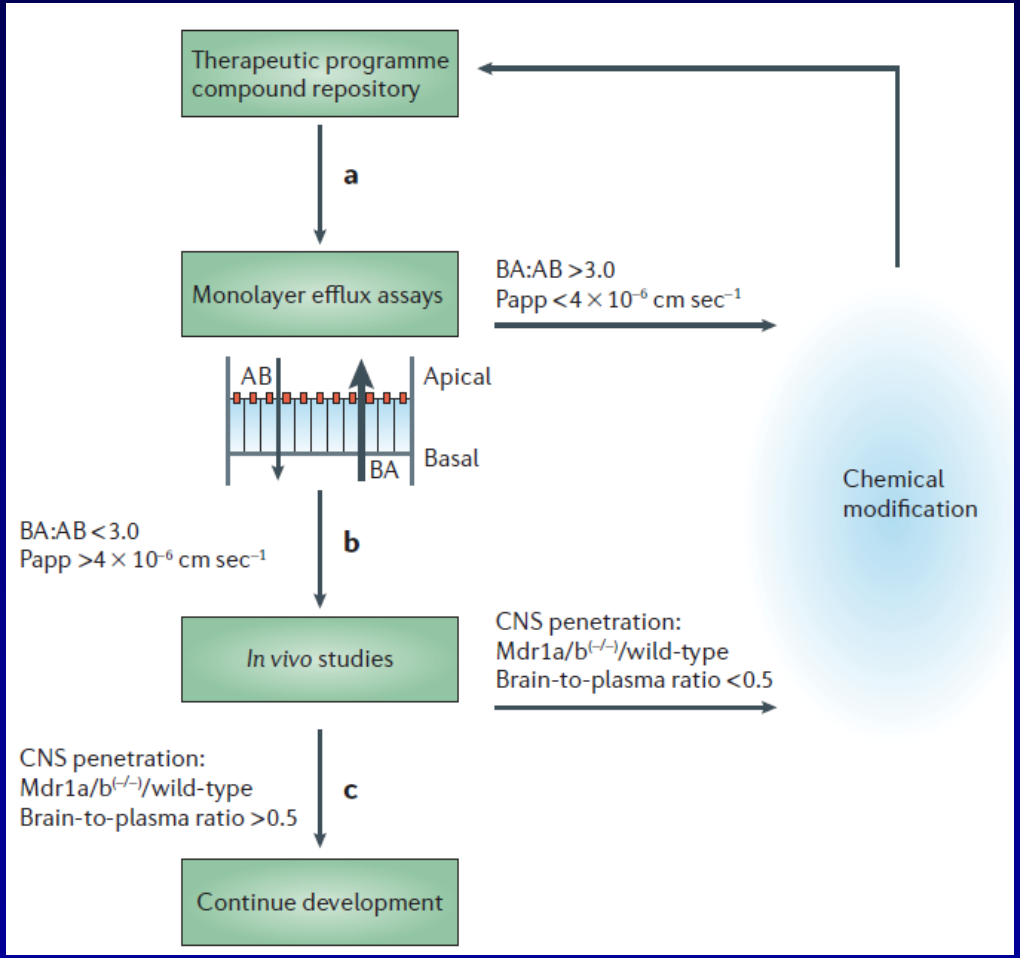


Figure 1. Major ABC transporters; *P*-glycoprotein (P-gp), BCRP, and MRP2, involved in the interaction with fluoroquinolones.

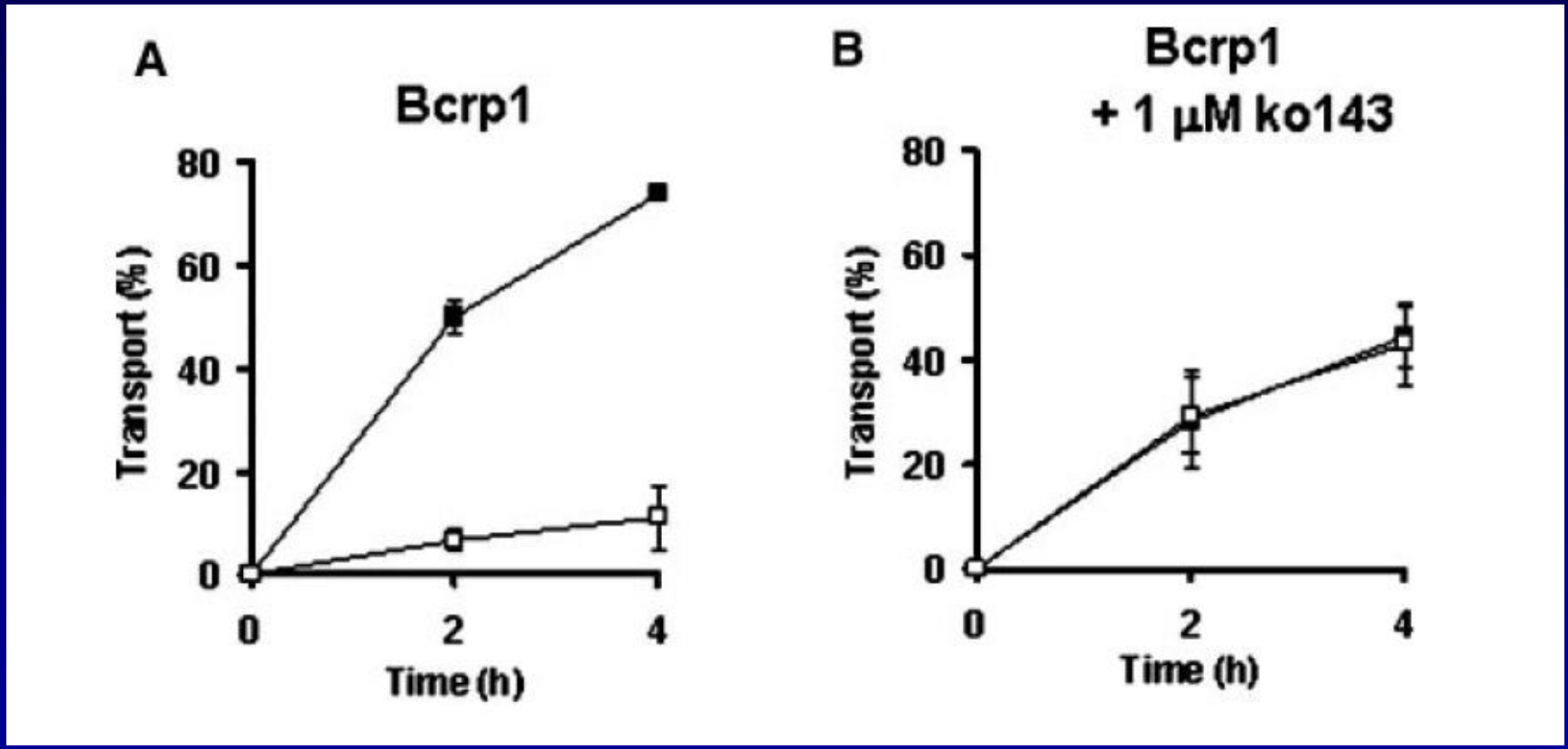
Alvarez A. J Pharmaceutical Science. 2007

P-gp in drug development



Szakack G. Nature Drug Discovery. 2006

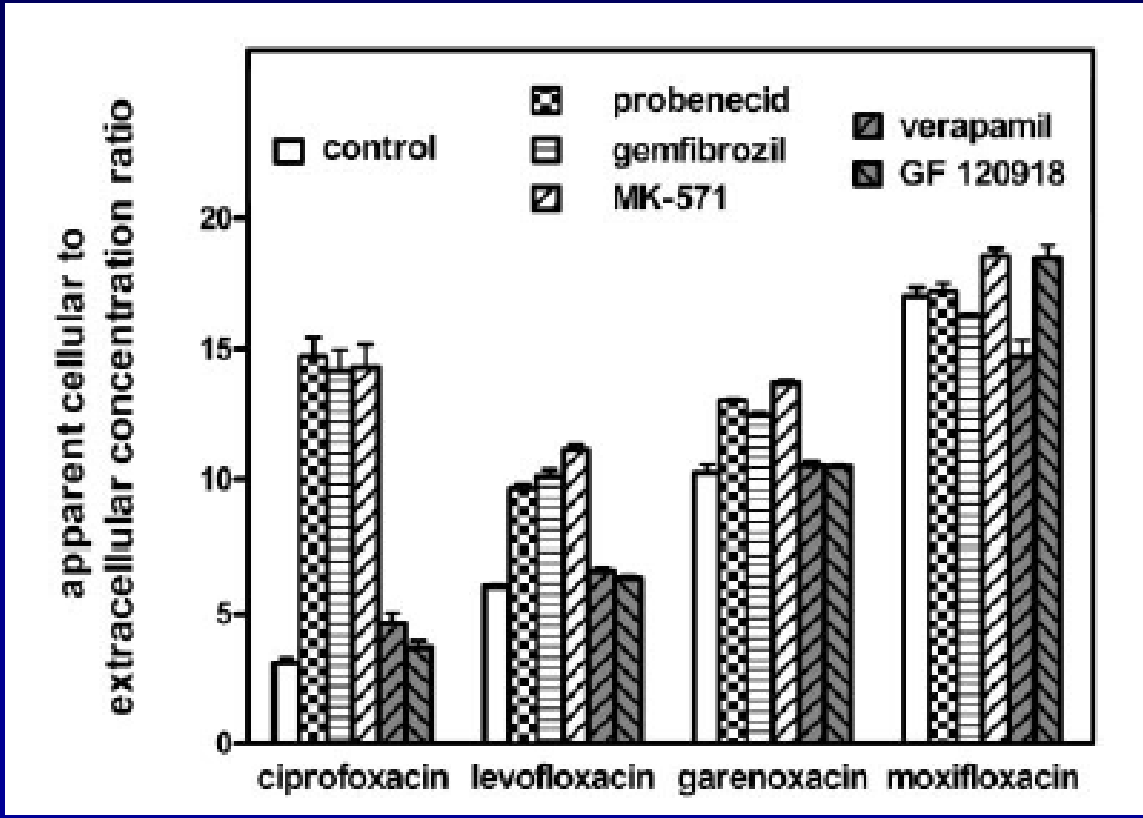
Enofloxacin penetration monolayers



Pulido M. J Vet Pharmacol Ther. 2006

Influence of Efflux Transporters on the Accumulation and Efflux of Four Quinolones (Ciprofloxacin, Levofloxacin, Garenoxacin, and Moxifloxacin) in J774 Macrophages

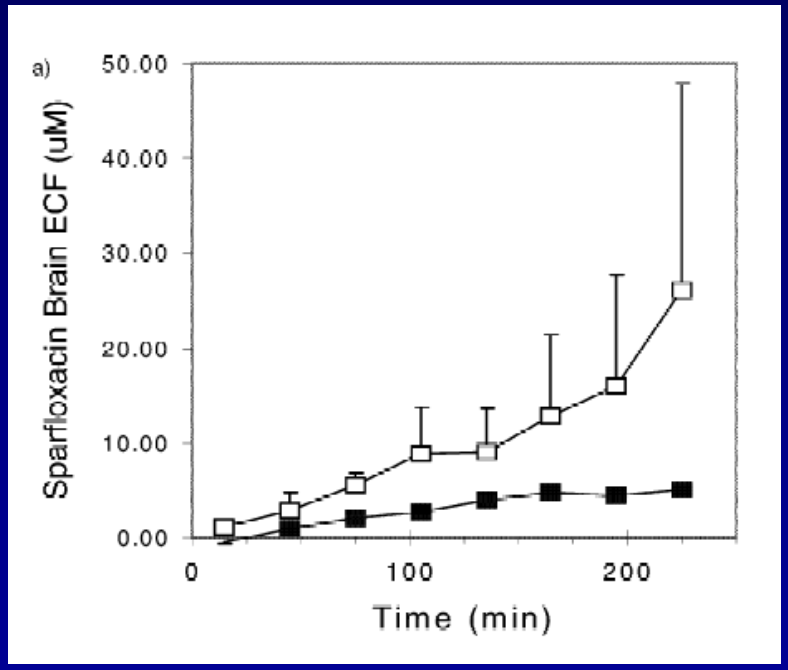
Jean-Michel Michot,† Cristina Seral,†‡ Françoise Van Bambeke, Marie-Paule Mingeot-Leclercq, and Paul M. Tulkens*

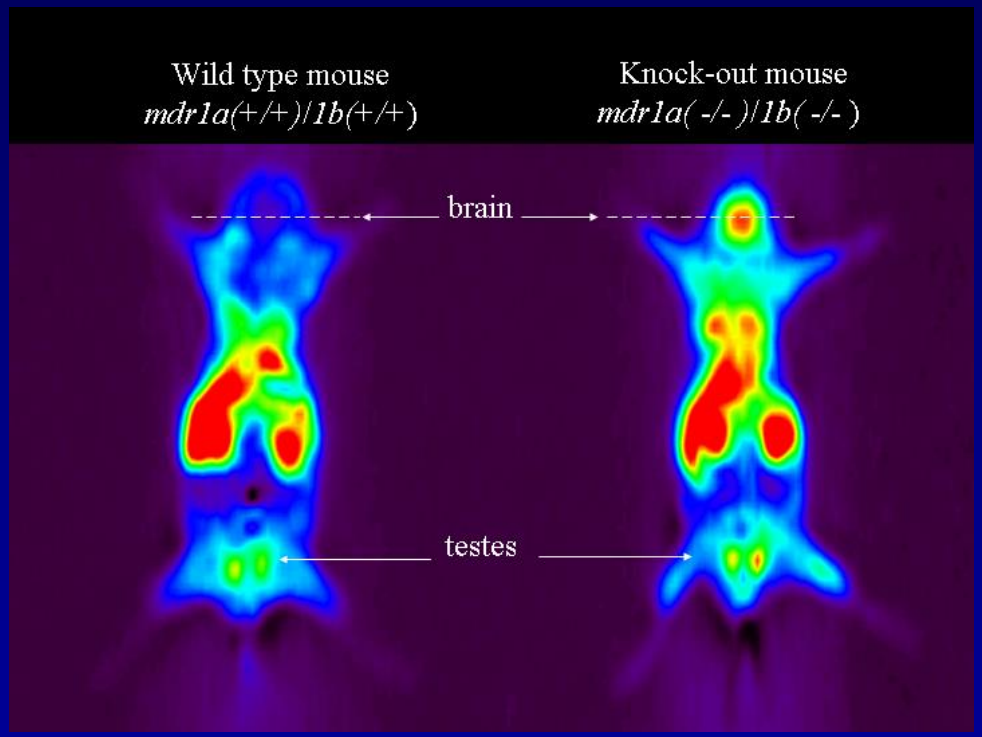


AAC. 2005

In vitro and in vivo investigations on fluoroquinolones; effects of the P-glycoprotein efflux transporter on brain distribution of sparfloxacin

Elizabeth C.M. de Lange[a,*], Sandrine Marchand[b], Dirk-Jan van den Berg[a], Inez C.J. van der Sandt[a], Albertus G. de Boer[a], Annie Delon[b], Serge Bouquet[b], William Couet[b]

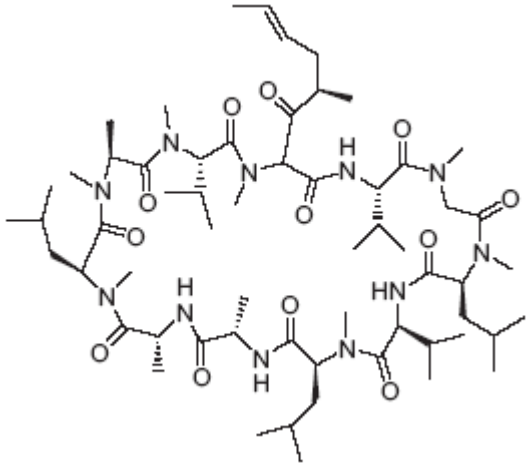




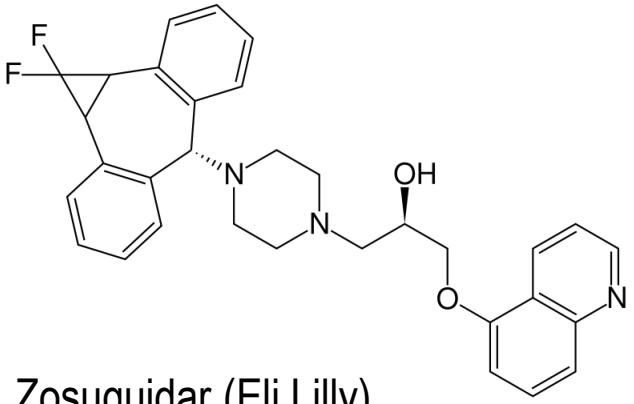
Eu J Pharmac Science. 2000

Luurtsema G. Nucl. Med. Biol. 2003

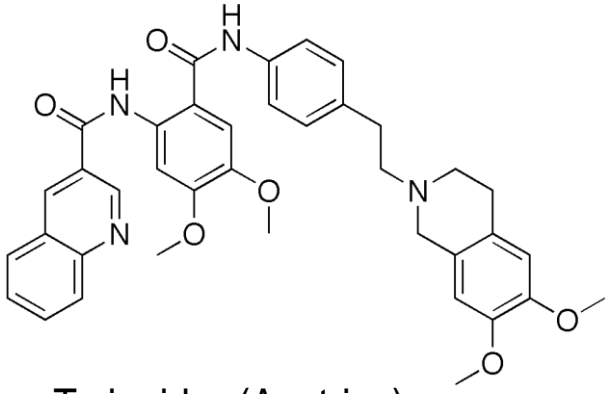
New-generation P-gp modulators



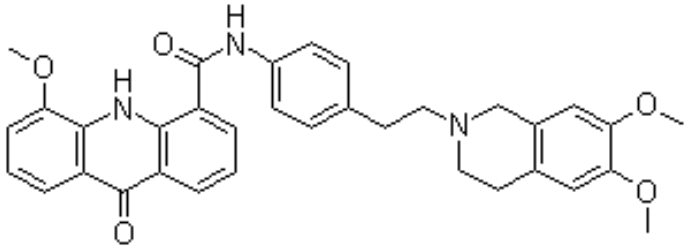
Valspodar (Novartis)



Zosuquidar (Eli Lilly)

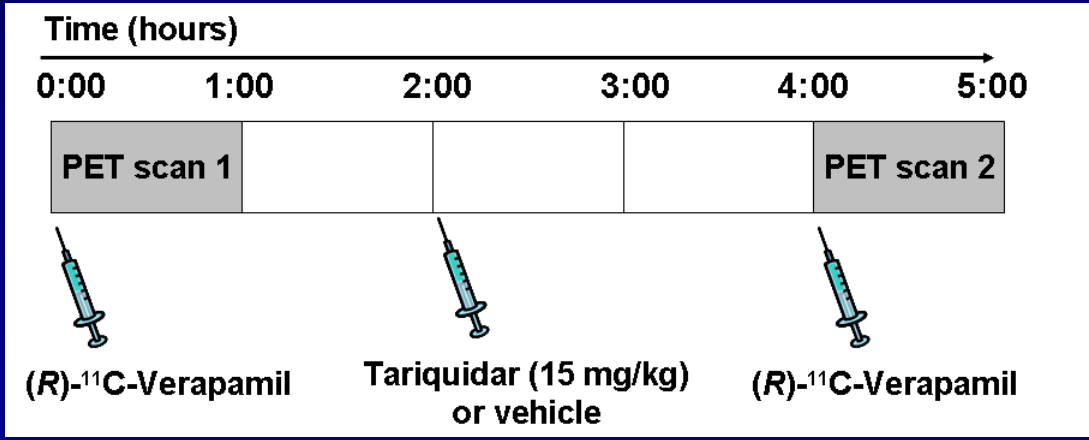


Tariquidar (Azatrius)



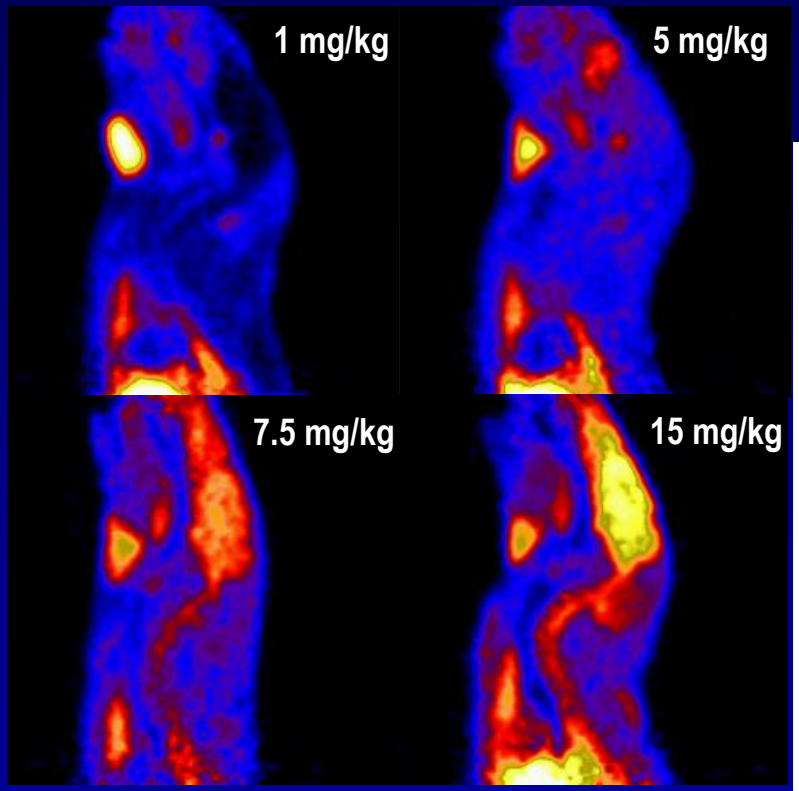
Elacridar (GSK)

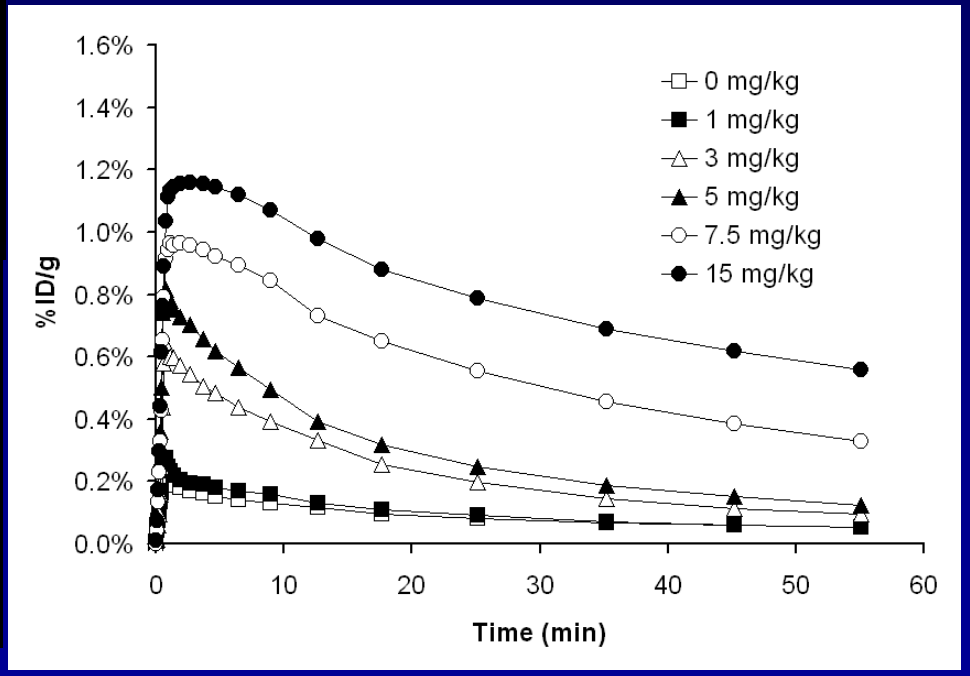
Double-scan protocol with (*R*)-[^{11}C]verapamil µPET and tariquidar



J. Bankstahl. J. Nucl. Med. 2008

Dose Response Tariquidar





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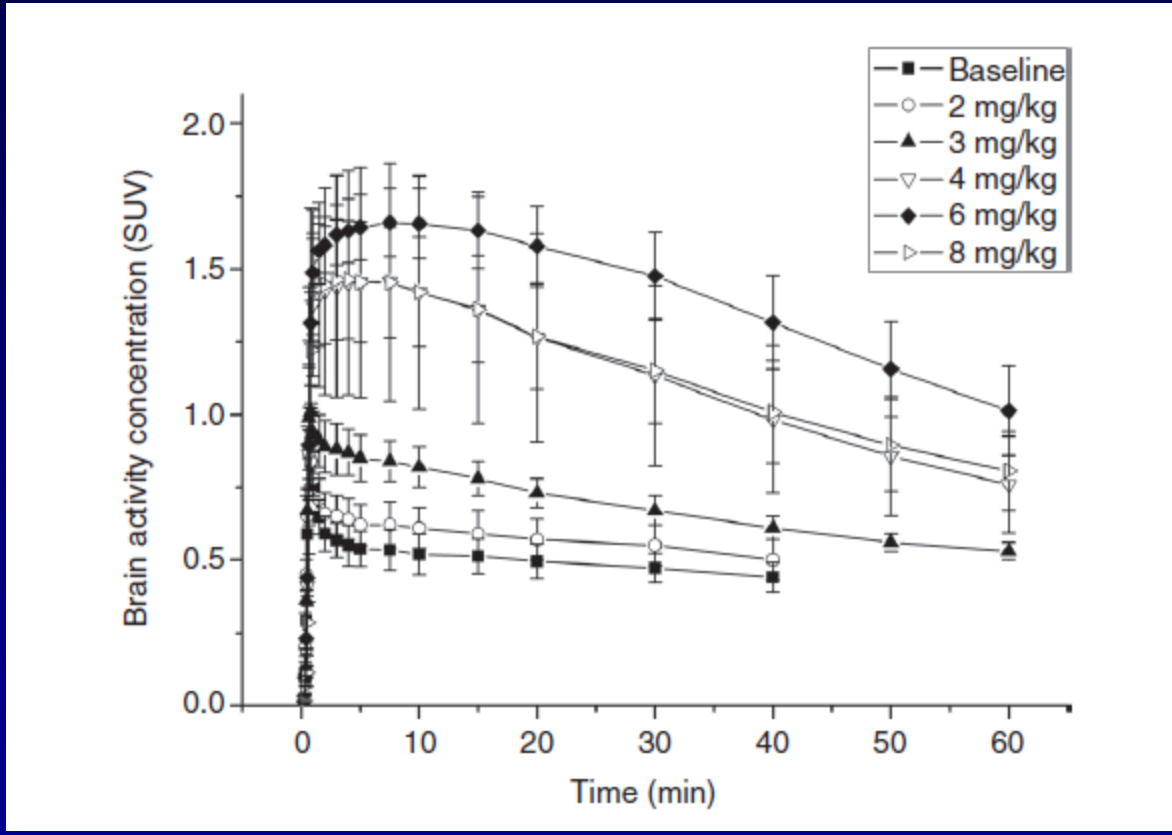
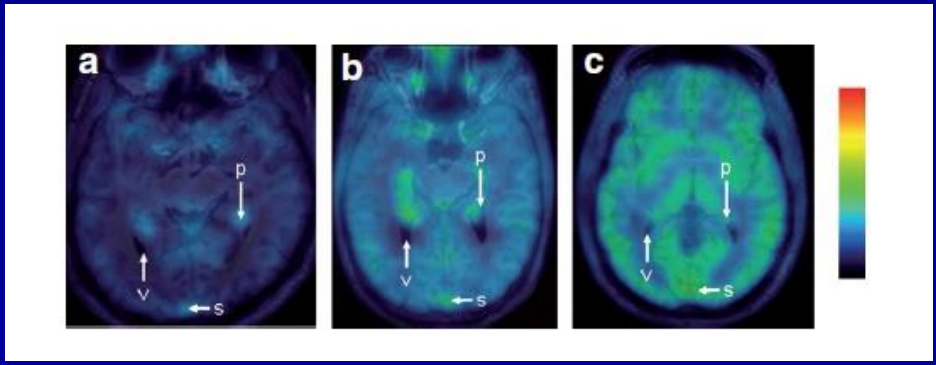
Human Brain uptake with Tariquidar





Bauer M. Clinical Pharm Ther 2011

EFFECT OF EFFLUX INHIBITION ON BRAIN UPTAKE OF ITRACONAZOLE IN MICE INFECTED WITH *CRYPTOCOCCUS NEOFORMANS*

FRÉDÉRIC IMBERT, MÉRYAM JARDIN, CHRISTINE FERNANDEZ, JEAN CHARLES GANTIER, FRANÇOISE DROMER, GABRIEL BARON, FRANCE MENTRE, LUDY VAN BEIJSTERVELDT, ERIC SINGLAS, AND FRANÇOIS GIMENEZ

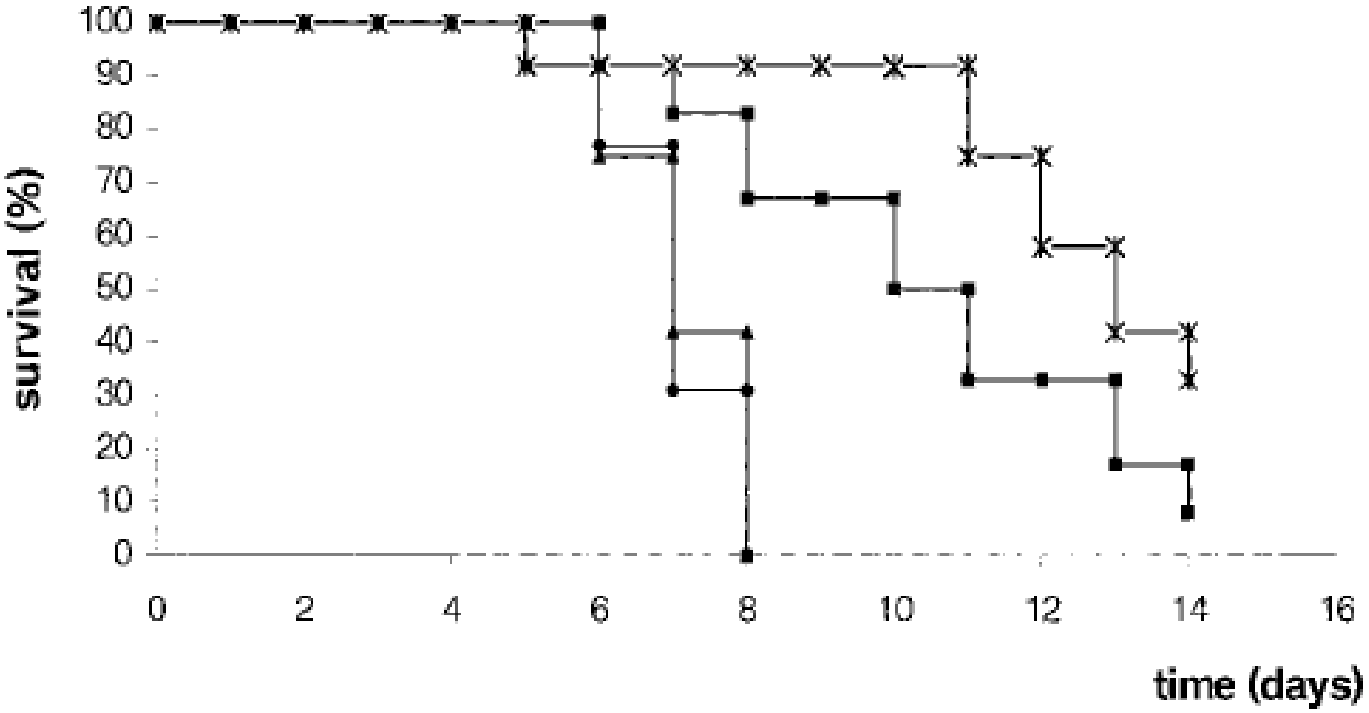


FIG. 5. *Survival curve in the four groups of BALB/c mice infected with 10^6 C. neoformans (H99 strain) and treated as follows: (▲) placebo GF120918 + placebo ITC; (●) GF120918 treatment + placebo ITC; (■) placebo GF120918 + ITC treatment; (✱) GF120918 treatment + ITC treatment (n = 12).*

Drug Metab Disp. 2002

Conclusion

- There is no „breakthrough“
- Toxicity
- Most promissing drugs either already used or developed to overcome P-GP
- TBC potentially first application area

The “Magic Bullet”?