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In silico studies of aminated thioxanthones: bacterial multidrug efflux pumps *vs* P-glycoprotein

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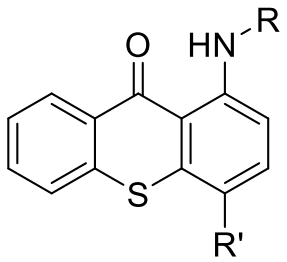
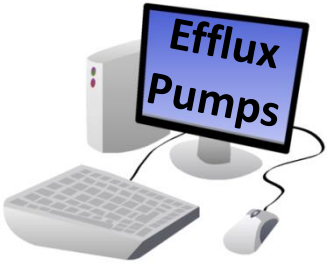
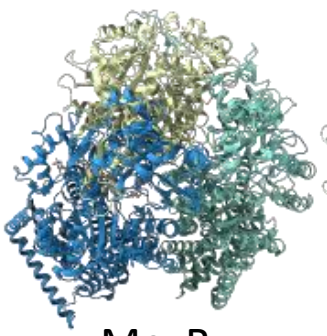
In silico studies of aminated thioxanthones: bacterial multidrug efflux pumps *vs* P-glycoprotein

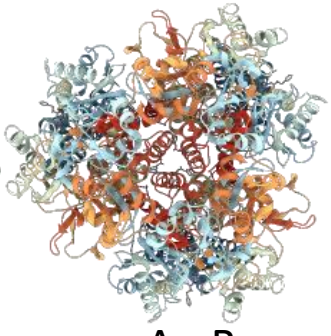


≈ 1000 aminated (thio)xanthones

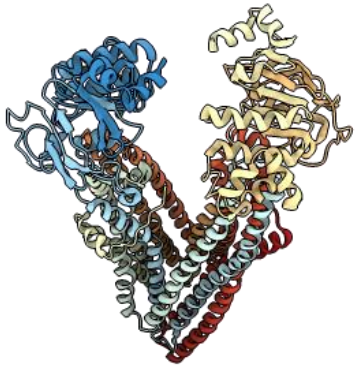




MexB



AcrB



P-glycoprotein

Abstract:

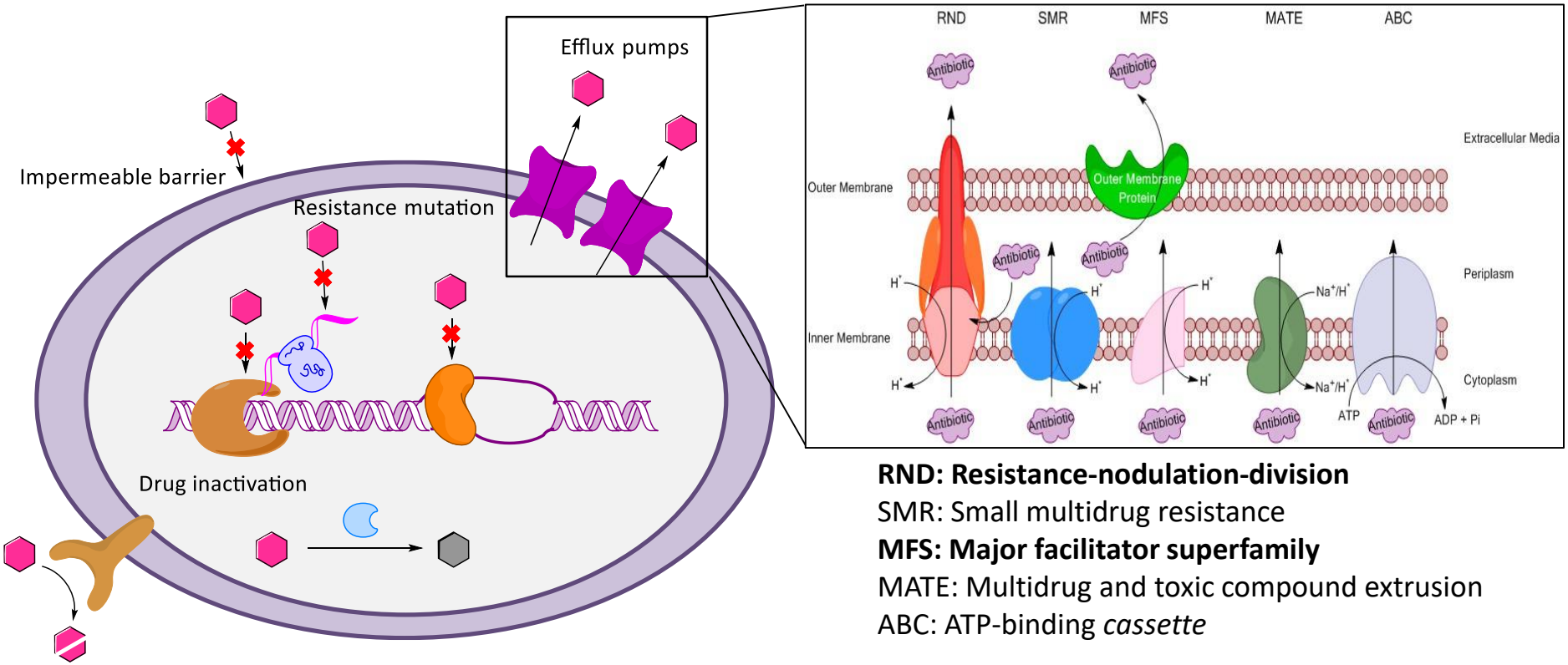
Antimicrobial resistance can arise from several reasons, among which is the overexpression of efflux pumps. This allows bacteria to develop multidrug resistance, through the extrusion of antimicrobial drugs. They can be divided into five families, being the resistance-nodulation-division (RND) family and the major facilitator superfamily (MFS) the most relevant. Efforts have been put towards a selective, efficient efflux pump inhibitor (EPI), but no EPI has yet been introduced in the therapeutic scenario.

The aim of this work was the design of a virtual library of approximately 1.000 aminated (thio)xanthones, the performance of docking studies in bacterial efflux pumps whose crystal structure has been elucidated and available, and in a model of the human P-glycoprotein (P-gp).

The compounds that will be selected for synthesis are the ones that virtually displayed good scores for the bacterial referred efflux pumps and lower scores for P-gp, since this would mean that, *in vivo*, these compounds would efficiently reduce antimicrobial resistance while not interfering with human detoxification pathways.

Keywords: thioxanthones; docking; bacterial efflux pumps; P-glycoprotein.

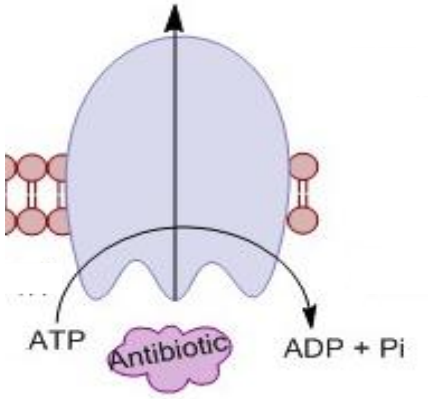
Introduction



RND: Resistance-nodulation-division
SMR: Small multidrug resistance
MFS: Major facilitator superfamily
MATE: Multidrug and toxic compound extrusion
ABC: ATP-binding *cassette*

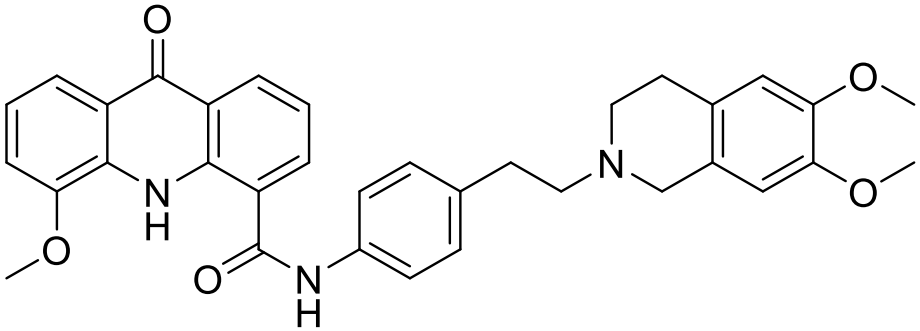
Mechanisms of antimicrobial resistance in a Gram-negative bacterial cell, with emphasis on efflux pumps (adapted from Allen *et al*. Nat Rev Micro. 2010;8(4):251-9 and Durães *et al*. Curr Med Chem. 2018)

Introduction



ABC transporters

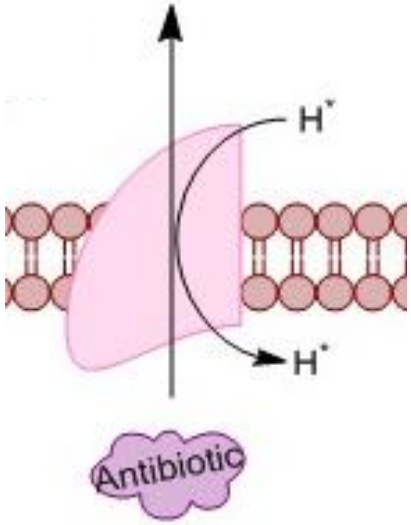
- Ubiquitous of all systems (eukaryotic and prokaryotic)
- Four conserved domains:
  - Two transmembrane domains
  - Two cytoplasmic domains - responsible for ATP binding
- Antibiotics, sugars, amino acids and vitamins are examples of substrates.



GG918, an example of an ABC inhibitor

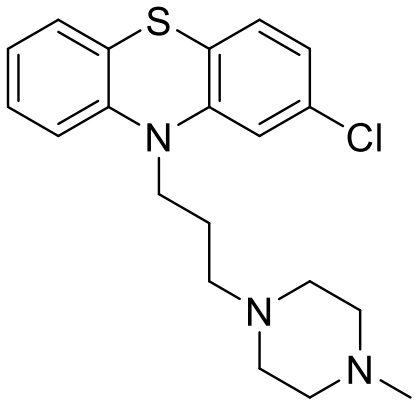
Durães *et al*. Curr Med Chem. 2018

Introduction



MFS transporters

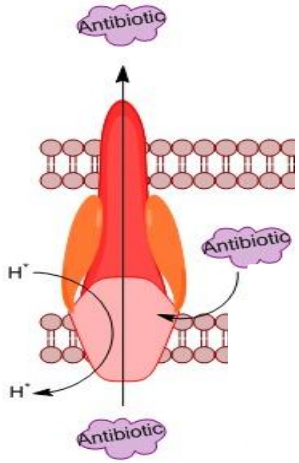
- Largest and most extensively studied family of transporters
  - Uniporters, symporters and antiporters
- Ions, carbohydrates, lipids, amino acids and nucleosides are substrates
- 12 transmembrane domains:
  - Four helices facing away from the interior cavity
  - Eight helices forming the internal cavity
- Most studied pump:
  - NorA (*Staphylococcus aureus*)



Prochlorperazine, an example of a MFS inhibitor

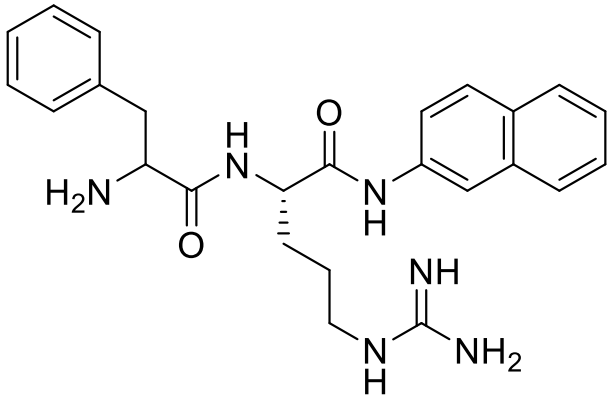
Durães *et al*. Curr Med Chem. 2018

Introduction



RND transporters

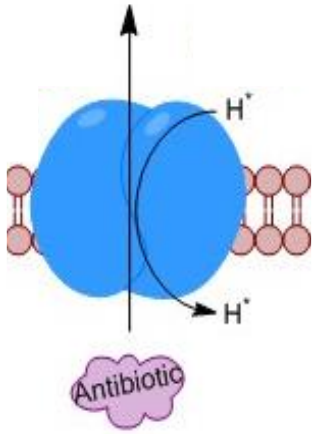
- Mostly present in Gram-negative bacteria
- Antibiotics, dyes, antiseptics and detergents are examples of substrates of these pumps
- Have a unique tripartite complex, constituted by a minimum of 12 transmembrane segments:
  - Transmembrane pump
  - Outer membrane channel
  - Periplasmic adaptor protein
- Most studied pumps:
  - AcrAB-TolC (Enterobacteriaceae)
  - MexAB-OprM (*Pseudomonas aeruginosa*)



Phenyl-arginine β-naphtylamide, an example of a RND inhibitor

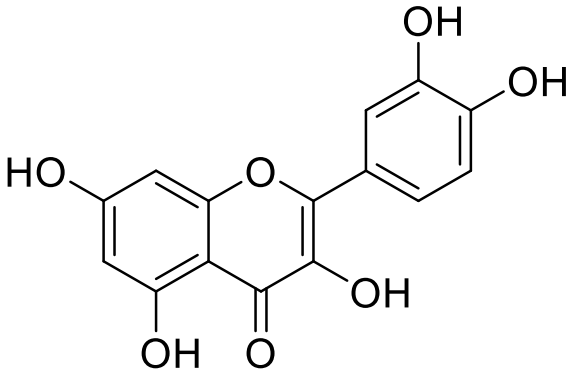
Durães *et al*. Curr Med Chem. 2018

Introduction



SMR transporters

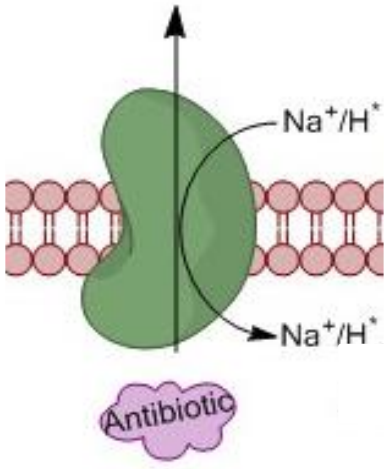
- Smallest drug efflux proteins known
  - Four transmembrane domains
- Exclusive to bacteria
- Efflux of lipophilic compounds:
  - Quaternary ammonium salts
  - Antibiotics
- Most studied pump:
  - EmrE (*Escherichia coli*)



Quercetin, an example of a SMR inhibitor

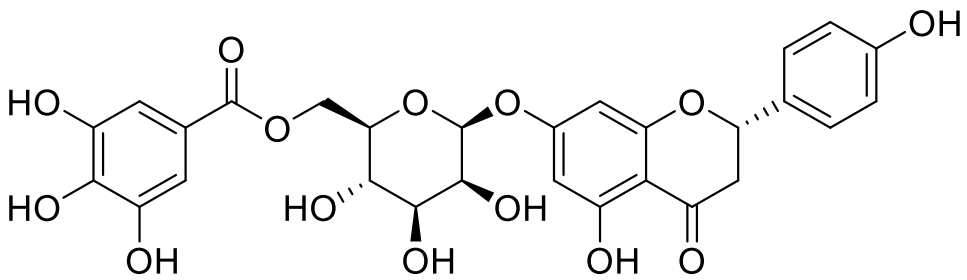
Durães *et al*. Curr Med Chem. 2018

Introduction



MATE transporters

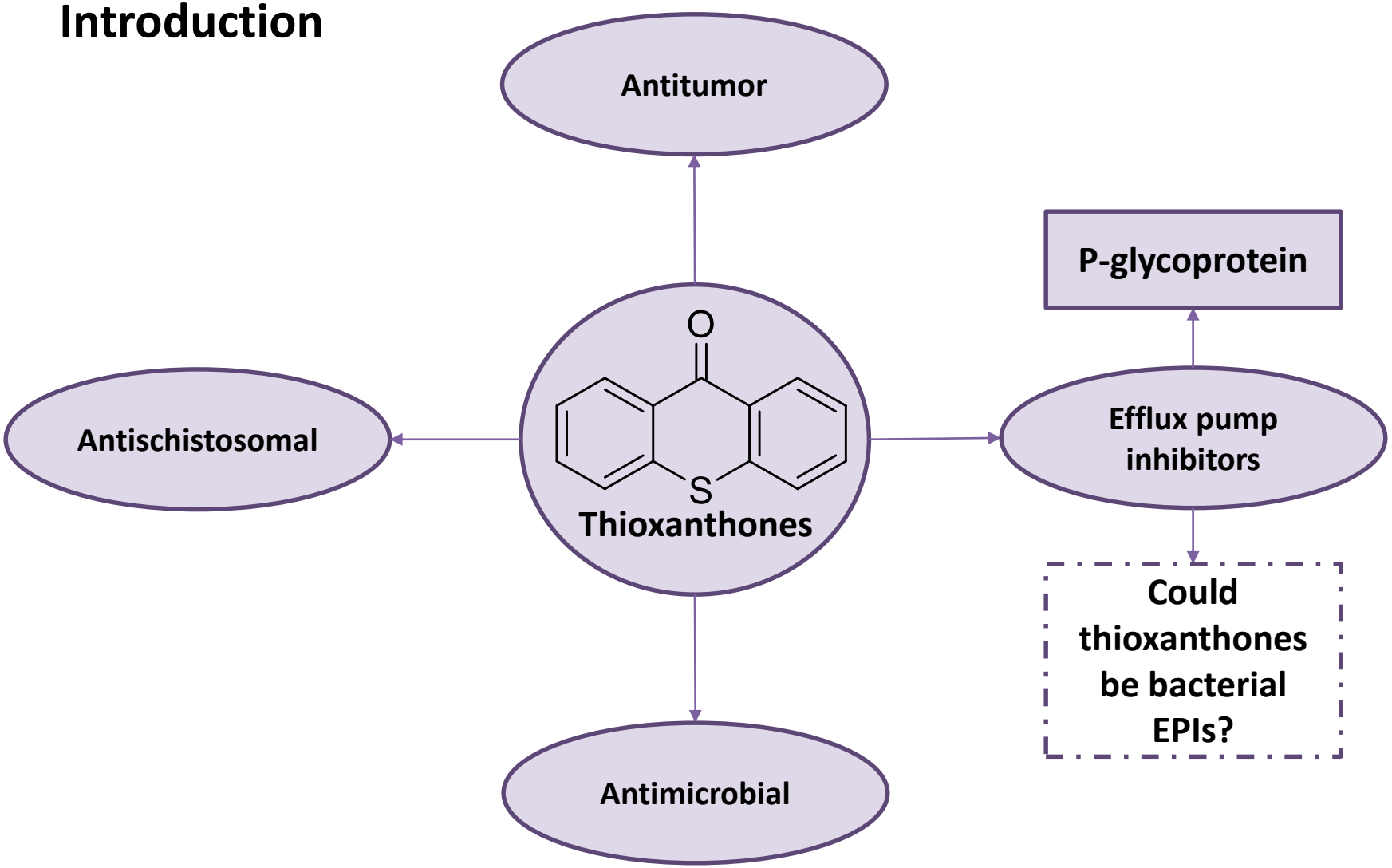
- Use the sodium gradient as energy source, as well as the proton gradient
- Twelve transmembrane helices
- Efflux of cationic, lipophilic compounds
- Most studied pumps:
  - NorM (*Neisseria* sp.)
  - MepA (*S. aureus*)



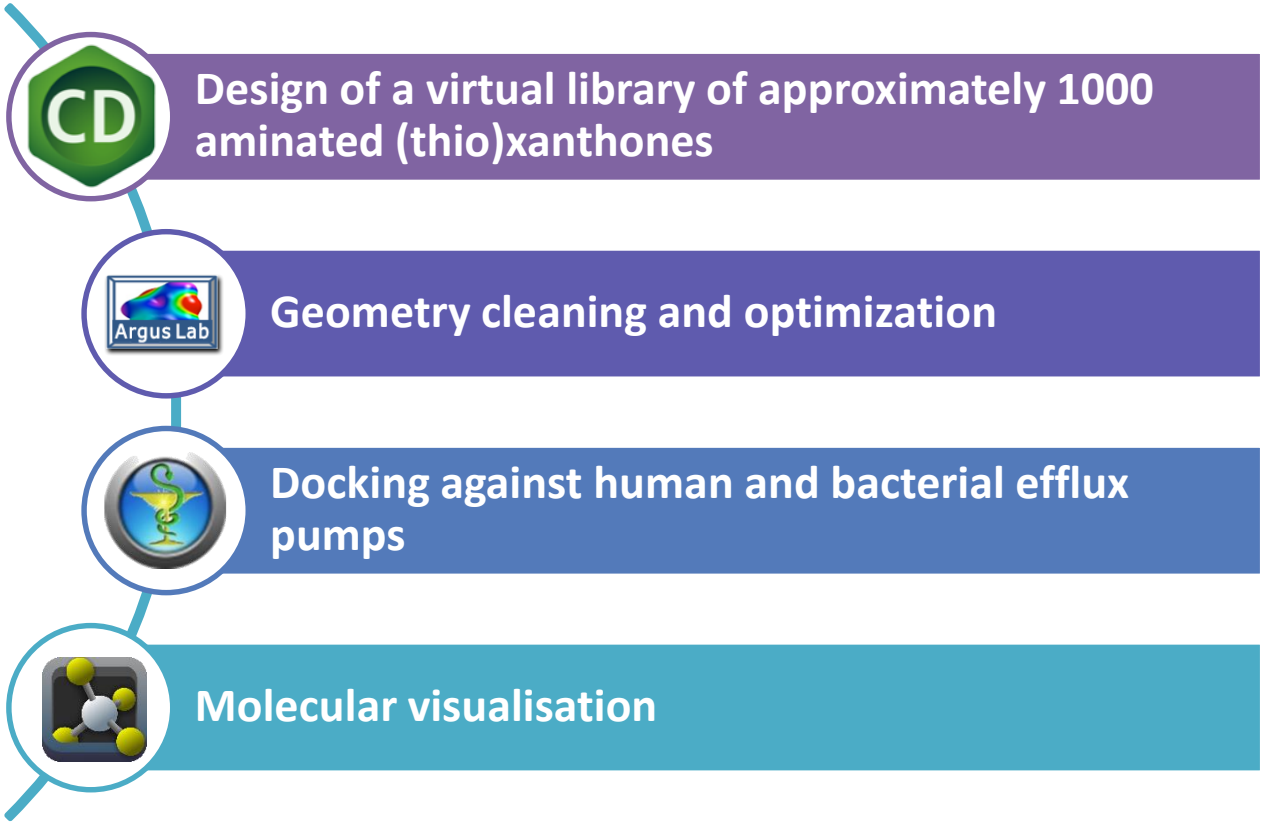
Prunin 7''-*O*-gallate, an example of a MATE inhibitor

Durães *et al*. Curr Med Chem. 2018

Introduction



Results and discussion



Results and discussion

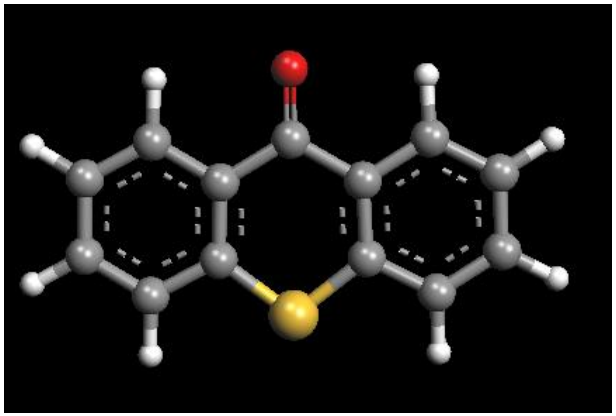
Design of a virtual library of approximately 1000 aminated (thio)xanthones

- **Software used: ChemDraw Professional 16.0**
- **Design of aminated thioxanthones based on a thioxanthone that had previously shown good results in modulating human efflux pumps**
- **Design of aminated xanthones based on a xanthone synthesized by our group**
- **Amines were chosen based on what was commercially available from three different suppliers**

Results and discussion

Geometry cleaning and optimization

- **Software used: ArgusLab 4.0.1**
- **Energy minimization – molecule reaches its most stable conformation**
- **Geometry optimization, using Hamiltonian mechanics – quantum mechanics, AM1**



Results and discussion

Docking against human and bacterial efflux pumps

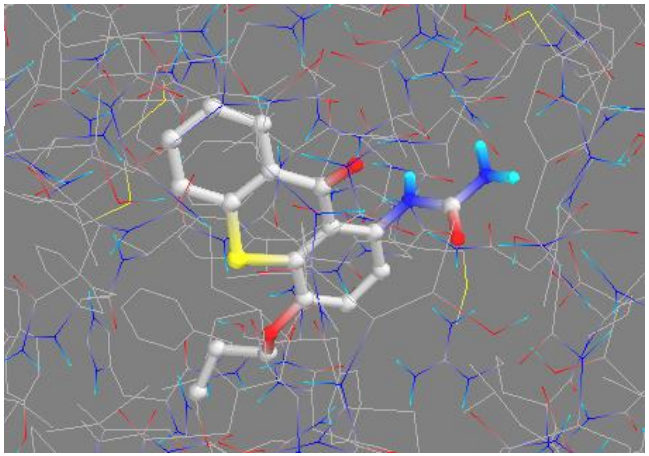
- Software used: PyRx 0.8
- Docking performed using AutoDock Vina
- Bacterial efflux pumps used were AcrB (PDB: 1T9Y) and MexB (PDB: 2V50), available in the Protein Data Bank
  - Docking in two different sites each, according to the described in literature
- Human efflux pump used was a model of P-glycoprotein
  - Docking into the transmembrane and nucleotide binding domains

Results and discussion

Docking against human and bacterial efflux pumps

- **Analysis of the docking scores – geometrical fit and favorable interactions**
  - **Lower binding energy for bacterial efflux pumps and higher for P-gp**

TXU1	2v50	MexB	-8.2
TXU1	1t9y	AcrB	-6.7
TXU1		P-glycoprotein	-7.4

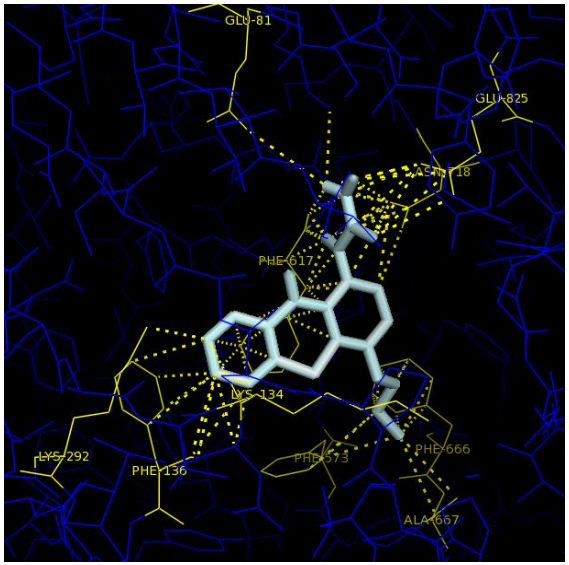


Results and discussion

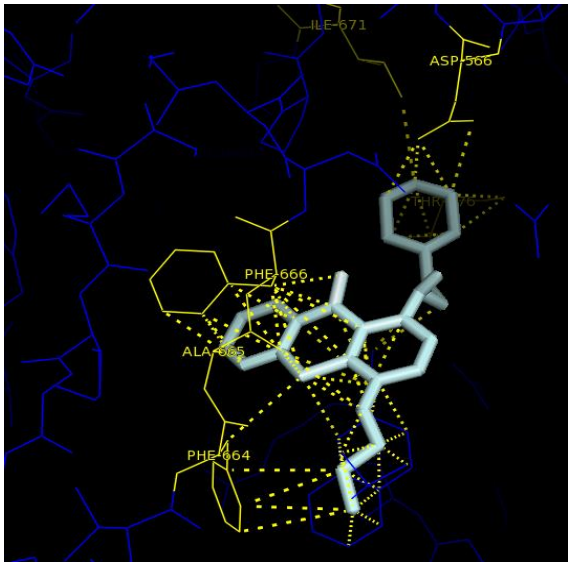
Molecular visualisation

- **Software used: PyMOL 1.1**

- **Visualisation of the binding of the ligand and the receptor**
  - **Interactions between efflux pump residues and the molecules**

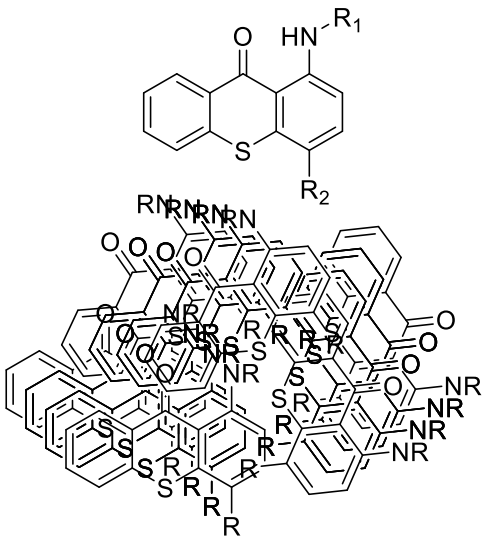


Thioxanthone interacting with MexB



Thioxanthone interacting with AcrB

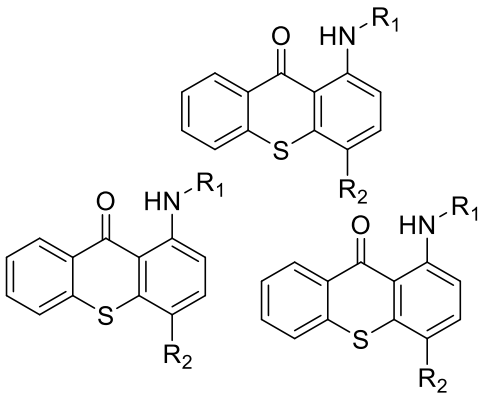
Results and discussion



1000 (thio)xanthones

Good docking scores for bacterial efflux pumps
Higher binding energy for human P-gp

Feasible synthesis
Drug likeness

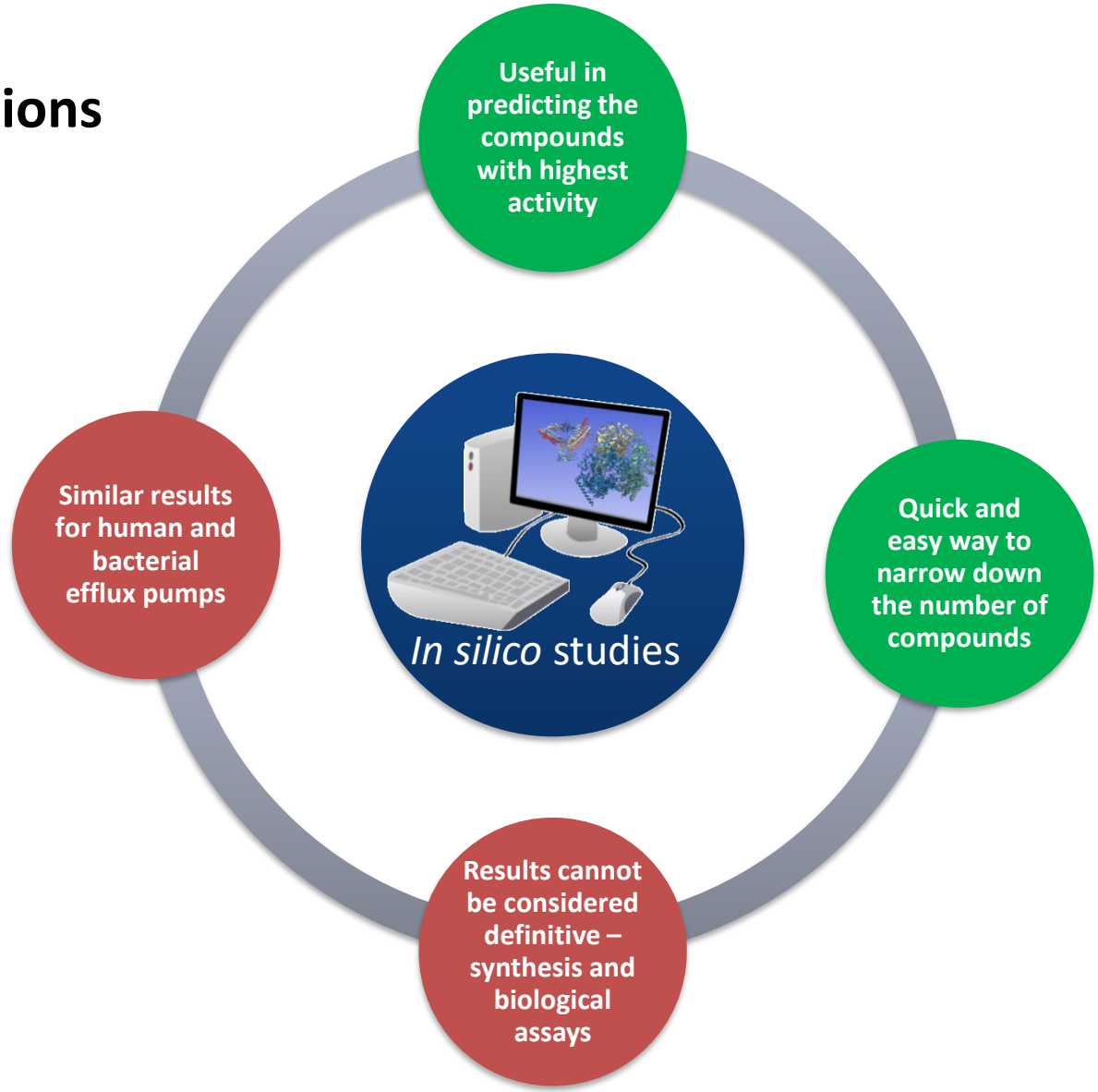


30 (thio)xanthones

Ongoing work

Synthesis

Conclusions



Acknowledgments

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