2nd International Electronic Conference on Medicinal Chemistry

1-30 November 2016

chaired by Dr. Jean Jacques Vanden Eynde

sponsored by pharmaceuticals

Studying the influence of stereochemistry in P-gp modulation: case-study with thioxantones

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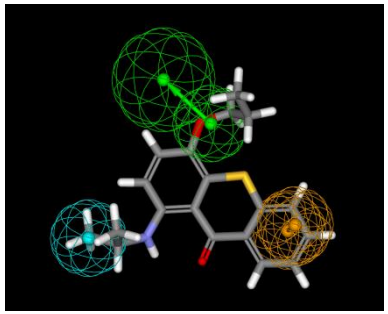
Studying the influence of stereochemistry in P-gp modulation: case-study with thioxantones

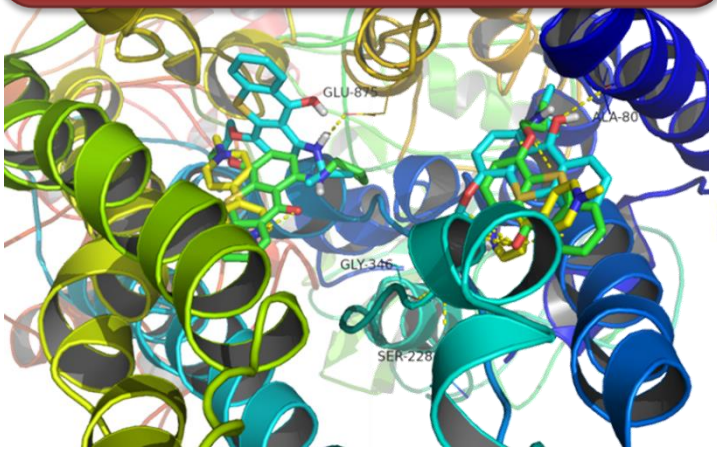
Graphical Abstract

Eight newly synthesized thioxanthonic derivatives

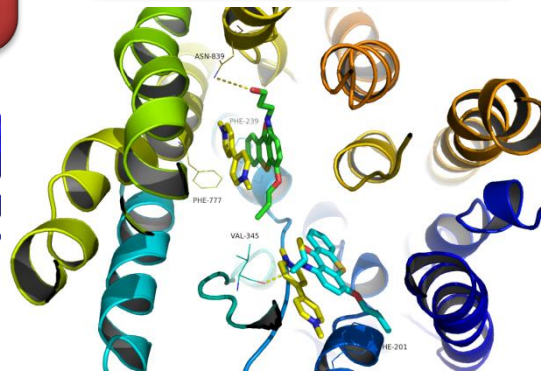


Combination of *in vitro* and *in silico* studies

- **Did not increase P-gp expression**
- **Increased Rho123 efflux**
- **Did not increase P-gp ATPase activity**



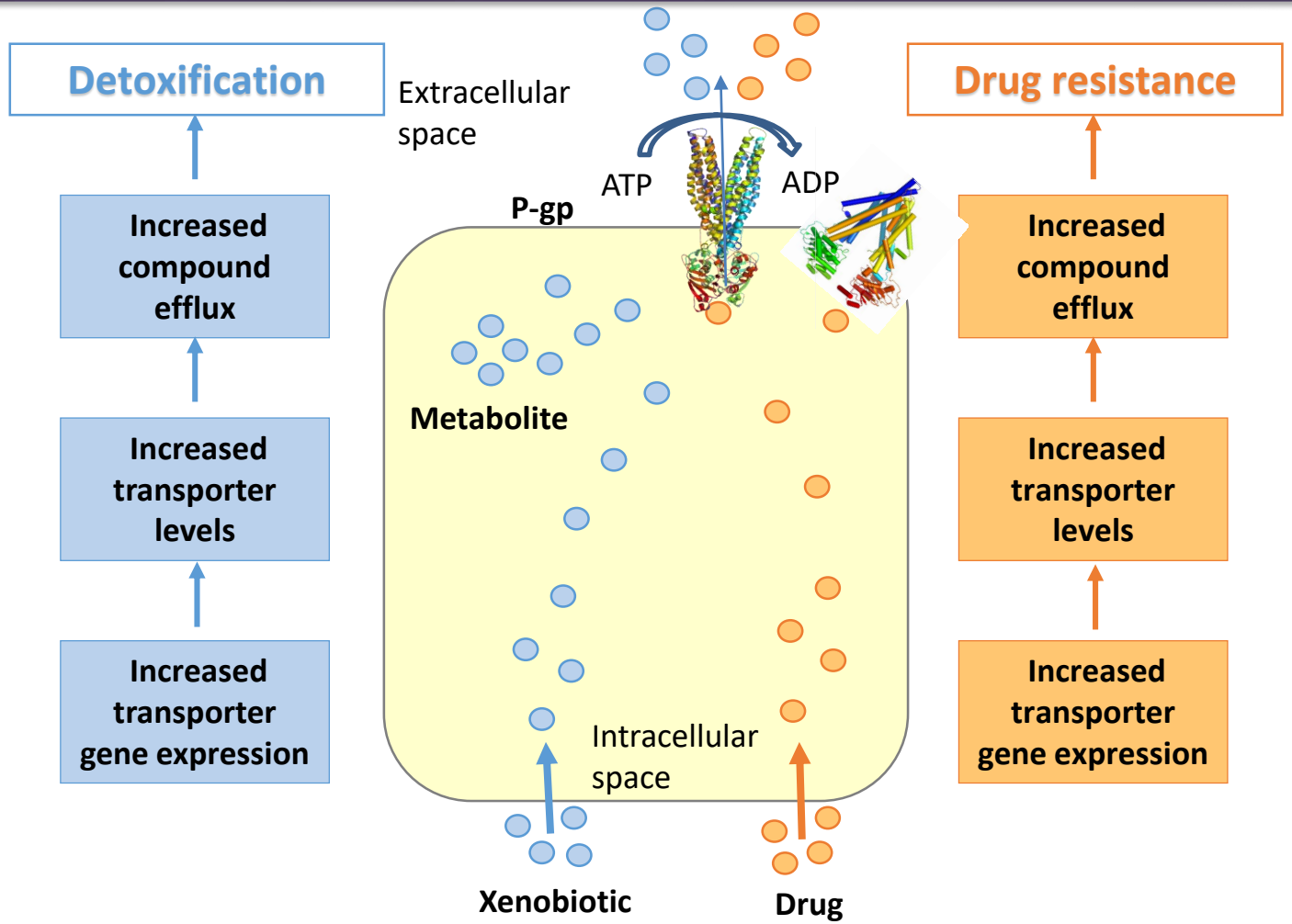
Potential new source of antidotes against PQ intoxications



Study the influence of stereochemistry

Abstract:
Chirality is an interesting geometric property and it is meaningful to explore the interactions between chiral small molecules and stereoselective biomacromolecules, with pre-clinical and clinical significance. Since the first observation of enantioselective binding to human-derived P-glycoprotein (P-gp) by mefloquine enantiomers , sparse stereoselectivity studies with P-gp modulators emerged. Recently, we have shown that newly synthesized (thio)xanthonic derivatives protect against toxic P-gp substrates acting as potent inducers/activators of this transporter. Now we aim to discover new P-gp modulators and enlightening the stereoselectivity of this ABC transporter face to this class of modulators.
Herein, we report the synthesis and characterization of a library of new chiral aminated thioxanthones in their enantiomeric pure form and *in silico* and *in vitro* preliminary results concerning their P-gp modulation behavior.
In silico docking studies in P-gp rat model anticipated enantioselectivity for these new derivatives. Thioxanthones cytotoxicity was evaluated by the Neutral Red uptake assay, in order to select a non-cytotoxic working concentration. The compounds were assessed for their influence in P-gp ATPase assay. The investigation of P-gp expression and activity allowed to discover new P-gp modulators. Nevertheless, no significant differences between enantiomeric pairs of thioxanthones were observed.
Keywords: antidotes, chirality, P-glycoprotein, thioxanthones

Importance of P-glycoprotein (P-gp) modulation in Pharmaceuticals



Special Issue "Can Membrane Transporters Contribute to Drug Discovery?"

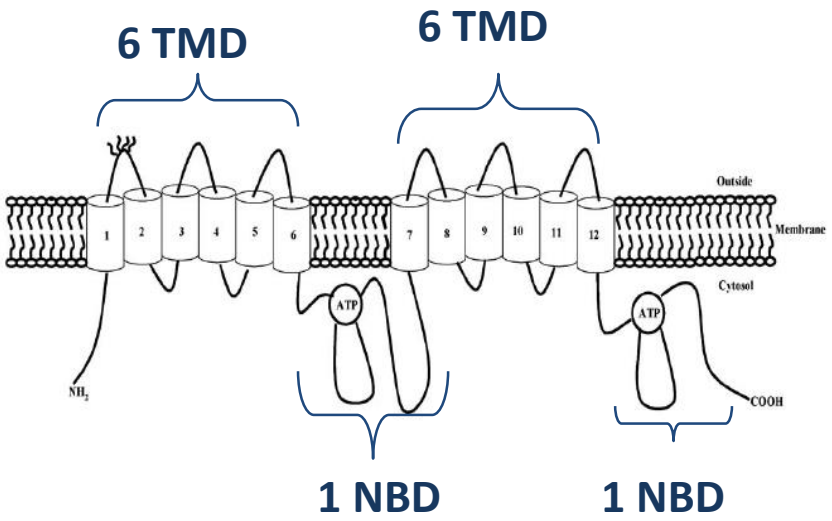
A special issue of *Molecules* (ISSN 1420-3049). This special issue belongs to the section "Medicinal Chemistry".

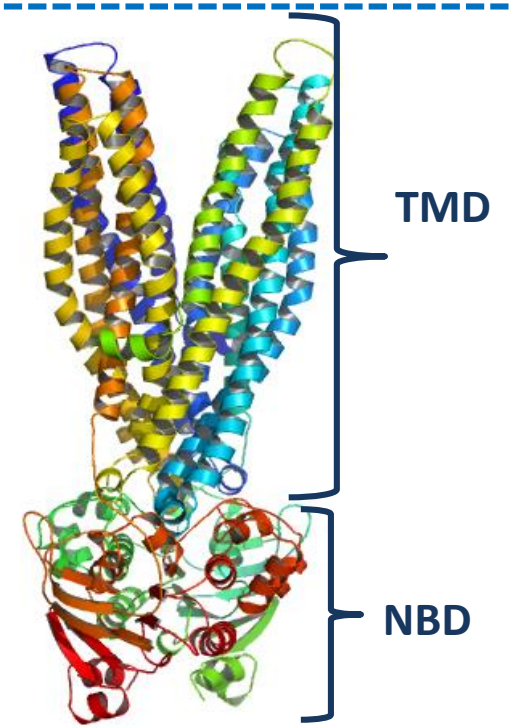
P-gp: The most studied among multidrug resistance (MDR) pumps

- 170 KDa membrane protein
- 1280 a.a.
- (2 repeating units of 610 a.a.

joined by a linker region of about 60 a.a.)

is ATP-binding cassette (ABC)super-family member and is a **membrane transporter** that actively extrudes a set of **structurally unrelated compounds** out of the cells, driven by ATP hydrolysis, conferring the **MDR phenotype in cancer**





NBD - Nucleotide binding domain

TMD -Transmembrane domain

There is still no human P-gp structure at atomic resolution

Andreia Palmeira, Emília Sousa, Helena Vasconcelos, Madalena Pinto, Miguel X. Fernandes. "Three decades of structure and ligand-based design of P-glycoprotein inhibitors", Curr Pharm Des. 2012

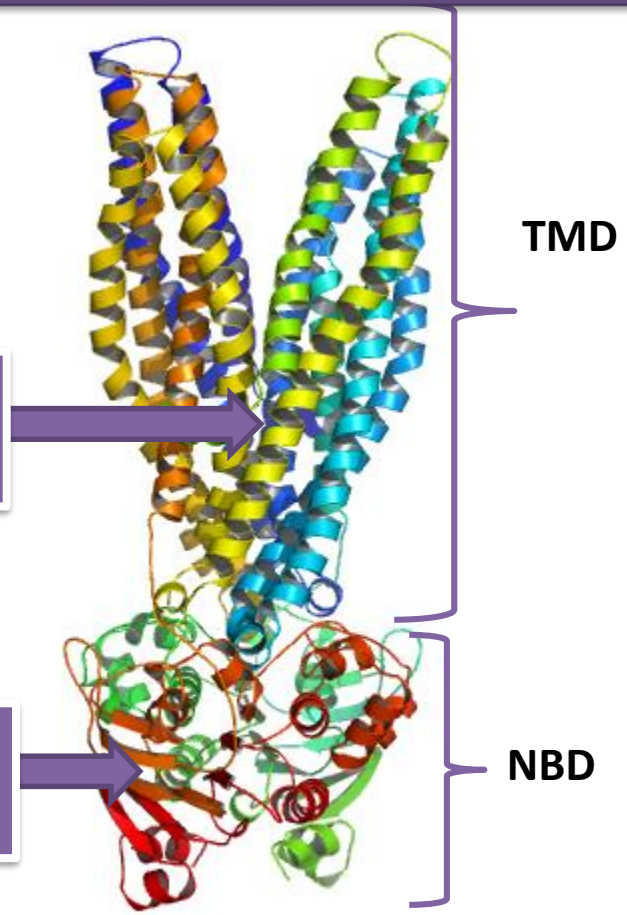
Compounds interacting with P-gp: inhibitors or activators

substrates (inhibitors/activators)

• **direct interaction** with one or more of the **drug-binding sites** on P-gp, thus blocking transport

inhibitors/activators

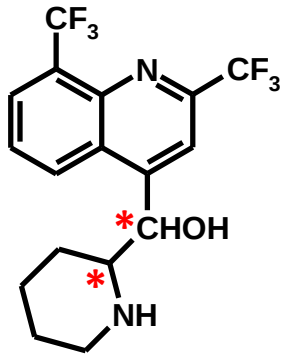
• **interaction with the binding of ATP to ATP-binding site** on P-gp, blocking ATP binding and hydrolysis

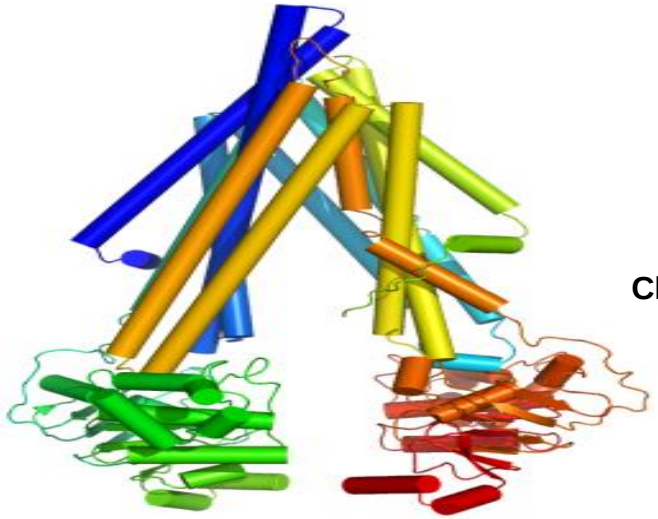


modulators

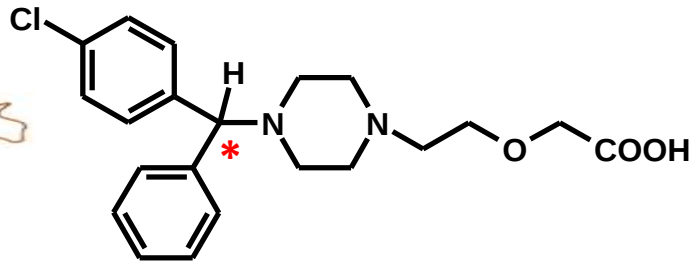
• **interaction with an allosteric residue** relevant for P-gp activity and translocation

Consequences of Chirality on P-glycoprotein





P-glycoprotein



Mefloquine (MQ)

[(+)-MQ >> (−)-MQ]
Blocking drugs uptake

Cetirizine

(*R*)-cetirizine upregulates P-gp expression

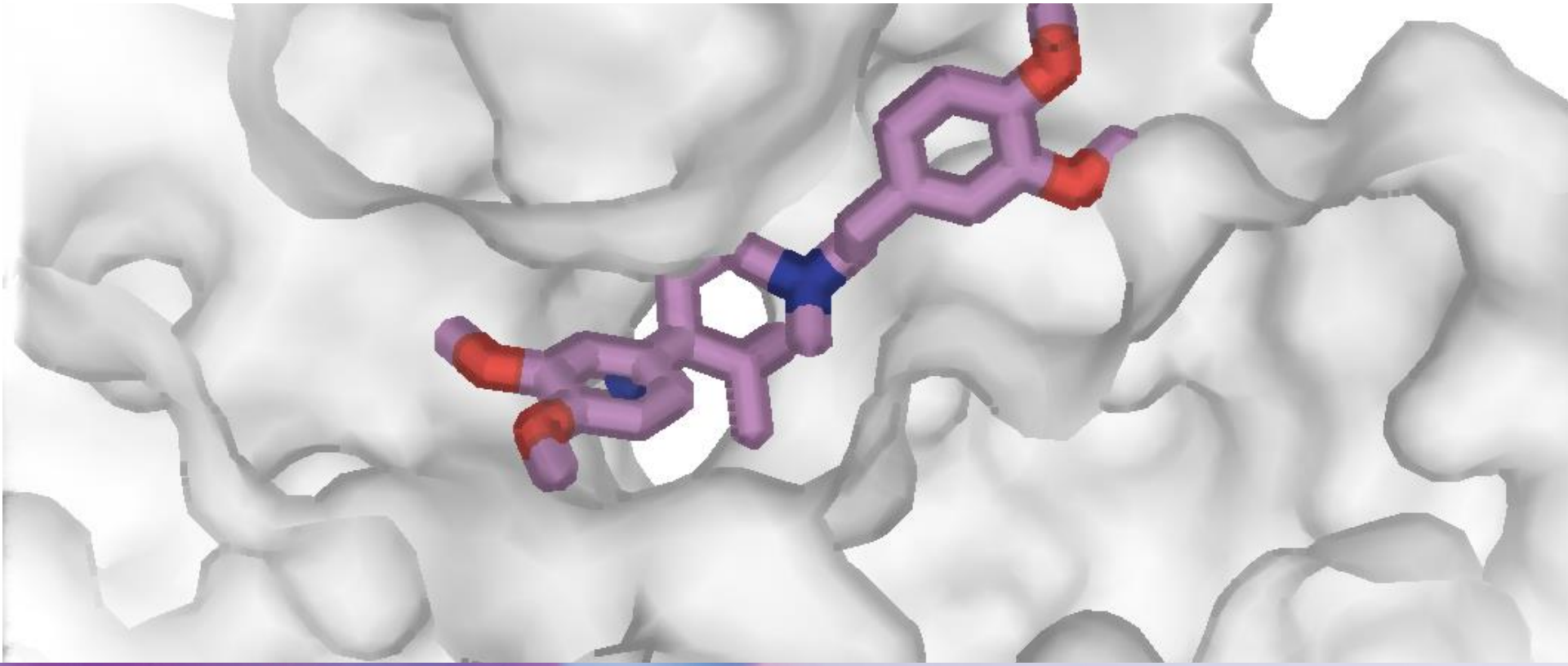
(*S*)-cetirizine down-regulates P-gp expression

Our initial goal in this research field: Overcoming resistances…

Dual inhibitors of P-glycoprotein and tumor cell growth: (Re)discovering thioxanthones

Biochemical Pharmacology 83 (2012) 57–68

Andreia Palmeira [a,b,c], M. Helena Vasconcelos [c,d], Ana Paiva [a,b], Miguel X. Fernandes [e], Madalena Pinto [a,b], Emília Sousa [a,b,*]



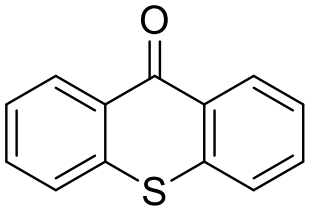
By serendipity

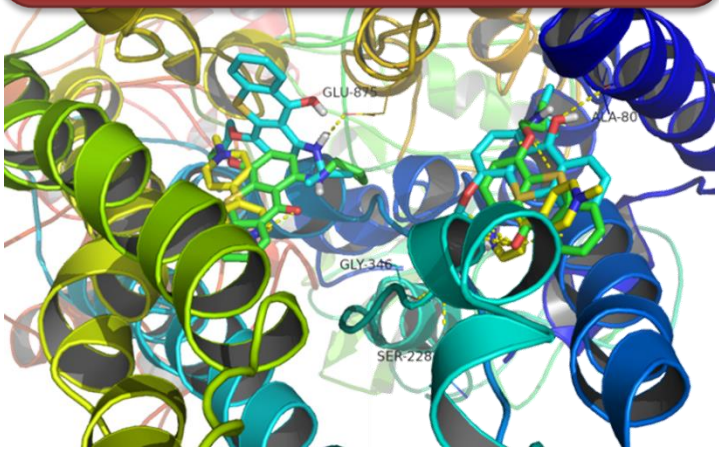
Discovery of P-glycoprotein activators

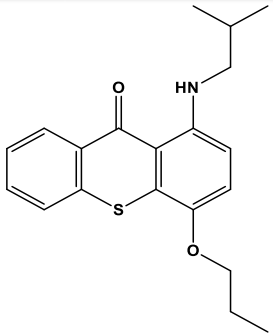
Library of (thio)xanthonic derivatives

- Increased P-gp expression
- Increased Rho123 efflux
- Increased P-gp ATPase activity

Significant reduction in PQ-induced cytotoxicity







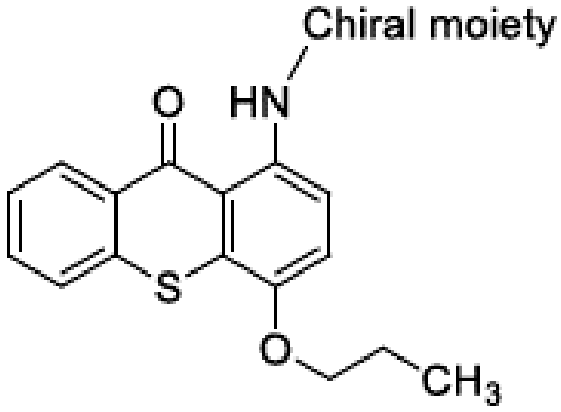
Combination of *in vitro* and *in silico* studies

Potential new source of antidotes against PQ intoxications

Silva, R., et al. P-glycoprotein induction in Caco-2 cells by newly synthetized thioxanthones prevents Paraquat cytotoxicity. Archives of Toxicology, 2015, 89(10):1783-800.

Aims

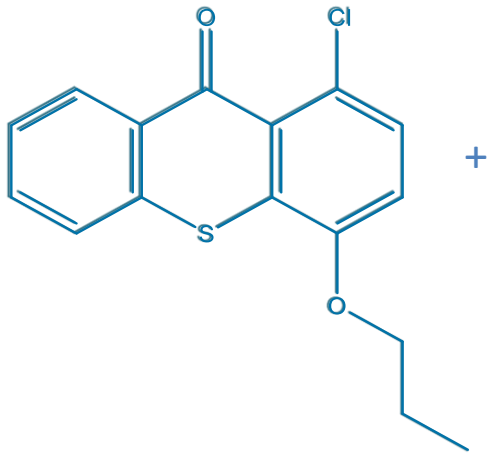
Synthesis and characterization of a library of new chiral aminated thioxanthones in their enantiomeric pure form



***In silico* and *in vitro* studies concerning their P-gp modulation behavior**

Results and discussion

Synthesis of new chiral aminated thioxanthones

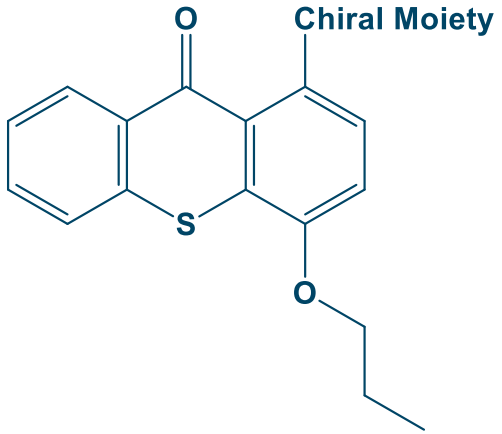


\+

Enantiomerically pure amino alcohol

CH_3OH, CuI_2, K_2CO_3

100 ^{0}C, 48h



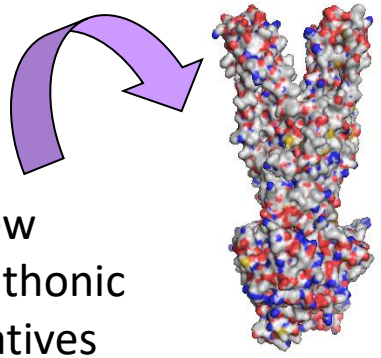
Chiral aminated thioxanthones 1-8

General scheme of *N*-arylation of 1-chloro-4-propoxythioxanthone

Results and discussion

In silico studies: docking scores using several softwares

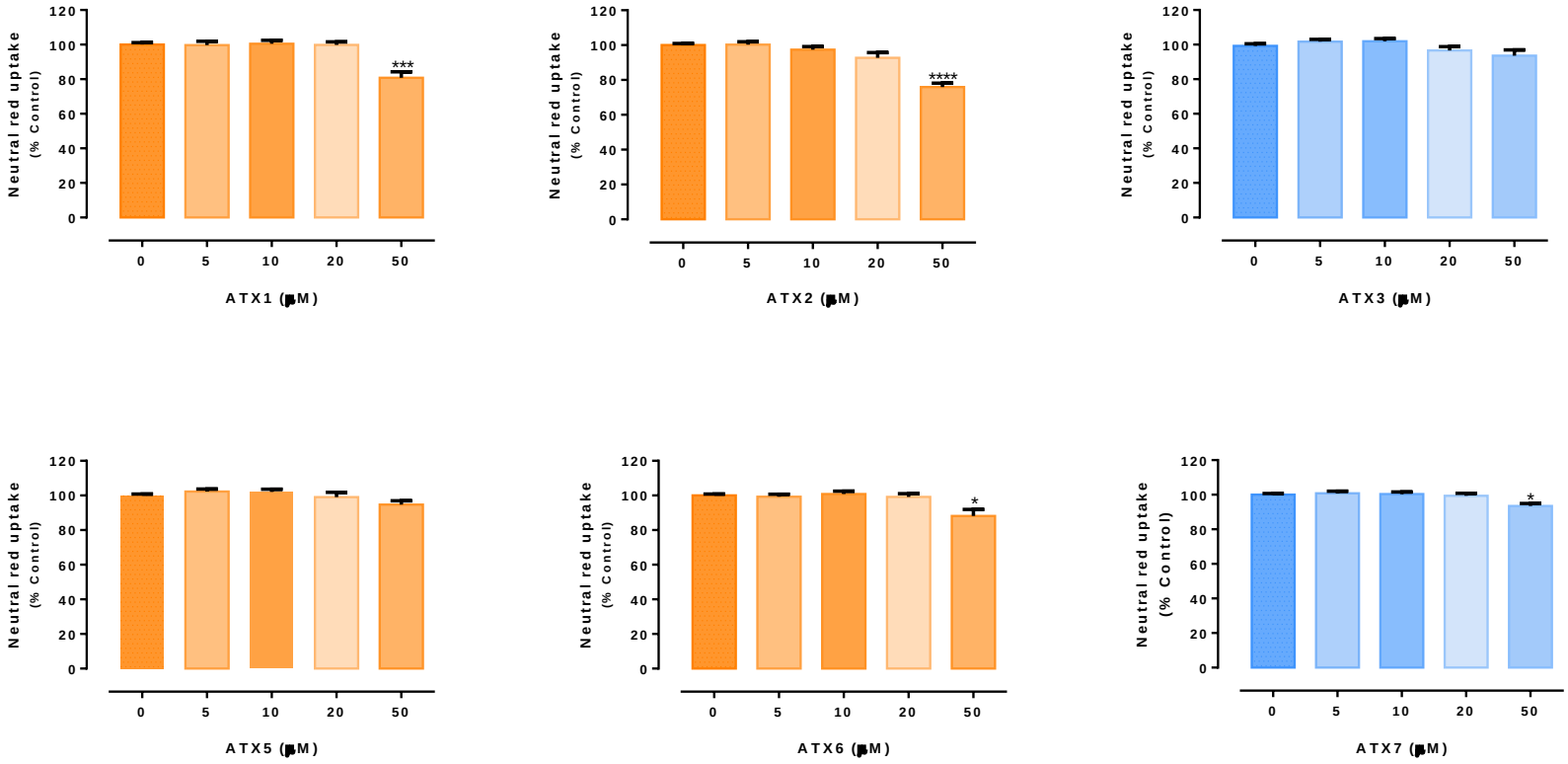
New thioxanthonic derivatives



			Surflex				
Ligand	Vina	FlexX	D_score	PMF_score	G_score	ChemScore	C-SCORE
ATx1-R	-6.6	-16.1426	33421.386	47.0529	-149.407	-31.1172	0.3456
ATx2-S	-6.6	-15.877	33256.2187	57.5923	-157.7654	-31.2803	0.6195
ATx3-R	-6.6	-12.9367	32923.759	36.5261	-141.8191	-27.2344	1.07
ATx4-S	-6.7	-13.2639	32269.1787	-10.9256	-132.7253	-29.2204	0.4771
ATx5-R	-7.3	-15.2651	36822.6008	96.7188	-174.0873	-32.4831	1.5495
ATx6-S	-7.1	-12.1209	36176.2518	87.9657	-170.2021	-30.7121	0.9091
ATx7-R	-6.9	-13.1639	35977.475	52.3755	-159.6309	-32.657	0.6761
ATx8-S	-7	-11.2137	35733.1555	41.5815	-162.152	-33.3319	0.9564

Results and discussion

Cytotoxicity



ATx 1 - ATx 8 (0 – 50 µM) cytotoxicity in Caco-2 cells evaluated by the Neutral Red uptake assay, 24 hours after exposure. Results are presented as mean ± standard error mean (SEM) from at least 5 independent experiments (performed in triplicate).

Results and discussion

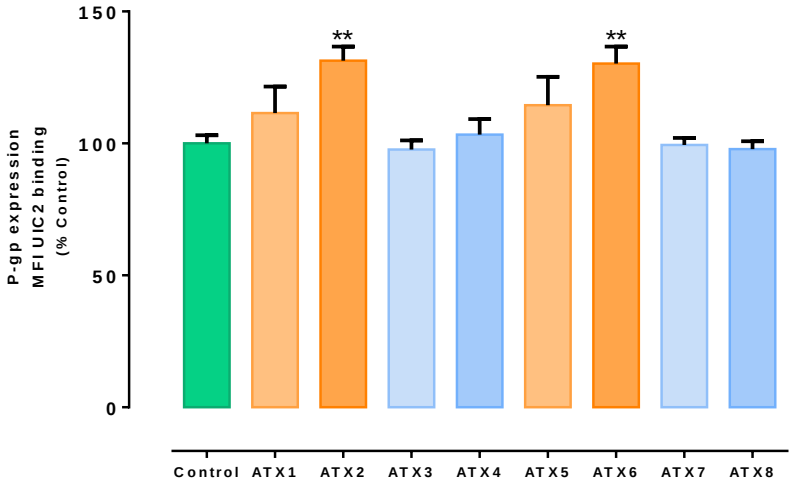
Flow cytometry analysis of P-glycoprotein expression

Incubation ATx 1-8 20 µM (24 h)

Trypsinization of cells

Incubation with P-gp antibody [UIC2] (30 min)

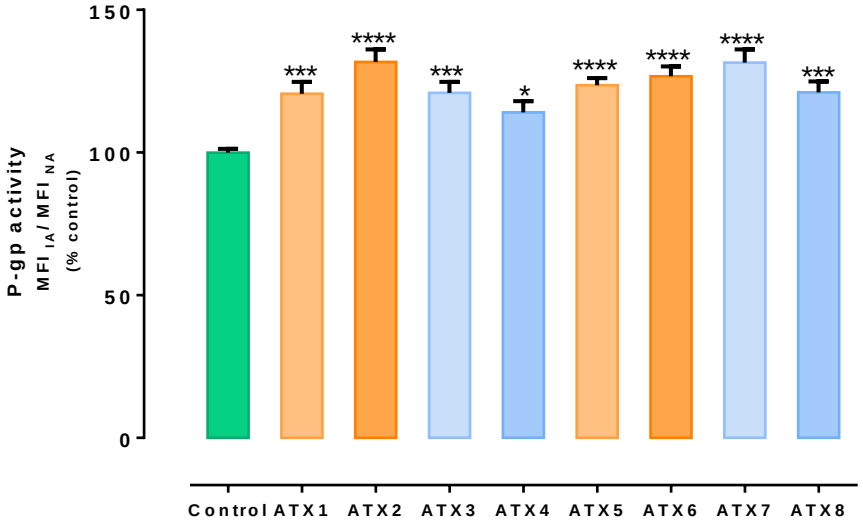
Mean of fluorescence intensity (MFI) of UIC2-PE antibody



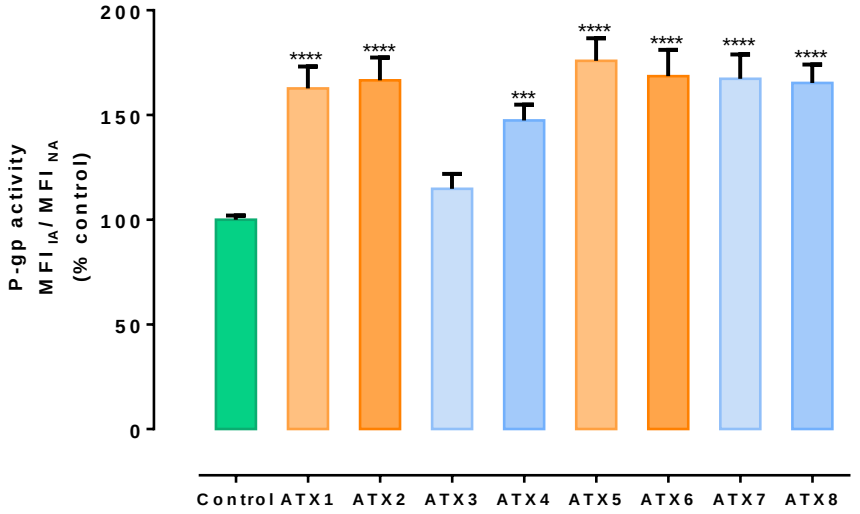
Results are presented as mean ± standard error mean (SEM) from 2 independent experiments (performed in duplicate).

Results and discussion

Evaluation of P-glycoprotein transport activity



RHO 123 accumulation in the presence of ATx's (20 µM) during the RHO123 accumulation phase.

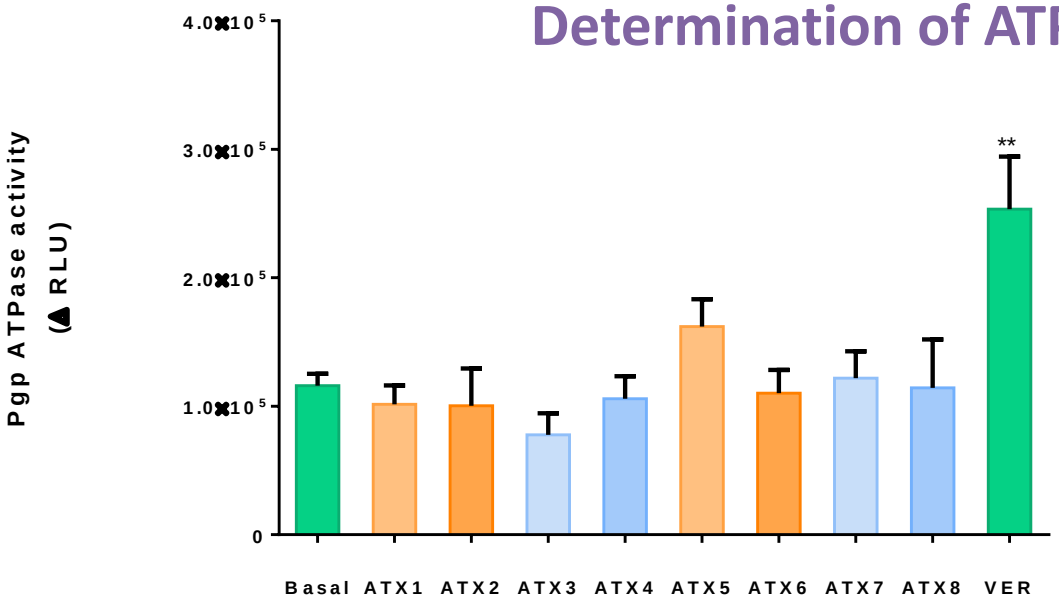


RHO 123 accumulation in Caco-2 cells exposed to ATx's (20 µM) for 24 hours.

$$\textbf{\textit{RHO 123 Accumulation}} = \frac{\text{MFI inhibited rhodamine 123 (RHO123) accumulation (IA)}}{\text{MFI normal RHO123 accumulation (NA)}}$$

Results and discussion

Determination of ATPase activity



Basal P-gp ATPase activity

ΔRLU basal = RLU Na_3VO_4 – RLU NT

Results are presented as mean ± standard error mean (SEM) from 2 independent experiments (performed in duplicate).

P-gp ATPase Activity in the presence of a test aminated thioxanthones (ATx)

ΔRLU ATx = RLU Na_3VO_4 – RLU ATX

NT = non-treated membranes
Na_3VO_4 = sodium orthovanadate used as a selective P-gp inhibitor
VER = verapamil used as positive control (P-gp substrate, stimulating P-gp ATPase activity while being transported)

Conclusions

- **Thioxanthonic derivatives and, particularly, aminated thioxanthones, have been characterized as P-gp modulators.**
- **Four enantiomeric pairs of thioxanthonic derivatives were synthesized, with success, in an enantiomerically pure form, using the Ullmann cross coupling reaction.**
- **Spectroscopic methods allowed the unambiguous elucidation of all the thioxanthonic derivatives (ATx 1-8) and the absolute values of specific rotation confirmed that no racemization occurred under these reaction conditions.**
- **ATx's 1-8 have the ability the increase the P-gp activity without interfering with its levels of expression and, therefore, can be characterized as P-gp activators.**
- **ATx 2 and ATx 7 demonstrated to be the more efficient P-gp activators, among all the aminated thioxanthones tested.**
- **No significant enantioselectivity was observed for all the tested enantiomeric pairs.**

Acknowledgments

This work was partially supported through national funds provided by: FCT - Foundation for Science and Technology and European Regional, Development Fund (ERDF) and COMPETE under the projects PEst-C/MAR/LA0015/2013, PTDC/MAR-BIO/4694/2014, and INNOVMAR - Innovation and Sustainability in the Management and Exploitation of Marine Resources, reference NORTE-01-0145-FEDER-000035, Research Line NOVELMAR.