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In silico studies of bacterial efflux pump inhibition by thioxanthones and their synergistic antibacterial activity

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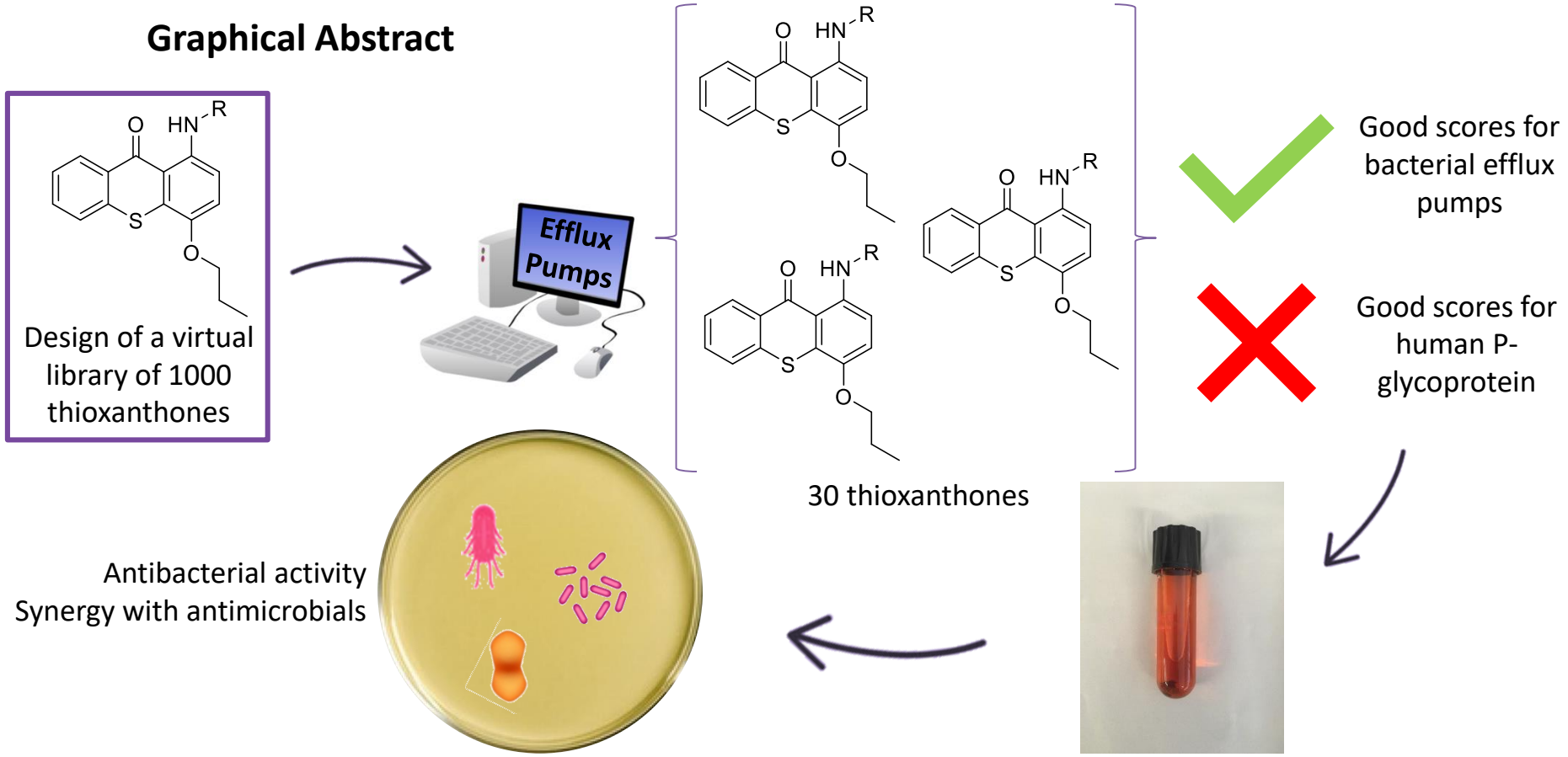
In silico studies of bacterial efflux pump inhibition by thioxanthones and their synergistic antibacterial activity

Graphical Abstract



Abstract:

Efflux pumps are transmembrane transporters, ubiquitous in bacteria, that can actively extrude several antimicrobial drugs from within cells into the external environment, allowing bacteria to develop multidrug resistance. Efforts have been put towards a selective, efficient efflux pump inhibitor (EPI), and although some progress has been achieved, no EPIs have been approved in the therapeutic scenario. This problem leads to the inefficacy of a large amount of antimicrobial drugs, with antimicrobial resistance posing one of the most urgent threats concerning health problems of our days.

Thioxanthones are heterocyclic, privileged structures with a dibenzo-γ-thiopyrone scaffold. Previous work by our group has demonstrated the potential of these compounds as human efflux pump modulators.

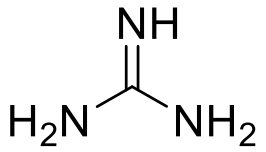
In this scope, a virtual library of approximately 1000 thioxanthones was designed, and *in silico* studies were performed. The compounds that displayed good docking scores were selected to be synthesized. The synthesis of thioxanthones was performed using a copper-catalysed Ullmann coupling.

Antibacterial activity and synergism assays with antibacterial drugs were performed, with two compounds displaying promising results in combination with antibacterial drugs, although with no relevant antimicrobial activity. Future studies will involve insights into the mechanism of synergy of promising compounds.

Keywords: efflux pumps; thioxanthones; antibacterial; synergy.

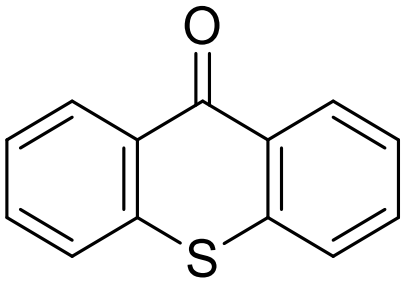
Introduction

Guanidine



Neutral, nitrogen-containing compounds
Present in bacterial efflux pump inhibitors

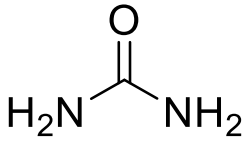
Thioxanthones



Dibenzo-γ-thiopyrone scaffold
Privileged structure
Diverse biological activities

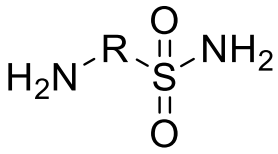
- Antitumor
- Antimicrobial
- Human efflux pump modulation

Urea



Bioisostere of guanidine
Present in bacterial efflux pump inhibitors

Sulphonamides



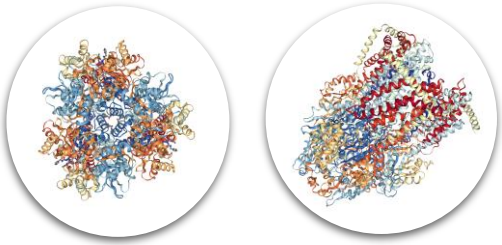
Antibacterial drugs

Palmeira A *et al*. *Biochem Pharmacol*. 2012;83(1):57-68; Tan CH *et al. Aust J Chem*. 2014;67(7):963-4; Durães F *et al*. *Curr Med Chem*. 2018;25(42):6030-69.

Aims

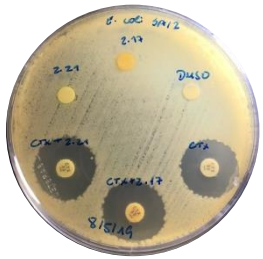
Design of a virtual library of 1000 aminated thioxanthones

In silico studies on bacterial and human efflux pumps



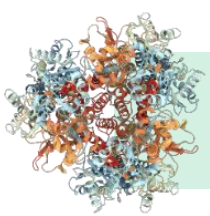
Synthesis of virtual hits for bacterial efflux pumps inhibitors

Antibacterial activity and synergy with antimicrobials

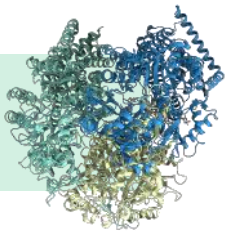


In silico studies

- Design of a virtual library of approximately 1000 aminated (thio)xanthones
- Geometry cleaning and optimization
- Docking against human (P-glycoprotein) and bacterial efflux pumps (AcrB and MexB)
- Molecular visualisation

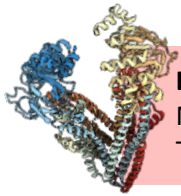


High scores



AcrB (PDB: 1T9Y)

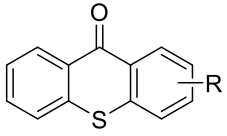
MexB (PDB: 2V50)

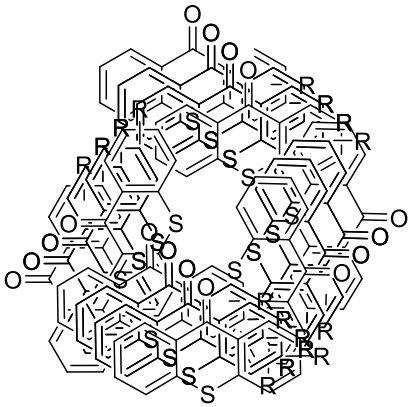


Low Scores
Nucleotide Binding Domain
Transmembrane Domain

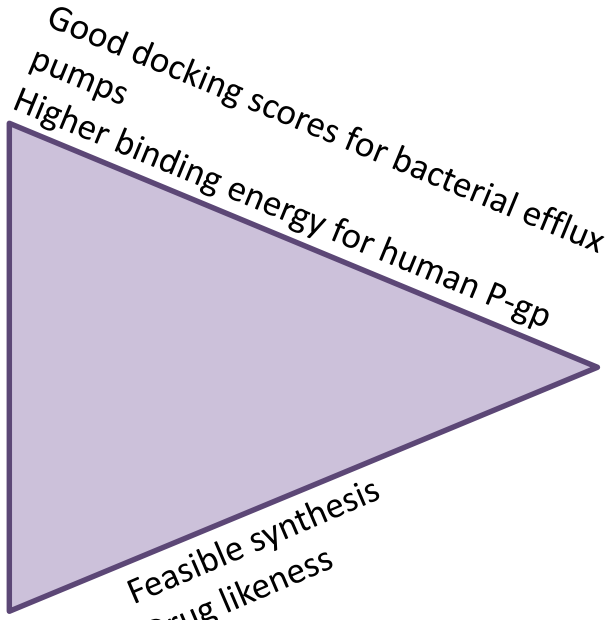
P-glycoprotein

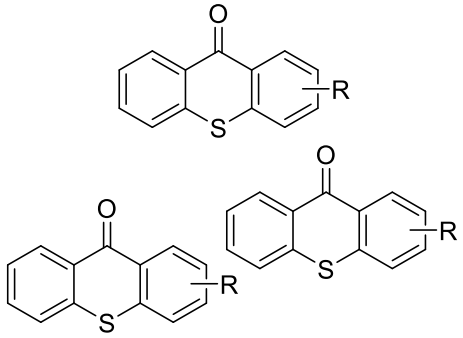
Docking results





1000 (thio)xanthones





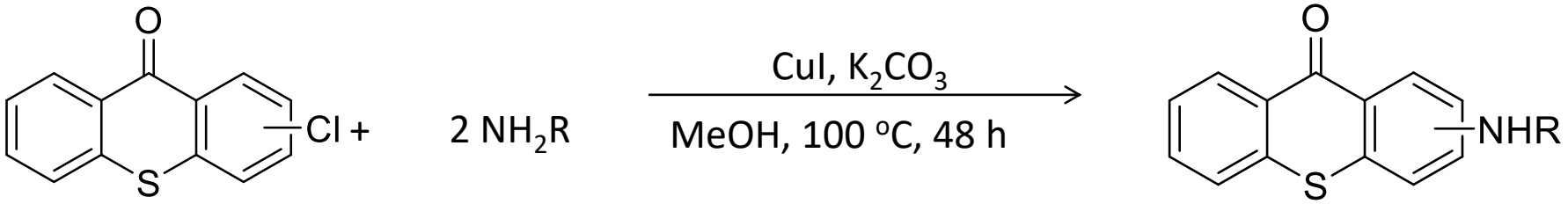
30 (thio)xanthones

		Estimated Binding Energy
TXP	P-glycoprotein	-7.4
TXP	AcrB	-7.8
TXP	MexB	-8.2

		Estimated Binding Energy
TXG318	P-glycoprotein	-9.9
TXG318	AcrB	3.9
TXG318	MexB	6.0

Synthesis

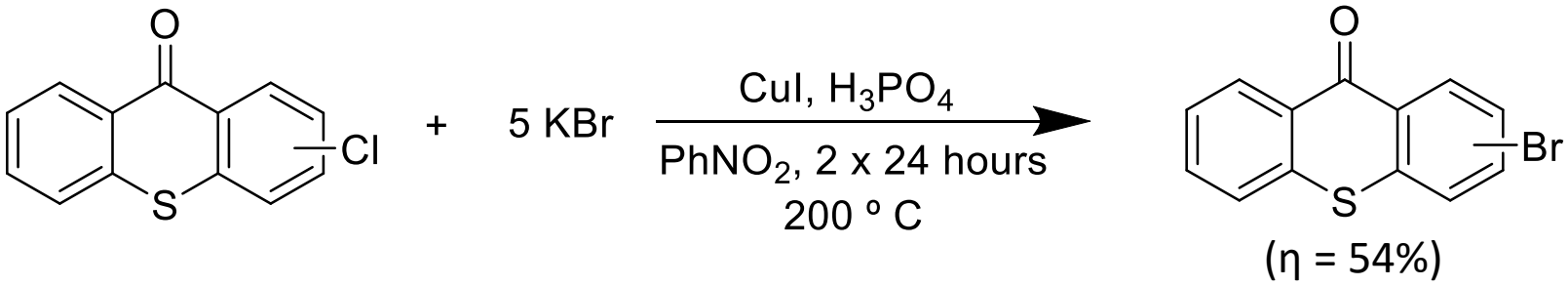
Copper-catalyzed Ullmann type C –N coupling



1 – 12

(η = Traces – 45%)

Synthesis



- Better leaving group than chlorine: better yields
- Possibility to couple with halogen amines

Biological assays

- Minimum Inhibitory Concentration (MIC), using susceptible strains:
  - *Escherichia coli* ATCC 25922
  - *Staphylococcus aureus* ATCC 29213
  - *Pseudomonas aeruginosa* ATCC 27853
  - *Enterococcus faecalis* ATCC 29121

- Synergy with antibiotics, using resistant strains:
  - Cefotaxime (CTX) and *E. coli* SA/2
  - Oxacillin (OXA) and *S. aureus* 66/1
  - Vancomycin (VAN) and *E. faecalis* B3/101

- Checkerboard assay:
  - Compounds that displayed synergy with antibiotics

Antibacterial activity

	MIC (µg/ml)			
Compound	***E. coli* ATCC 25922**	***P. aeruginosa* ATCC 27853**	***E. faecalis* ATCC 29212**	***S. aureus* ATCC 29213**
1	>16	>16	>16	>16
2	>16	>16	>16	>16
3	>64	>64	>64	>64
4	>64	>64	>64	>64
5	>16	>16	>16	>16
6	ND	ND	ND	ND
7	>64	>64	32	64
8	>64	>64	>64	>64
9	>64	>64	>64	>64
10	>64	>64	>64	>64
11	>64	>64	>64	>64
12	>64	>64	>64	>64

Synergy with antibiotics

E. coli SA/1		*S. aureus* 66/1		*E. faecalis* B3/101	
CTX (10 mg/ml) + Compound	MIC (µg/ml)	OXA (5 mg/ml) + Compound	MIC (µg/ml)	VAN (10 mg/ml) + Compound	MIC (µg/ml)
CTX	512	OXA	128	VAN	1024
1 (64 µg/ml)	512	1 (64 µg/ml)	128	1 (64 µg/ml)	1024
2 (16 µg/ml)	512	2 (16 µg/ml)	128	2 (16 µg/ml)	1024
3 (16 µg/ml)	512	3 (16 µg/ml)	128	3 (16 µg/ml)	1024
4 (64 µg/ml)	512	4 (64 µg/ml)	128	4 (64 µg/ml)	1024
5 (16 µg/ml)	512	5 (16 µg/ml)	128	5 (16 µg/ml)	64
6 (64 µg/ml)	512	6 (64 µg/ml)	128	6 (64 µg/ml)	1024
7 (64 µg/ml)	512	7 (64 µg/ml)	128	7 (64 µg/ml)	1024
8 (64 µg/ml)	32	8 (64 µg/ml)	128	8 (64 µg/ml)	1024
9 (64 µg/ml)	512	9 (64 µg/ml)	128	9 (64 µg/ml)	1024
10 (64 µg/ml)	128	10 (64 µg/ml)	128	10 (64 µg/ml)	1024
11 (64 µg/ml)	128	11 (64 µg/ml)	128	11 (64 µg/ml)	1024
12 (64 µg/ml)	512	12 (64 µg/ml)	128	12 (64 µg/ml)	1024

Checkerboard assay

- Performed in the compounds that displayed synergy with antibiotics
- Results show that 1 µg/ml can reduce the MIC of CTX to 64 µg/ml, in *E. coli* SA/2.

	CTX	*E. coli* SA/2						
	512							
	256	256 + 1	256 + 2	256 + 4	256 + 8	256 + 16	256 + 32	
	128	128 + 1	128 + 2	128 + 4	128 + 8	128 + 16	128 + 32	
	64	64 + 1	64 + 2	64 + 4	64 + 8	64 + 16	64 + 32	
	32	32 + 1	32 + 2	32 + 4	32 + 8	32 + 16	32 + 32	
	16	16 + 1	16 + 2	16 + 4	16 + 8	16 + 16	16 + 32	
Comp. 8			2	4	8	16	32	64

Growth

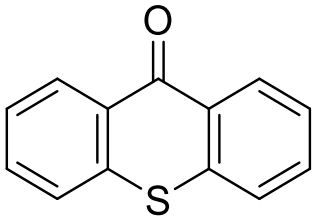
Checkerboard assay

- Regarding *E. faecalis* B3/101, compound 5 reduced the MIC of vancomycin to 512 µg/ml, when at a concentration of 1 µg/ml

	VAN		*E. faecalis* B3/101					
	1024							
	512	512 + 0.25	512 + 0.5	512 + 1	512 + 2	512 + 4	512 + 8	
	256	256 + 0.25	256 + 0.5	256 + 1	256 + 2	256 + 4	256 + 8	
	128	128 + 0.25	128 + 0.5	128 + 1	128 + 2	128 + 4	128 + 8	
	64	64 + 0.25	64 + 0.5	64 + 1	64 + 2	64 + 4	64 + 8	
	32	32 + 0.25	32 + 0.5	32 + 1	32 + 2	32 + 4	32 + 8	
Comp. 5			0.5	1	2	4	8	16

Growth

Conclusions



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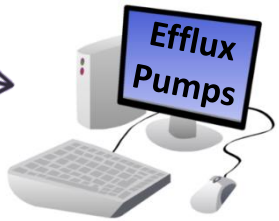
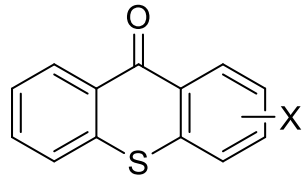
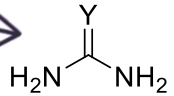
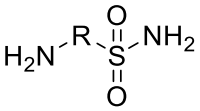




X = Br, Cl



Y = NH, O



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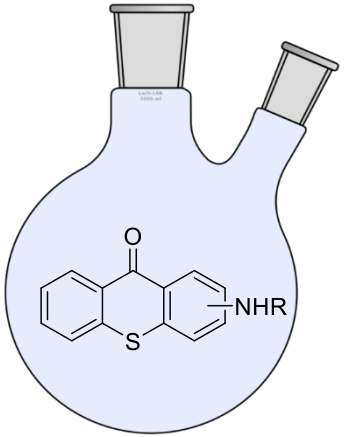

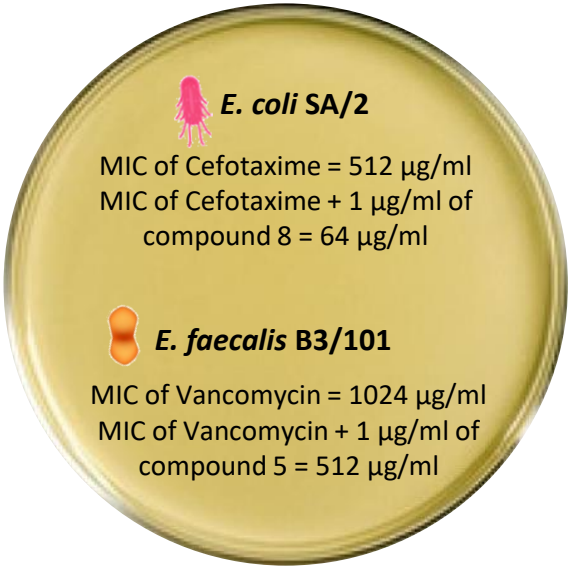

Future work

Insights into the mechanism of action of synergy with antibiotics

Acknowledgments

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