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Chiral derivatives of xanthones: synthesis, enantiomeric purity and enantioselectivity in the reversal antimicrobial resistance mechanisms

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pharmaceuticals MDPI

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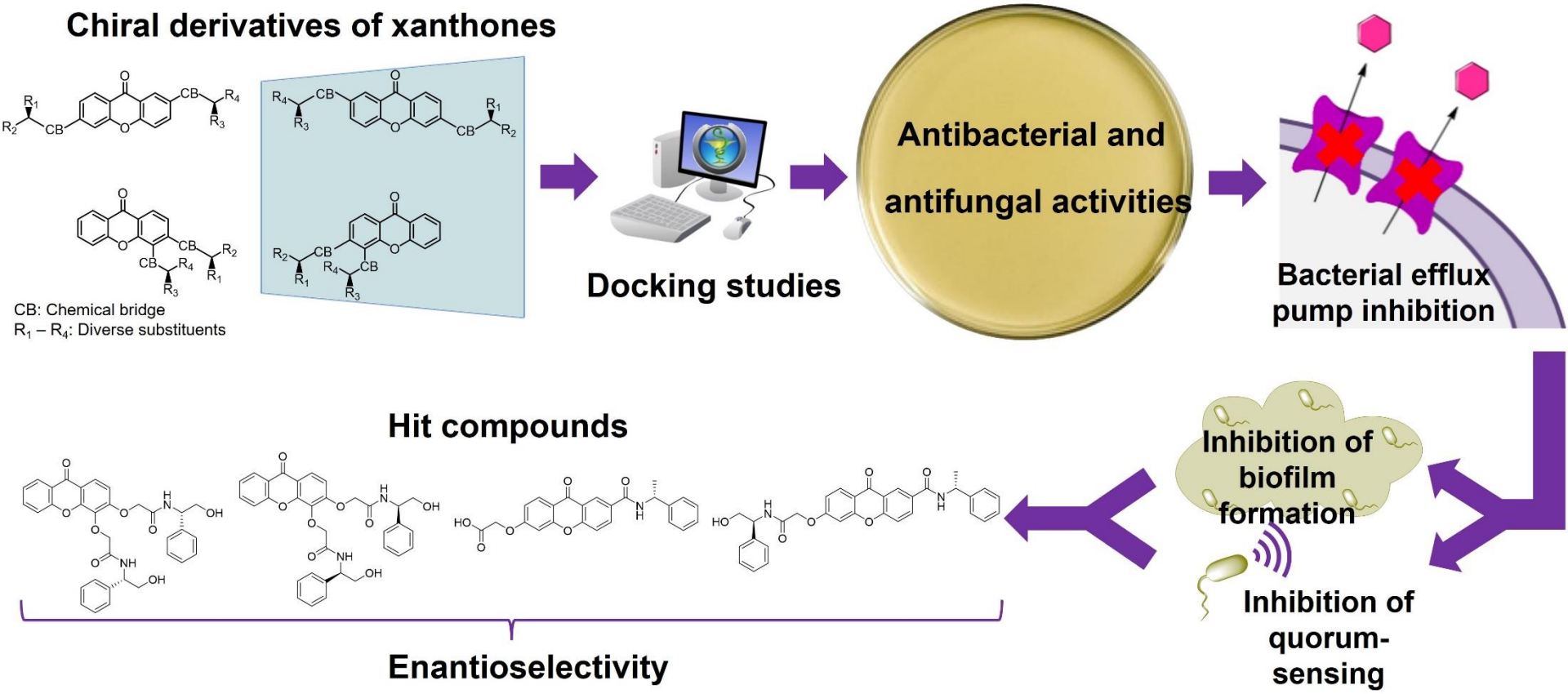
Chiral derivatives of xanthones: synthesis, enantiomeric purity and enantioselectivity in the reversal antimicrobial resistance mechanisms

Graphical Abstract

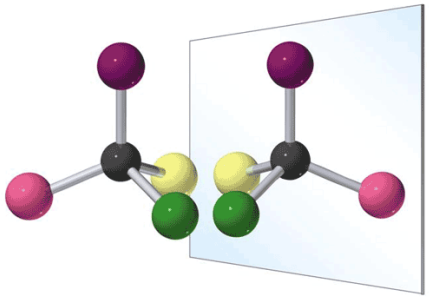


Abstract:

The design of peptidomimetic small molecules, such as amino acid substituted xanthones, has become an attractive research field. The strategy of linking molecules with xanthone scaffold to peptide moieties demonstrated to be successful for the development of new antimicrobial agents. Our group has already described xanthones as promising antimicrobials, and as inhibitors of antimicrobial resistance mechanisms. Enantioselectivity studies associated with biological activities were also performed by us, and for some chiral derivatives of xanthones (CDXs) differences were found for the respective enantiomers.
Herein, a small library of CDXs was synthesized and their enantiomeric purity was evaluated by chiral liquid chromatography. Enantiomeric ratio values higher than 99% were achieved. The potential of CDXs as antimicrobial agents, and their application to improve the activity of common antibiotics or to reverse bacterial mechanism of resistance were studied. In addition, to gain a better insight on how the active compounds bind to the bacterial efflux pumps, *in silico* studies were performed. Hit compounds were suggested and, in some cases, enantioselectivity was evident.

Keywords: antimicrobial resistance; bacterial efflux pumps; chiral; docking; enantioselectivity; xanthones

Chiral molecules and Enantioselectivity



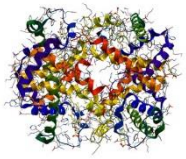
Chiral molecule

ENANTIOSELECTIVITY

Enantiomers

Biotargets

(D-sugars, L-amino acids)



DIFFERENT BIOLOGICAL/ PHARMACOLOGICAL ACTIVITIES

Review

Enantioselectivity in Drug Pharmacokinetics and Toxicity: Pharmacological Relevance and Analytical Methods

Maria Miguel Coelho [1], Carla Fernandes [1,2], Fernando Remião [3] and Maria Elizabeth Tiritan [1,2,4,*]

Molecules **2021**, *26*, 3113. https://doi.org/10.3390/molecules26113113 MDPI

M.E. Tiritan, A.R. Ribeiro, C. Fernandes, M. Pinto, Chiral Pharmaceuticals.
In *Kirk-Othmer Encyclopedia of Chemical Technology*: John Wiley & Sons, Inc., **2016**, 1-28.

Trends in the development of chiral drugs

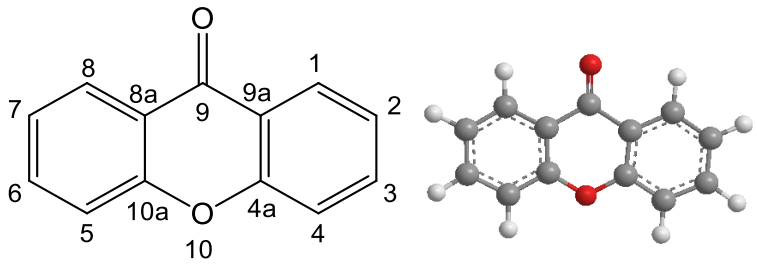
In 2021, 20 out of 35 pharmaceuticals approved by the FDA were chiral.

In 2021, from the ten best selling drugs, nine were chiral; among them, seven were complex molecules with intrinsic chirality; the other two were small molecules being one commercialized as single enantiomer.

Chirality can be considered as one of the major topics in the design, discovery, development and marketing of new drugs.

Top companies and drugs by sales in 2021, March **2022**, in https://www.nature.com/articles/d41573-022-00047-9.
https://www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/novel-drug-approvals-2021 (acessed 17 April 2022)

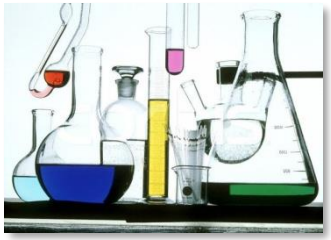
Xanthone derivatives



9*H*-xanthen-9-one (dibenzo-*γ*-pyrone)

NATURAL

SYNTHETIC



via benzophenone or aryl ether intermediates, Grover, Shah and Shah, others

a scaffold able to provide potent and selective ligands for a range of different biological targets through modification of functional groups

Large diversity of biological and pharmacological activities

A.C.S. Veríssimo, D.C. G. A. Pinto, A.M. S. Silva, *Mar. Drugs*, **2022**, *20*, 347.
A.I. Shagufta, *Eur J Med Chem,* **2016**, *116, 267-280.*
M.M.M. Pinto, M.E. Sousa, M.S J. Nascimento, *Curr.Med. Chem.*, **2005**, *12,* 2517-2538.

Xanthone derivatives

molecules

MDPI

Review

From Natural Products to New Synthetic Small Molecules: A Journey through the World of Xanthones

Madalena M. M. Pinto [1,2,*], Andreia Palmeira [1,2,†], Carla Fernandes [1,2,†], Diana I. S. P. Resende [1,2,†], Emília Sousa [1,2,†], Honorina Cidade [1,2,†], Maria Elizabeth Tiritan [1,2,3,†], Marta Correia-da-Silva [1,2,†] and Sara Cravo [1,2,†]

Molecules **2021**, *26*, 431. https://doi.org/10.3390/molecules26020431

Chiral derivatives of xanthones (CDXs)

Chiral derivatives of xanthones

Review

Synthetic Chiral Derivatives of Xanthones: Biological Activities and Enantioselectivity Studies

Carla Fernandes [1,2,*], Maria Letícia Carraro [1,†], João Ribeiro [1,†], Joana Araújo [1,†], Maria Elizabeth Tiritan [1,2,3,*] and Madalena M. M. Pinto [1,2]

Molecules **2019**, *24*, 791; doi:10.3390/molecules24040791

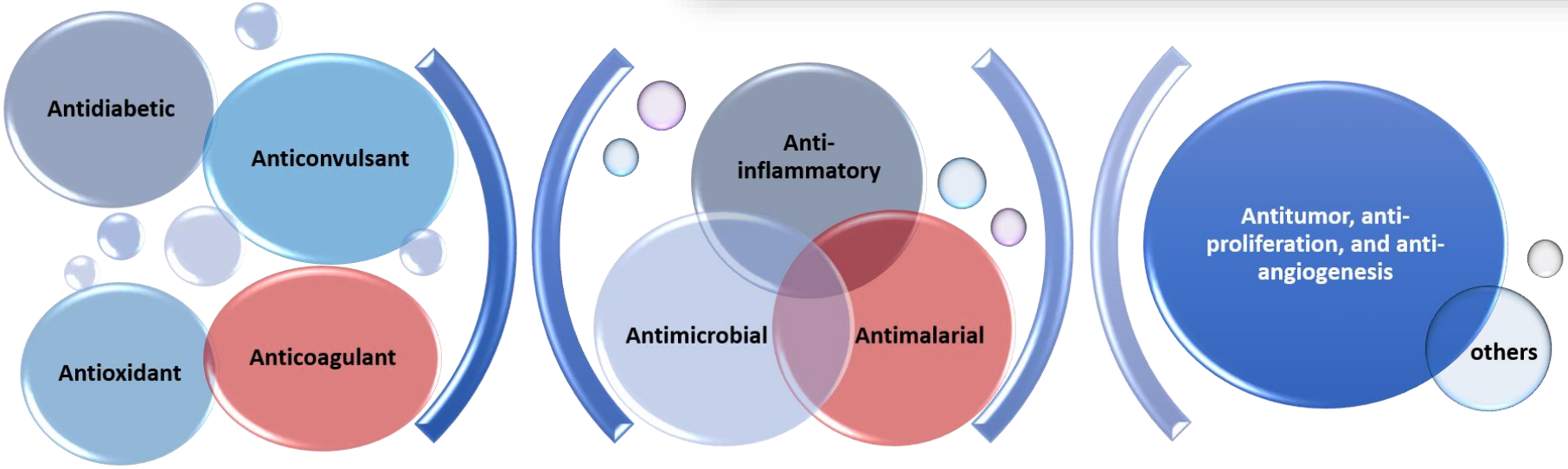
ENANTIOSELECTIVITY

Synthetic chiral derivatives of xanthones

inspired in naturally occurring xanthones

binding chiral moieties to the xanthone scaffold

SEVERAL BIOLOGICAL ACTIVITIES



Chiral derivatives of xanthones

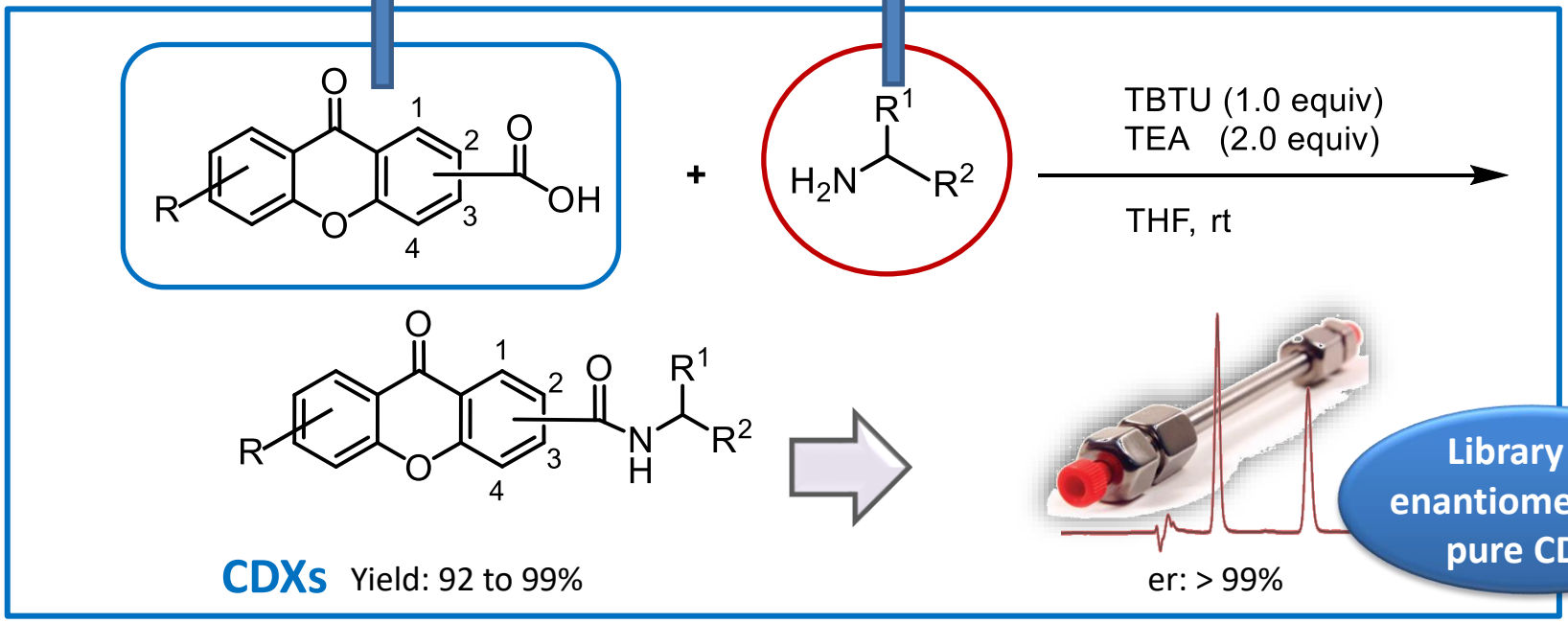
Synthetic CDXs binding chiral moieties to the xanthone scaffold

Carboxyxanthone derivatives

Amines, amino alcohols, amino esters, amino acids…

Chiral pool strategy

C. Fernandes, *et al., Pharmaceuticals*, **2017**, 10, 50.
C. Fernandes, *et al., Bioorg. Med. Chem.*, **2014**, 22, 1049.
C. Fernandes, *et al., Eur. J. Med. Chem.*, **2012**, 55, 1.



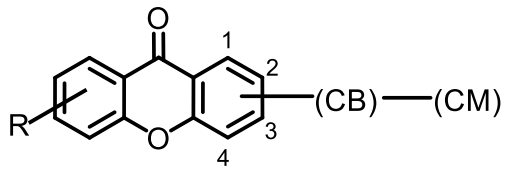
CDXs Yield: 92 to 99%

er: > 99%

TBTU: *O*-(Benzotriazol-1-yl)-*N*-*N*-*N'*-*N'*-tetramethyluronium tetrafluoroborate; TEA: Triethylamine; THF: Tetrahydrofuran; er : enantiomeric ratio.

Strategy for enantioselectivity studies

Synthesis

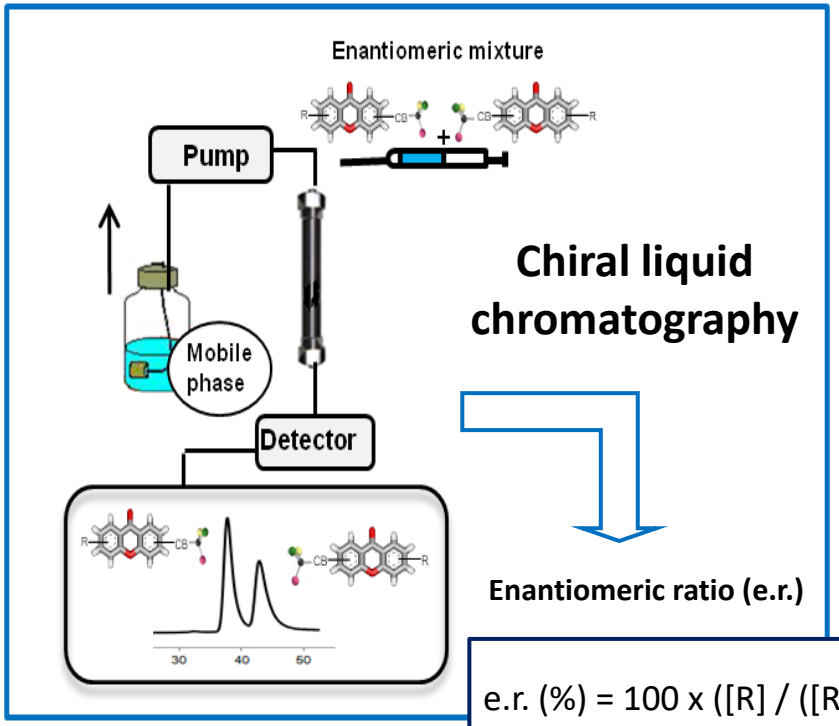


R – diverse substituents; CB – chemical bridge; CM – chiral moiety

Chiral derivatives of xanthones (CDXs)

BOTH SINGLE ENANTIOMERS

Evaluation of enantiomeric purity



Chiral liquid chromatography

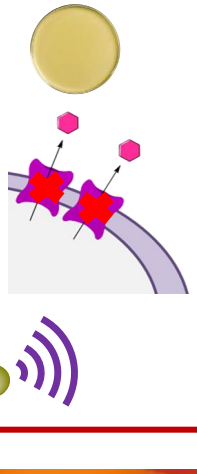
Enantiomeric ratio (e.r.)

e.r. (%) = 100 x ([R] / ([R]+[S])
or
= 100 x ([S] / ([S]+[R])

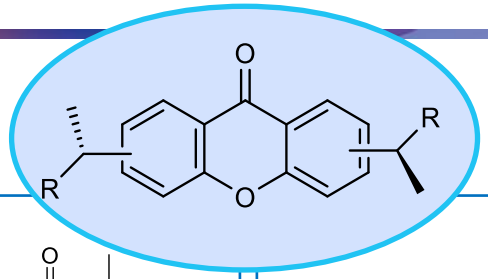
[*S*] and [*R*] are the area of the peak of each enantiomer

Biological screening

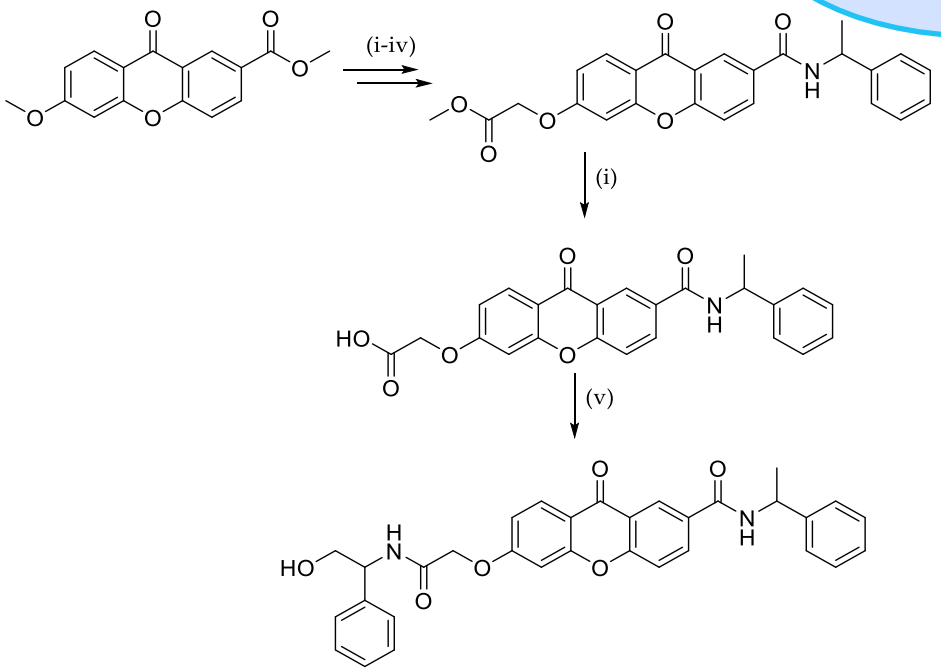
- **Antibacterial activity**
- **Bacterial efflux pump inhibition**
- **Synergy with antimicrobials**
- **Inhibition of biofilm formation**
- **Quorum-sensing inhibition**



Synthesis

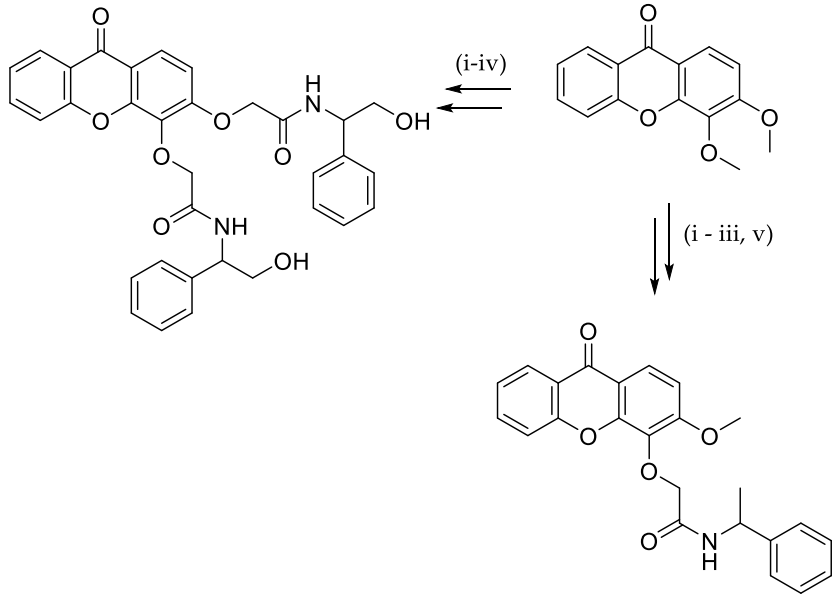


2,6-derivatives



(i) NaOH 5M, CH_2Cl_2:CH_3OH (1:1 *v/v*), rt, 5–22 h; (ii) (*R*)-(+) or (*S*)-(−)-(α)-methylbenzylamine, TBTU, anhydrous THF, TEA, rt, 1 h; (iii) $Et_2NCH_2SH.HCl$, NaO*t*Bu, anhydrous DMF, reflux, N_2, 4 h (iv) $BrCH_2COOCH_3$, K_2CO_3, anhydrous acetone, 3 h; (v) (*S*)-(−) or (*R*)-(+)-2-phenylglycinol, TBTU, anhydrous THF, TEA, rt, 3 h.

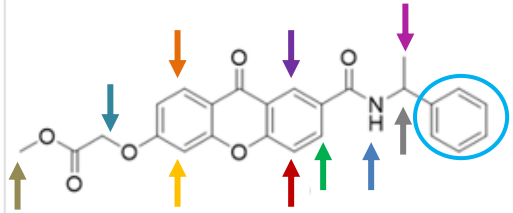
3,4-derivatives



(i) $AlCl_3$, anhydrous toluene, 70 ° C, 40 min; (ii) $BrCH_2COOCH_3$, K_2CO_3, anhydrous acetone, 4–24 h; (iii) NaOH 5M, CH_2Cl_2:CH_3OH (1:1 *v/v*), rt, 5–24 h; (iv) (*R*)-(−) or (*S*)-(+)-2-phenylglycinol, TBTU, anhydrous THF, TEA, rt, 5 h; (v) (*R*)-(+) or (*S*)-(−)-(α)-methylbenzylamine, TBTU, TEA, anhydrous THF, 2 h.

Structure elucidation

Example



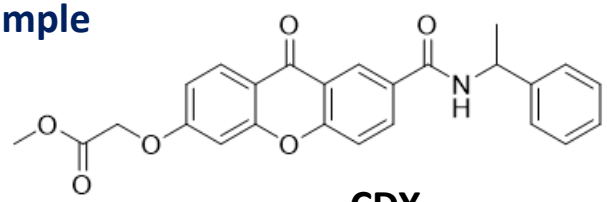
CDX

^{1}H NMR (300.13 MHz, DMSO-d_6)

m.p.: > 330 °C. $[\alpha]_D^{25^\circ C}$ - 686.0 (c = 0.86 × 10^{-3} gmL^{-1} in CH_3OH); IR ν_{max} (cm^{-1}) (KBr): 3431.7, 3295.6, 1759.3, 1648.4, 1617.9, 1540.7, 1479.7, 1348.7, 1213.5, 1067.7, 828.6, 781.6; HRMS (ESI+): m/z $[C_{24}H_{19}NO_6 + H]+$ calcd. for $[C_{24}H_{20}NO_6]$: 432.14471; found 432.14410

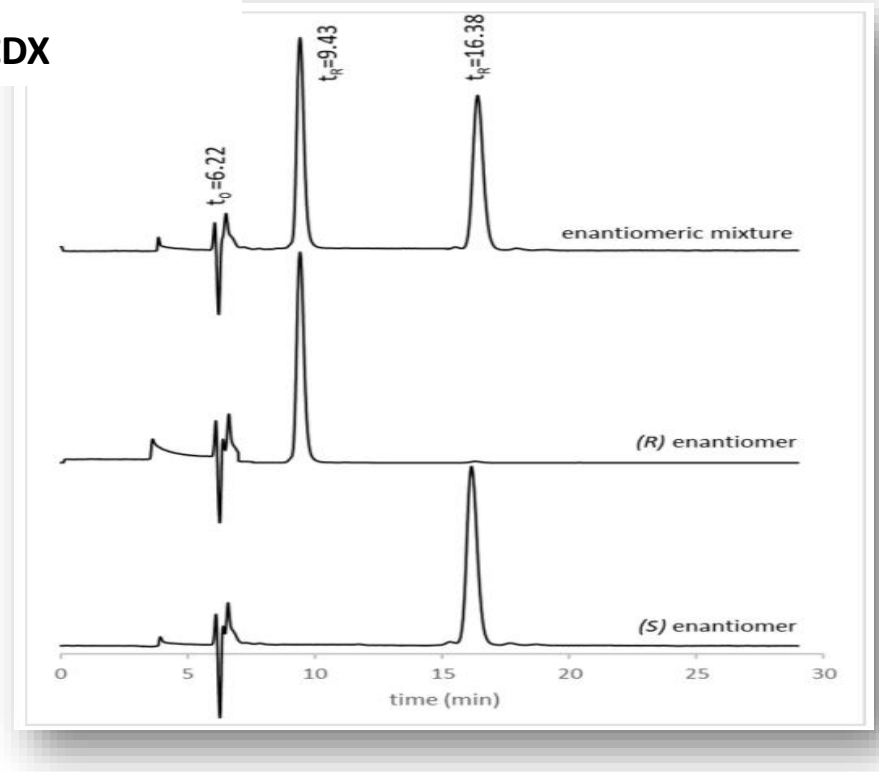
Enantiomeric purity

Example



CDX

Chromatograms for the enantioseparation at optimized chromatographic conditions



Column: (*S*,*S*)-Whelk-O1®
Mobile phase: CH_3CN/CH_3OH (50:50 *v*/*v*)
Flow rate: 1.0 mL/min
Detection: UV at 254 nm

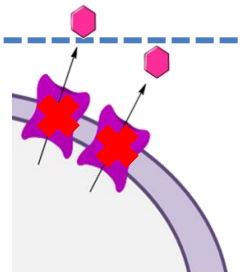
e.r. > 99% for both enantiomers

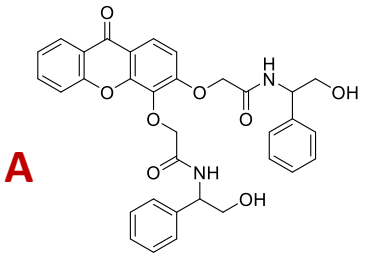
Enantiosselectivity studies

Antibacterial activity ✖ No active compounds

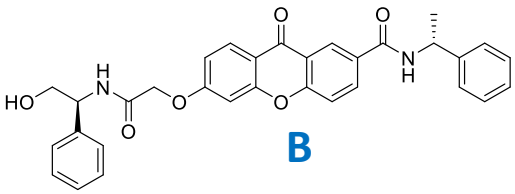
Bacterial efflux pump inhibition

Evaluate the capability to modify the accumulation of ethidium bromide (EB), a known efflux pump substrate, that can increase fluorescence when bound to DNA.

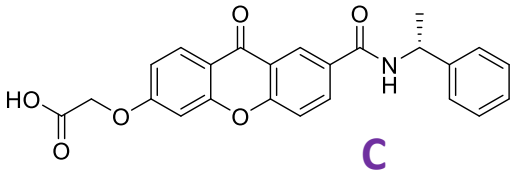




R,*R* enantiomer active against *S. aureus* 272123
S,*S* enantiomer active against *S.* Typhimurium SL1344 (SE03)

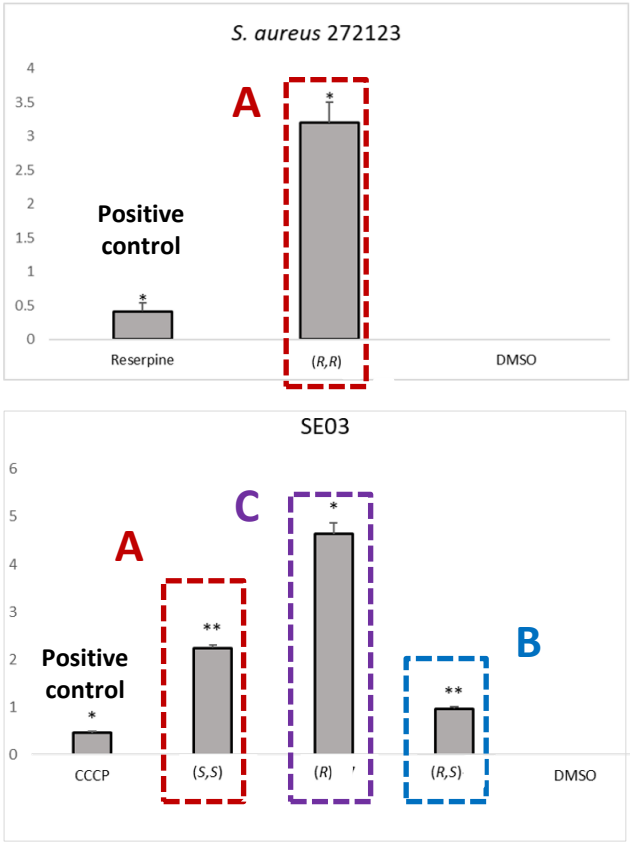


R,*S* enantiomer against *S.* Typhimurium SL1344 (SE03)
R,*R* enantiomer inactive



R enantiomer against *S.* Typhimurium SL1344 (SE03)
S enantiomer inactive

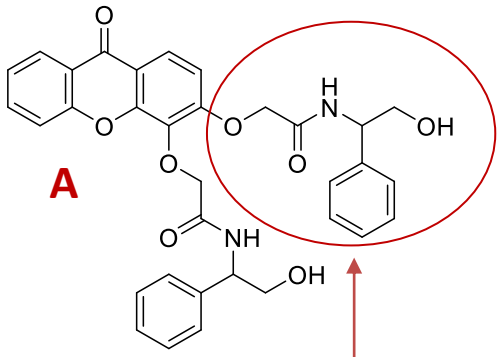
Relative fluorescence index (RFI) of active CDXs



CCCP: Carbonyl cyanide 3-chlorophenylhydrazone

Enantiosselectivity studies

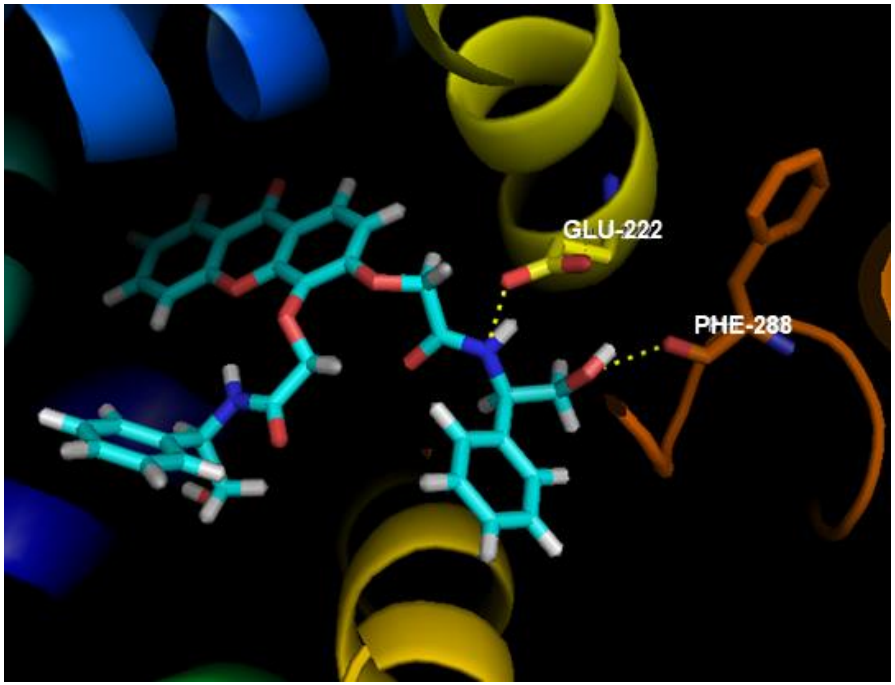
Bacterial efflux pump inhibition



R,R **enantiomer active against *S. aureus* 272123 (SE03)**

In silico study

AutoDock Vina
PyMol

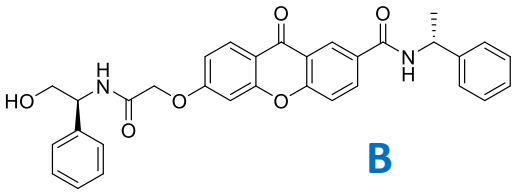


Molecular visualization of interaction of *R,R* enantiomer with key residues in the binding core region of the homology model of NorA.

The substituent in the C3 position is predicted to play an important role in binding to this portion of the efflux system.

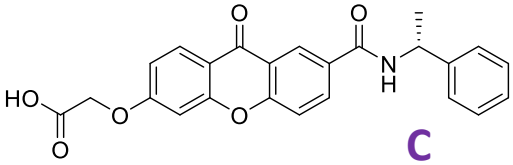
Enantiosselectivity studies

Bacterial efflux pump inhibition



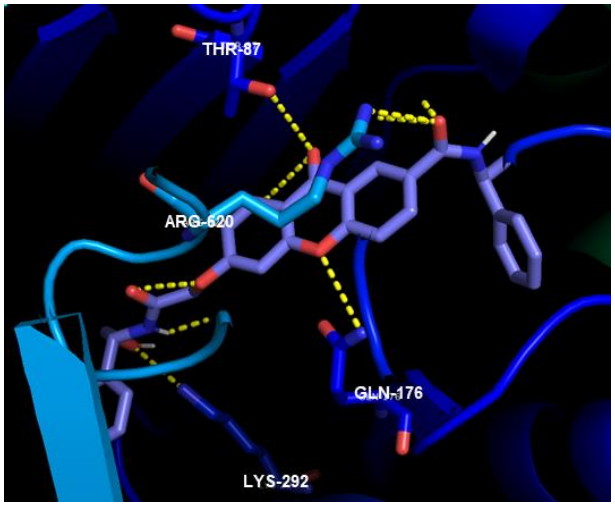
B

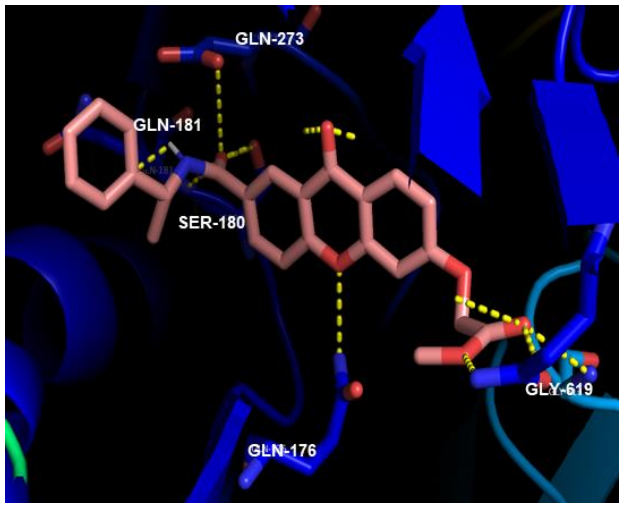
***R,S* enantiomer active against *S*. Typhimurium SL1344 (SE03)**



C

***R* enantiomer active against *S*. Typhimurium SL1344 (SE03)**





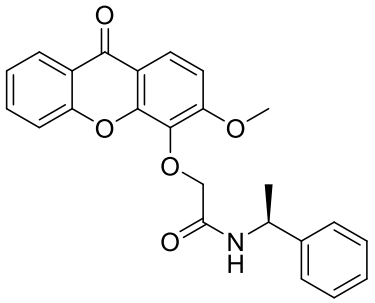
In silico study

AutoDock Vina
PyMol

Molecular visualization of interaction of *R,S* enantiomer of **B** and *R* enantiomer of **C** with the substrate binding site of AcrB.

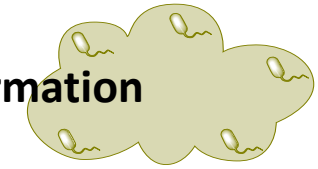
Enantiosselectivity studies

Synergy with antimicrobials against resistant bacteria



S enantiomer decreased the MIC of cefotaxime by 4-fold in *E. coli* SA/2
R enantiomer showed no activity

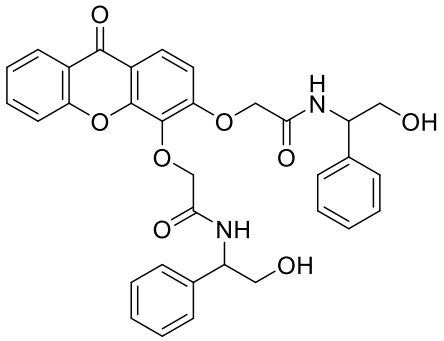
Inhibition of biofilm formation



None of the compounds had greater effect than reserpine (positive control)

Enantiosselectivity studies

Quorum-sensing inhibition



Both enantiomers active against *C. violaceum* CV026 + *Sphingomonas paucimobilis* Ezf 10-17

Compound	Quorum sensing inhibition (mm) ± SD		
	S. marcescens	wt85	EZF + CV026
(*S,S*)-enantiomer	0	0	47 ± 0.1
(*R,R*)-enantiomer	0	0	31 ± 0.8
Promethazine (Positive control)	18 ± 0.8	40 ± 0.1	41 ± 0.5

wt85: *Chromobacterium violaceum* wild-type 85; **EZF**: *Sphingomonas paucimobilis* Ezf 10-17; **CV026**: *C. violaceum* CV026

The inhibition of QS was observed as the reduction in pigment production and measured in millimeters (mm).

Conclusions

A small library of CDXs as single enantiomers was synthesized.

High enantiomeric purity was obtained, with e.r. values higher than 99%.

No CDXs were active against reference strains of bacteria and fungi.

Selected CDXs were evaluated for their potential to inhibit bacterial efflux pumps and active compounds were found, one of them inhibited efflux pumps in the Gram-positive model tested and other three were active in the Gram-negative strain used.

One CDX showed synergy with antimicrobials.

Both enantiomers of one CDXs demonstrated quorum-sensing inhibition.

Enantioselectivity was observed in different biological assays.

Acknowledgements

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