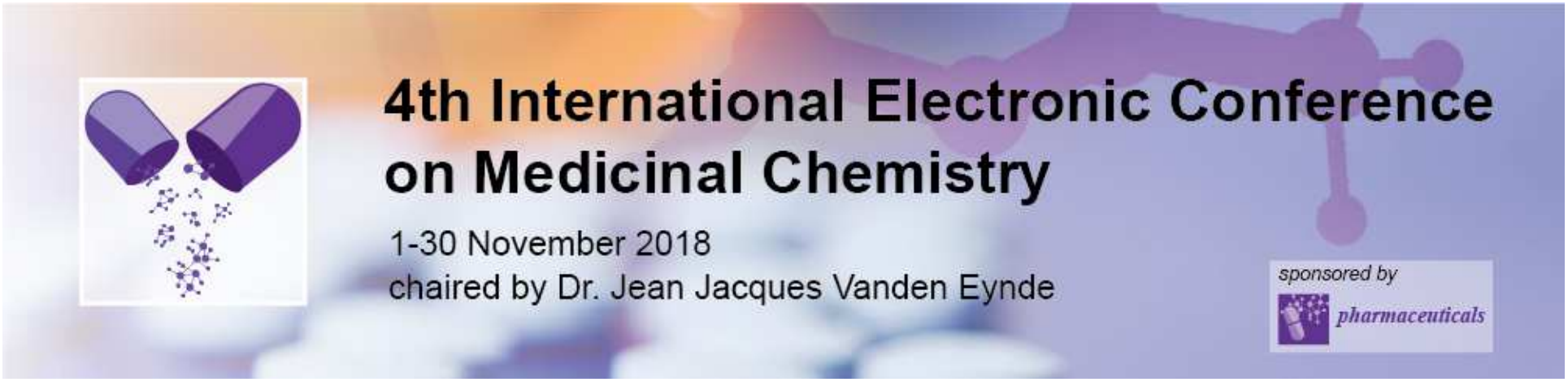

Dual Application of Chiral Derivatives of Xanthones: in Medicinal Chemistry and Liquid Chromatography

Carla Fernandes[1,2]*, Ye Zaw Phyo[1,3], João Ribeiro[2], Sara Cravo[1,2], Maria Elizabeth Tiritan[1,2,4], Artur M.S. Silva[5], Anake Kijjoa[1,3], Madalena M.M. Pinto[1,2]

[1] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Universidade do Porto, Matosinhos, Portugal
[2] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal
[3] ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal
[4] CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Porto, Portugal
[5] Departamento de Química & QOPNA, Universidade de Aveiro, Aveiro, Portugal

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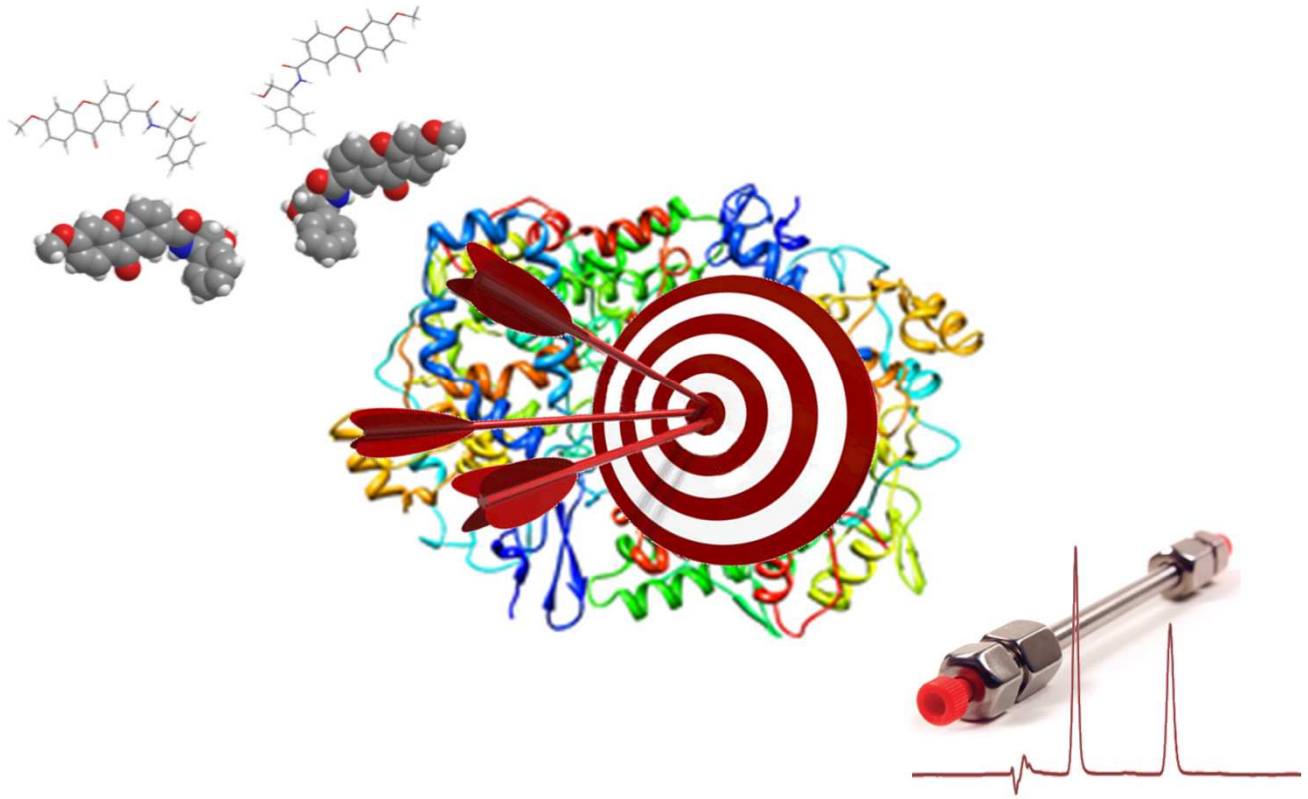
U.PORTO FACULDADE DE FARMÁCIA UNIVERSIDADE DO PORTO

ciimar Centro Interdisciplinar de Investigação Marinha e Ambiental

Dual Application of Chiral Derivatives of Xanthones: in Medicinal Chemistry and Liquid Chromatography

Graphical Abstract



Abstract:

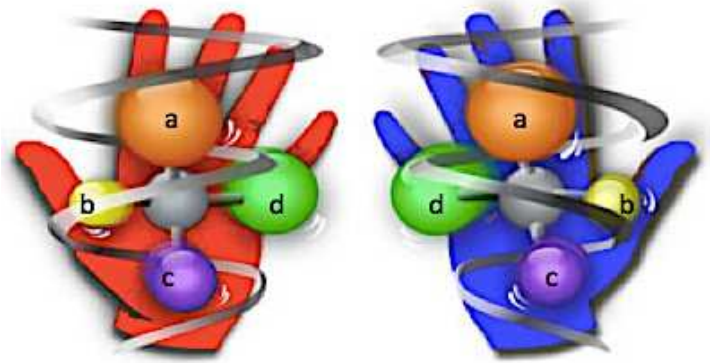
Over several years, xanthone derivatives have been the core of several studies, essentially due their wide range of biological and pharmacological activities. Recently, chiral derivatives of xanthones (CDXs) have come to arouse great interest considering enantioselectivity studies associated with biological activities as well as selectors for chiral stationary phases (CSPs) in liquid chromatography (LC).
From the perspective of Medicinal Chemistry, some CDXs synthetized by our group revealed interesting biological activities. Besides the potential as new drugs, CDXs afford promising LC enantioresolution results.
In a continuation of our study, new enantiomerically pure CDXs were synthetized for biological activity evaluation as well as selectors for new CSPs, confirming that CDXs have important applications not only in the field of Medicinal Chemistry but also for analytical applications.

Keywords: chiral derivatives of xanthones; biological activity; chiral stationary phases; liquid chromatography; enantioselectivity

INTRODUCTION

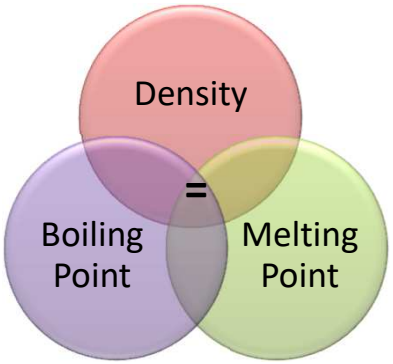
CHIRAL MOLECULES

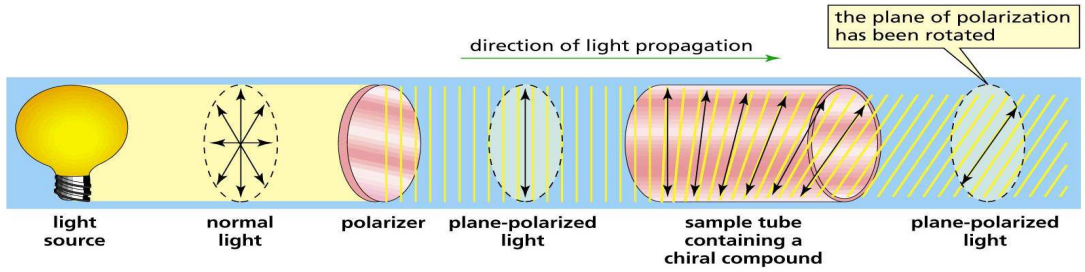
ENANTIOMERS

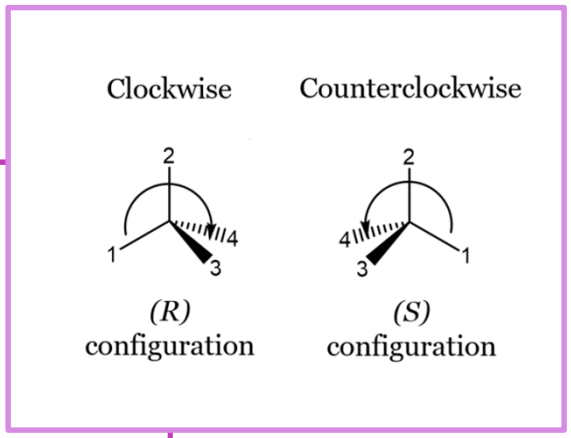


(-) Levorotatory

(+) Dextrorotatory



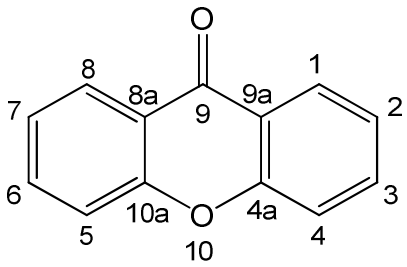


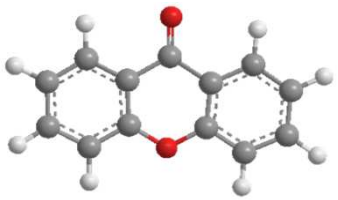


M.E. Tiritan, A.R. Ribeiro, C. Fernandes, M. Pinto, Chiral Pharmaceuticals. In Kirk-Othmer Encyclopedia of Chemical Technology: John Wiley & Sons, Inc., **2016**, 1-28.

INTRODUCTION

XANTHONE DERIVATIVES





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

NATURAL (TERRESTRIAL and MARINE) → From higher plants, fungi, lichens, bacteria, and crude oils

SYNTHETIC → Molecular diversity

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

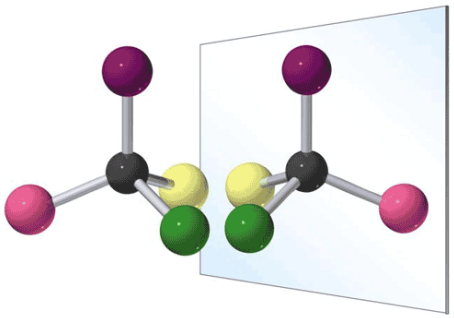
Chiral derivatives of xanthones (CDXs)

A.I. Shagufta, *Eur J Med Chem* ***2016****, 116, 267-280.*
K-S. Masters, S. Bräse, *Chem. Rev.*, **2012**, *112,* 3717–3776.
M.M.M. Pinto, M.E. Sousa, M.S J. Nascimento, *Curr.Med. Chem.*, **2005**, *12,* 2517-2538.

INTRODUCTION

WHY WORKING WITH CDXs?

- TO EXPLORE "**CHEMICAL AND BIOLOGICAL SPACES**"
- TO EXPLORE **CHIRALITY**

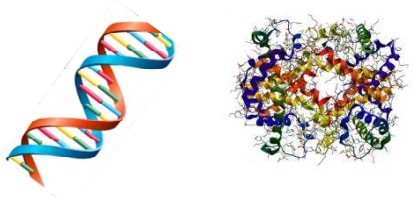


Chiral molecule

ENANTIOSELECTIVITY

Enantiomers

Biotargets
(D-sugars, L-amino acids)



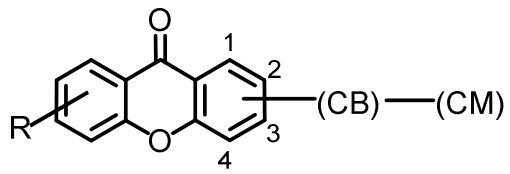
DIFFERENT BIOLOGICAL/ PHARMACOLOGICAL ACTIVITIES

- TO EXPLORE OTHER **APPLICATIONS**

AIMS

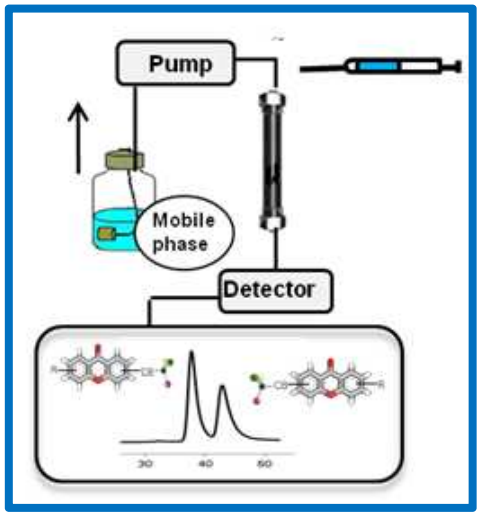
STRATEGY

I. SYNTHESIS AS SINGLE ENANTIOMERS



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

Chiral derivatives of xanthones (CDXs)

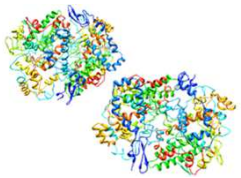


II. EVALUATION OF ENANTIOMERIC PURITY

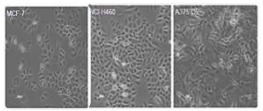
Liquid Chromatography (LC) using chiral stationary phases (CSPs)

III. BIOLOGICAL SCREENING

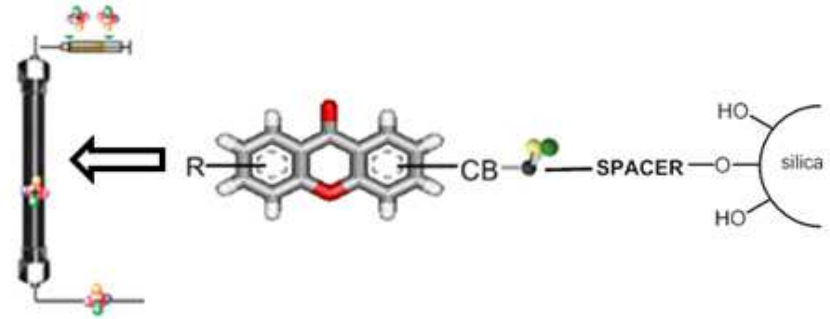
Inhibition of cyclooxygenases (COX-1 and COX-2)



Inhibition of on human tumor cell lines

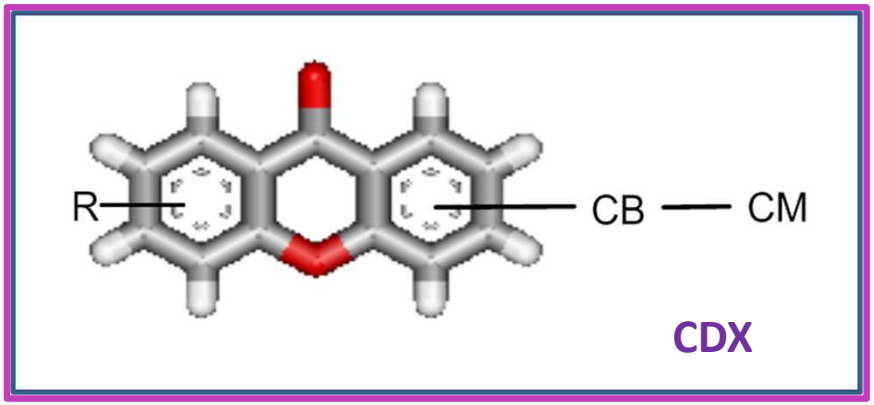


IV. DEVELOPMENT OF CSPS FOR LC



RESULTS AND DISCUSSION

I. SYNTHESIS



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

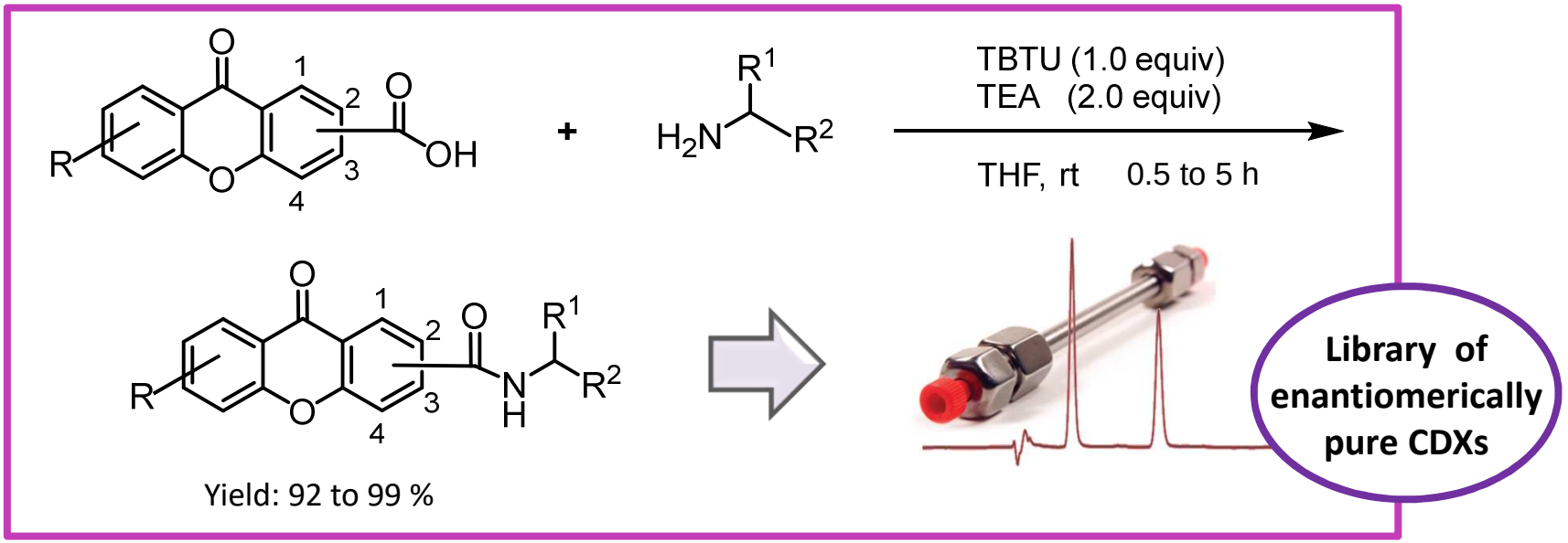
CDX = Chiral Derivatives of Xanthones

SYNTHETIC PROCEDURE

- highly efficient → - excellent yields; - without racemization; - short reaction times; - broad-scope applicability
- mild conditions
- operational simplicity
- easily scale-up for both enantiomers

RESULTS AND DISCUSSION

I. SYNTHESIS



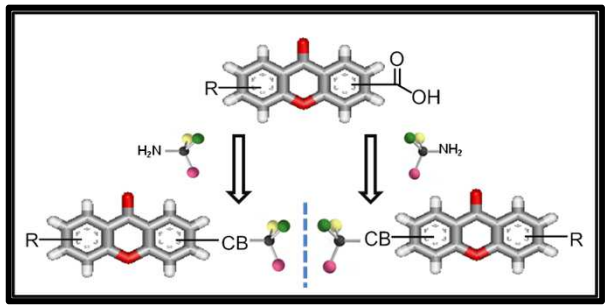
CDX: Chiral derivative of xanthone;TBTU: *O*-(Benzotriazol-1-yl)-*N*-*N*-*N'*-*N'*-tetramethyluronium tetrafluoroborate;
TEA: Triethylamine; THF: Tetrahydrofuran.

C. Fernandes, K. Masawang, M.E. Tiritan, E. Sousa, V. Lima, C. Afonso, H. Bousbaa, W. Sudprasert, M. Pedro, M. Pinto, *Bioorg. Med. Chem.* **2014**, 22, 1049-1062.
C. Fernandes, L. Oliveira, M.E. Tiritan, L. Leitão, A. Pozzi, J.B. Noronha-Matos, P. Correia-de-Sá, M.M. Pinto, *Eur. J. Med. Chem.*, **2012**, 55, 1-11.

RESULTS AND DISCUSSION

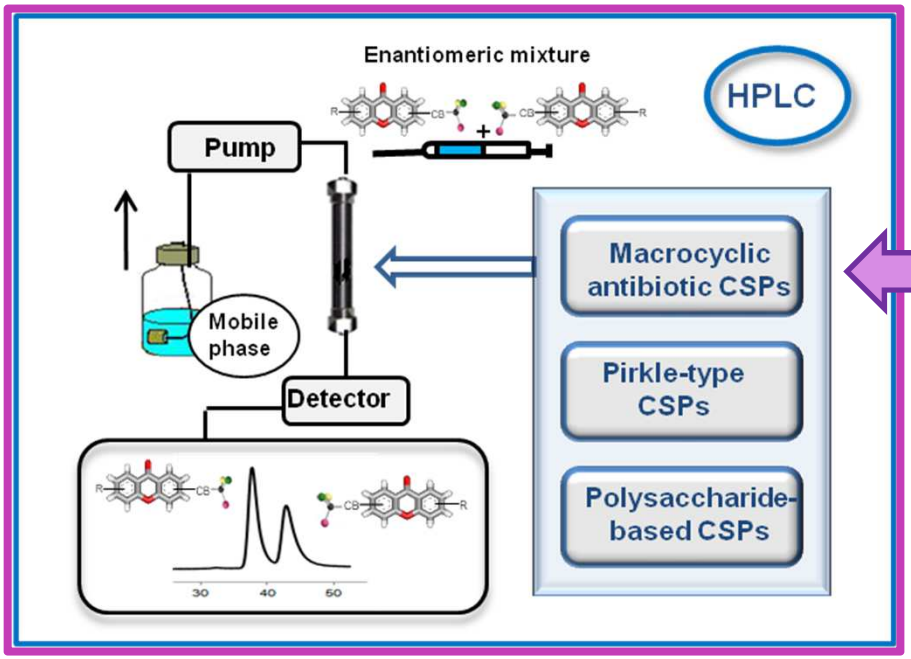
II. ENANTIOMERIC PURITY

LIBRARY OF CDXS



Next step

RESOLUTION AND EVALUATION OF ENANTIOMERIC RATIO



DIFFERENT TYPES OF CHIRAL STATIONARY PHASES (CSPS)

RESOLUTION AND DETERMINATION OF ENANTIOMERIC RATIO

Review article

Journal of Chromatography A

Enantiomeric ratios: Why so many notations?☆

Maria E. Tiritan[a,b,c], Carla Fernandes[b,c], Alexandra S. Maia[a], Madalena Pinto[b,c], Quezia B. Cass[d,*]

Journal of Chromatography A, 1569 (2018) 1–7

Enantiomeric ratio (e.r.)

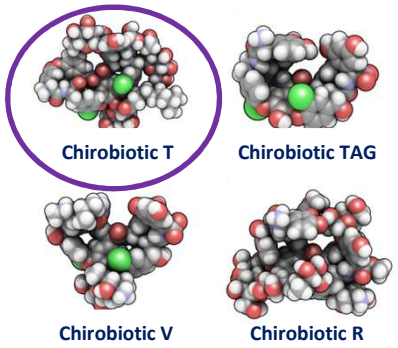
e.r. (%) = 100 x ([R] / ([R]+[S])
or
= 100 x ([S] / ([S]+[R])

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

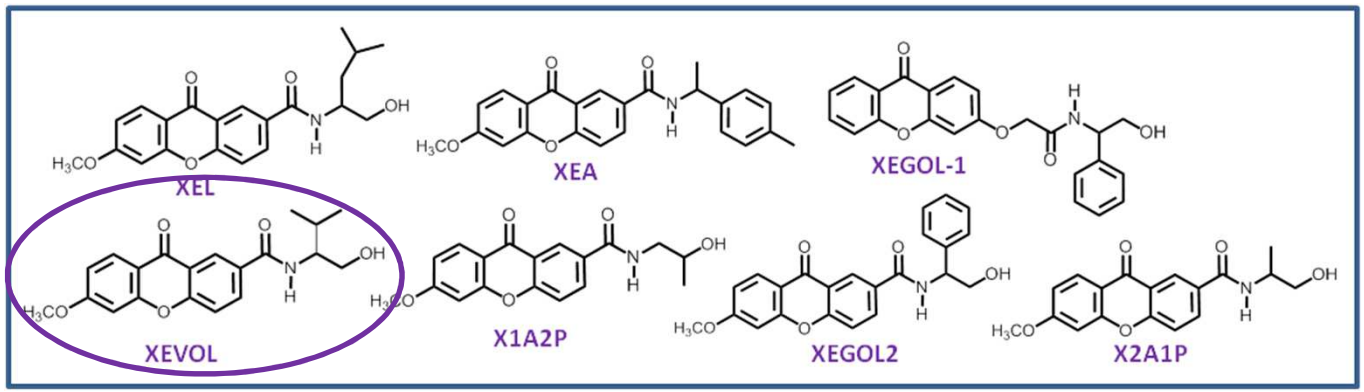
RESULTS AND DISCUSSION

II. ENANTIOMERIC PURITY

Macrocyclic antibiotic CSPs

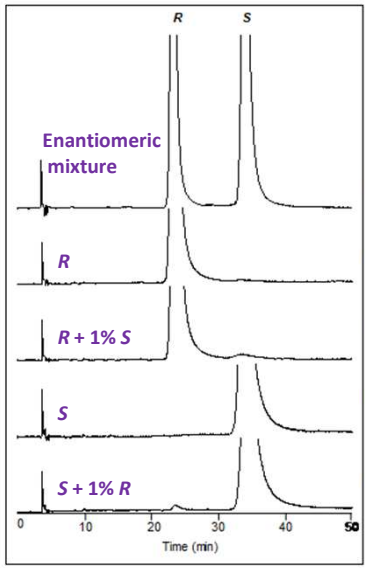


Enantiomeric mixtures of CDXs



Best enantioresolution

Enantiomeric Mixture	Chirobiotic	Mobile phase	k_1	α	R_S
XEGOL-1	TAG	MeOH/AcOH/TEA: 100/0.5/0.5	0.79	1.18	0.80
XEGOL-2	R	Hex/EtOH: 50/50	2.13	1.67	2.50
X2A1P	T	Hex/EtOH: 80/20	8.96	1.26	1.50
XEVOL	T	Hex/EtOH: 80/20	5.25	1.47	2.06
XEL	R	Hex/EtOH: 50/50	0.97	1.36	1.53
XEA	V	MeOH/AcOH/TEA: 100/0.01/0.01	0.53	1.39	0.92

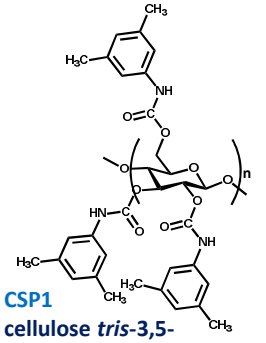


e.r. > 99%

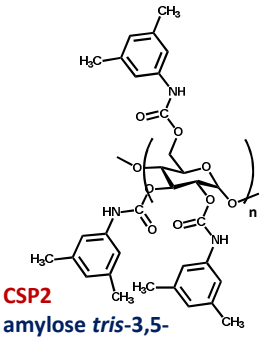
C. Fernandes, M.E. Tiritan, Q. Cass, V. Kairys, M.X. Fernandes, M. Pinto, *J. Chromatogr. A*, 1241, **2012**, 60-68.

RESULTS AND DISCUSSION

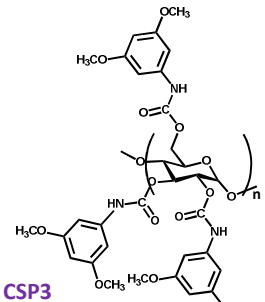
II. ENANTIOMERIC PURITY



CSP1
cellulose *tris*-3,5-dimethylphenylcarbamate

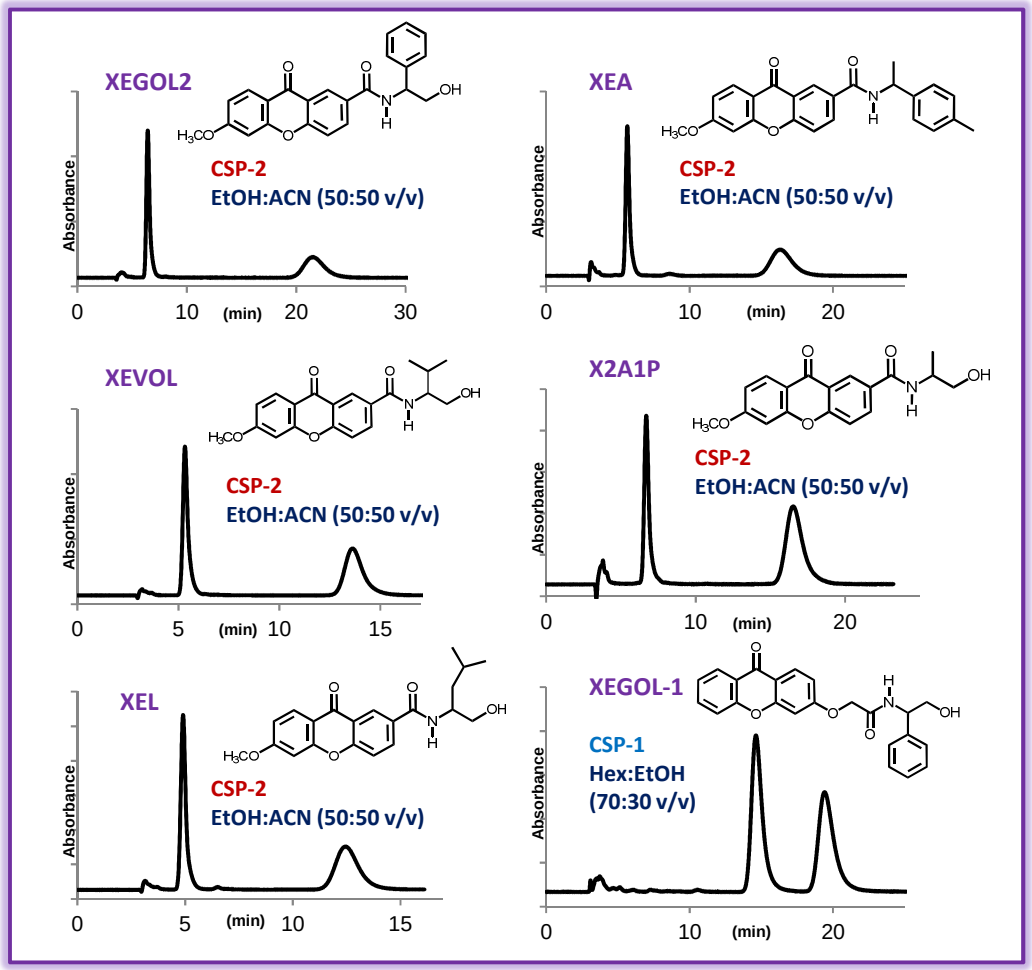


CSP2
amylose *tris*-3,5-dimethylphenylcarbamate

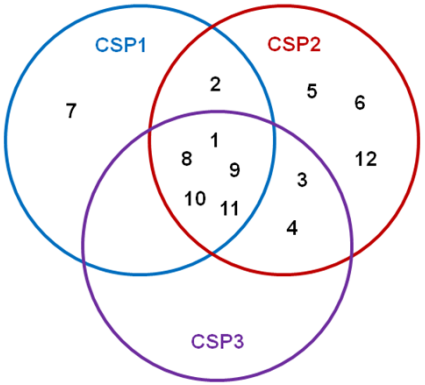


CSP3
amylose *tris*-3,5-dimethoxyphenylcarbamate

Polysaccharide-based CSPs



$1.43 \leq \alpha \leq 12.41$
$1.48 \leq R_S \leq 10.29$



CDXs 1-12
$R_S \geq 1.00$

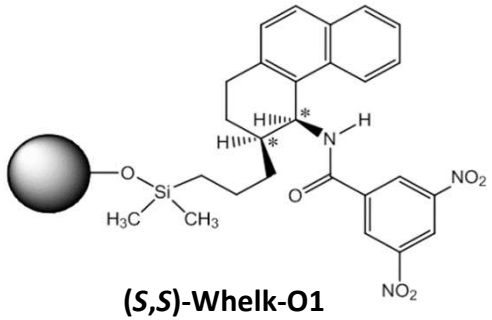
e.r. > 99%

C. Fernandes, P. Brandão, A. Santos, M.E. Tiritan, C. Afonso, Q.B. Cass, M.M. Pinto, *J. Chromatogr. A*, **2012,** 1269, 143-153.

RESULTS AND DISCUSSION

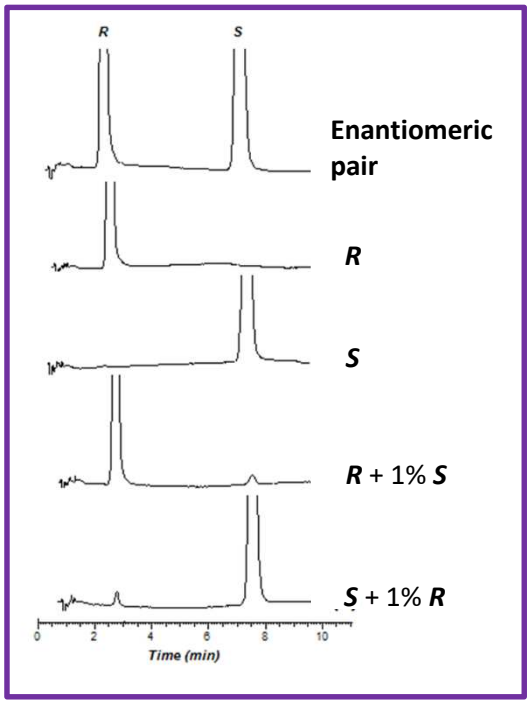
II. ENANTIOMERIC PURITY

Pirkle-type CSPs

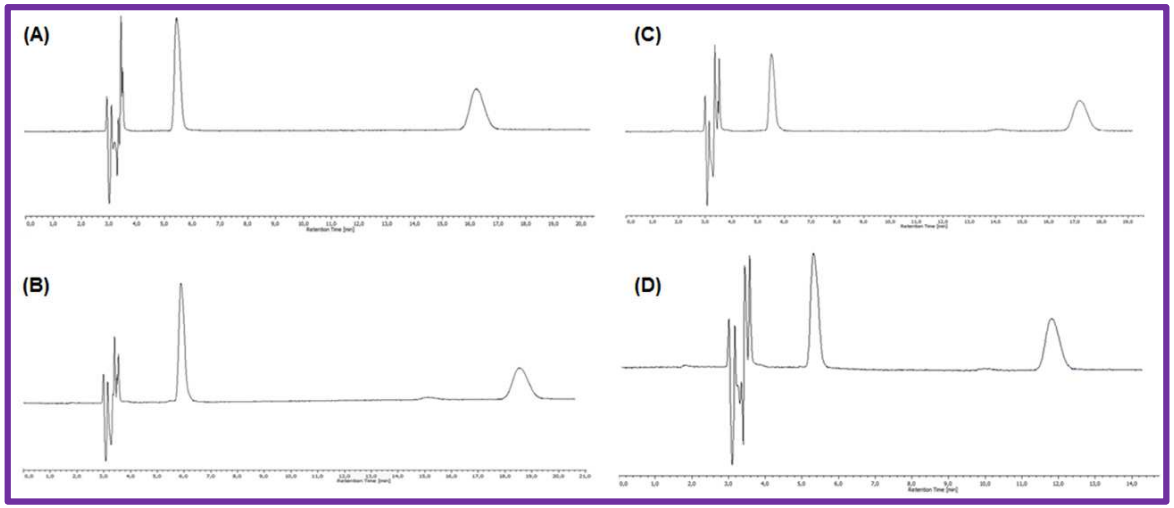


(*S*,*S*)-Whelk-O1

EXAMPLE



e.r. > 99%



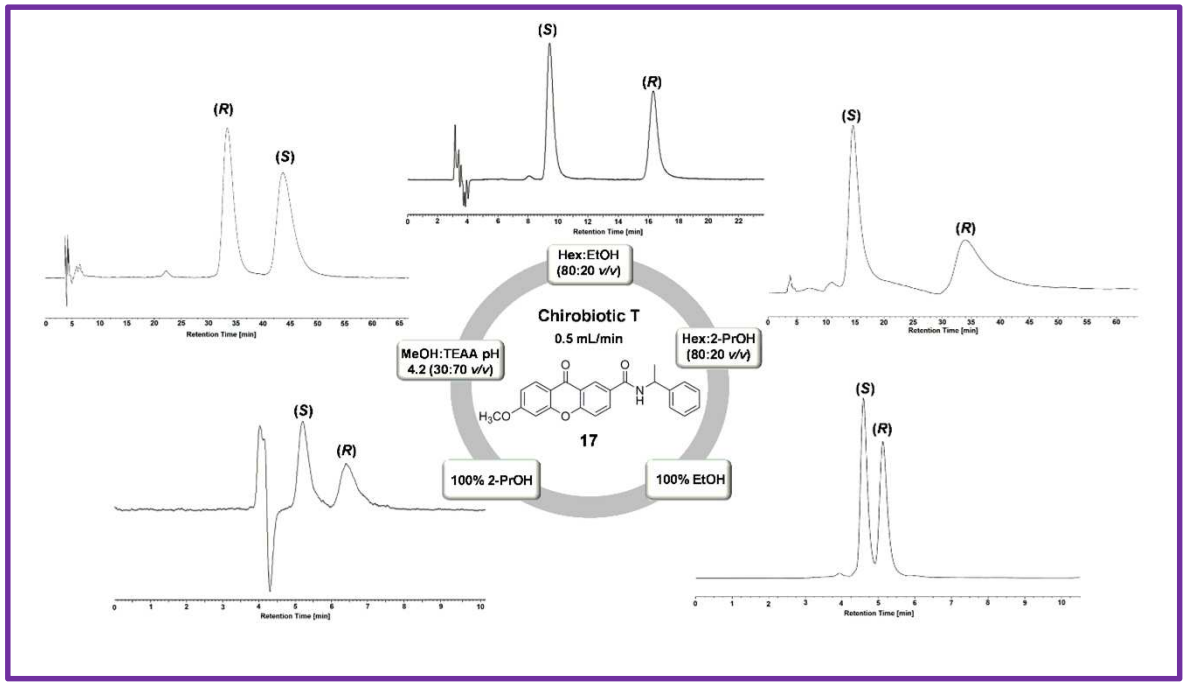
(A-D) LC chromatograms of enantiomeric pairs of new CDXs

ACN/MeOH (50:50 *v/v*), Flow rate 1.0 mL/min, detection wavelength 254 nm.

M.L. Carraro, A. Palmeira, M.E. Tiritan, C. Fernandes, M.M.M. Pinto, *Chirality*, **2017**, 1–10.

II. ENANTIOMERIC PURITY

Other example with macrocyclic antibiotic CSPs



Chromatograms of the enantioseparation of analyte **17** on Chirobiotic T column using different mobile phases.

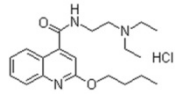
Flow rate 0.5 mL/min, detection wavelength 254 nm.

Y. Phyo, S. Cravo, A. Palmeira, M.E. Tiritan, A. Kijjoa, M.M.M. Pinto, C. Fernandes, *Molecules*, **2018,** 23, 142, doi:10.3390/molecules23010142.

RESULTS AND DISCUSSION

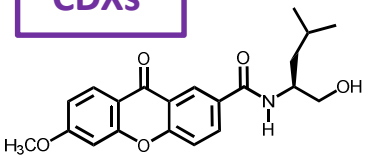
III. BIOLOGICAL SCREENING

Molecular moieties structurally very similar to aminoamide type local anaesthetics

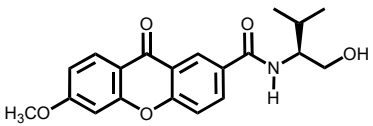


dibucaine

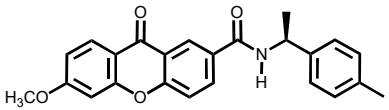
CDXs



XEL-L



XEVOL-L



XEA-S

EFFECT ON THE AMPLITUDE OF COMPOUND ACTION POTENTIALS

Rat sciatic nerve

Active

- low micromolar range (0.1 to 3 µM)
- nerve conduction blockade might result from an action on Na^+ ionic currents
- acting in a similar manner to local anaesthetic drugs

NERVE CONDUCTION BLOCKADE ACTIVITY

C. Fernandes, L. Oliveira, M.E. Tiritan, L. Leitão, A. Pozzi, J.B. Noronha-Matos, P. Correia-de-Sá, M.M. Pinto, *Eur. J. Med. Chem.*, **2012**, 55, 1-11.

RESULTS AND DISCUSSION

III. BIOLOGICAL SCREENING

INHIBITION OF GROWTH OF HUMAN TUMOR CELL LINES

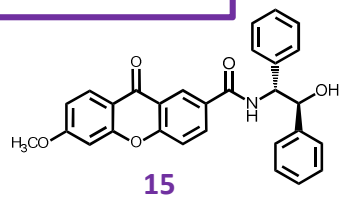
THE MOST ACTIVE

Compound	GI_{50} (μM) A375-C5	MCF-7	NCI-H460
3	>150	>150	85.88 ± 5.30
4	>150	91.91 ± 6.27	42.62 ± 1.77
15	32.15 ± 2.03	22.55 ± 1.99	14.05 ± 1.82
16	51.69 ± 5.77	36.54 ± 2.95	24.88 ± 1.37
31	>150	>150	>150
Doxorubicin	130.00 ± 25.20*	60.30 ± 1.20*	19.60 ± 1.90*

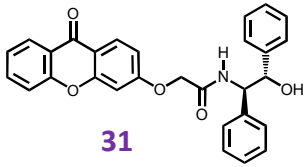
*Results are expressed in nM

A375-C5 (melanoma),
MCF-7 (breast adenocarcinoma)
NCI-H460 (non-small cell lung cancer)

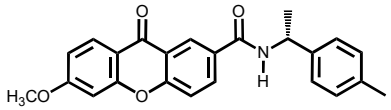
Structures of CDXs



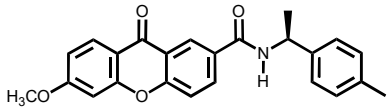
15



31



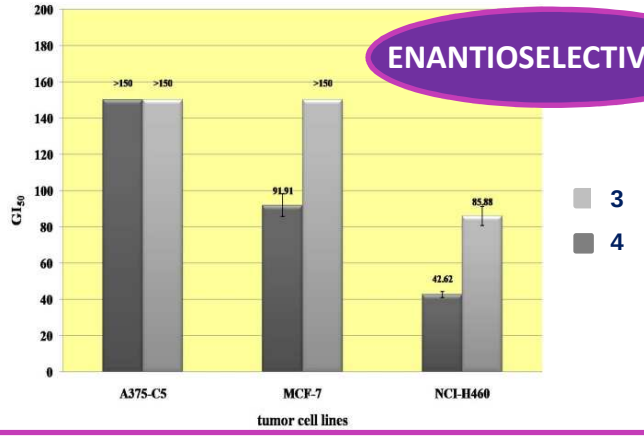
3



4

GI_{50} of enantiomeric pair of CDXs 3 and 4

ENANTIOSELECTIVITY



C. Fernandes, K. Masawang, M.E. Tiritan, E. Sousa, V. Lima, C. Afonso, H. Bousbaa, W. Sudprasert, M. Pedro, M. Pinto, *Bioorg. Med. Chem.* **2014**, 22, 1049-1062.

RESULTS AND DISCUSSION

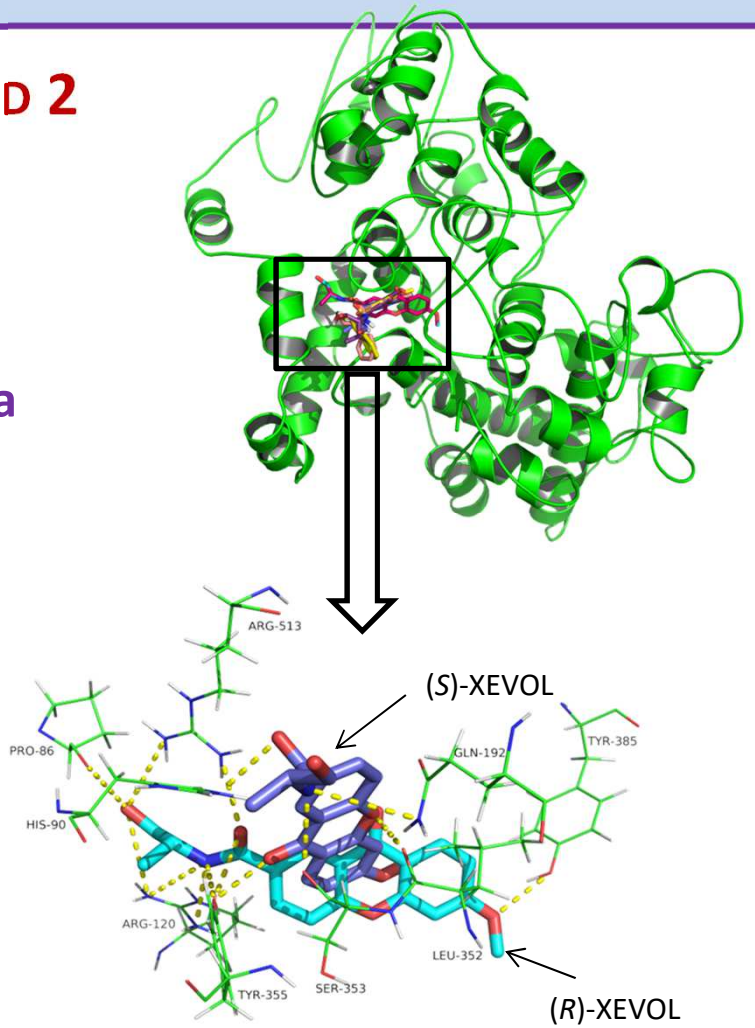
III. BIOLOGICAL SCREENING

INHIBITION OF CICLOXYGENASES (COXS) 1 AND 2

in silico **studies and** ***in vitro*** **assays**

COX-2 binding energy (Kcal/mol)		
Known ligands examples	Diclofenac	-7.9
	Indomethacin	-7.9
	Celecoxib	-11.5
	Valecoxib	-9.5
Ligands from database		-9.3
Decoys from database		-7.6
(*R*)-XEGOL2		-7.8
(*S*)-XEGOL2		-8.0
(*R*)-X2A1P		-6.9
(*S*)-X2A1P		-7.5
(*R*)-XEVOL		-6.5
(*S*)-XEVOL		-7.0

Docking data



C. Fernandes, A. Palmeira, I.I. Ramos, C. Carneiro, C. Afonso, M.E. Tiritan, H. Cidade, P.C.A.G. Pinto, M.L.M.F.S. Saraiva, S. Reis, M.M.M. Pinto, *Pharmaceuticals*, **2017**, 10, 50; doi:10.3390/ph10020050.

RESULTS AND DISCUSSION

IV. DEVELOPMENT OF CSPS FOR LC

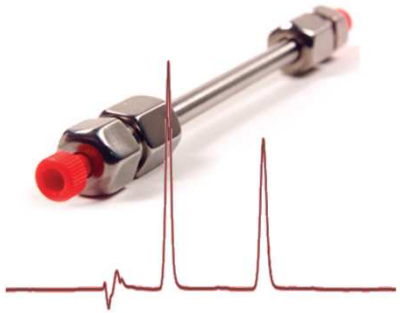
INSPIRATION

Chromatographia (2013) 76:871–897
DOI 10.1007/s10337-013-2469-8

REVIEW

Small Molecules as Chromatographic Tools for HPLC Enantiomeric Resolution: Pirkle-Type Chiral Stationary Phases Evolution

Carla Fernandes · Maria Elizabeth Tiritan · Madalena Pinto



Chiral Stationary Phases Based on Small Molecules: An Update of the Last 17 Years

Carla Fernandes,[1,2] Ye' Zaw Phyo,[3] Ana Sofia Silva,[1] Maria Elizabeth Tiritan,[1,2,4] Anake Kijjoa,[2,3] and Madalena M.M. Pinto[1,2]

Separation & Purification Reviews, 47: 89–123, 2018

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DOI: https://doi.org/10.1080/15422119.2017.1326939

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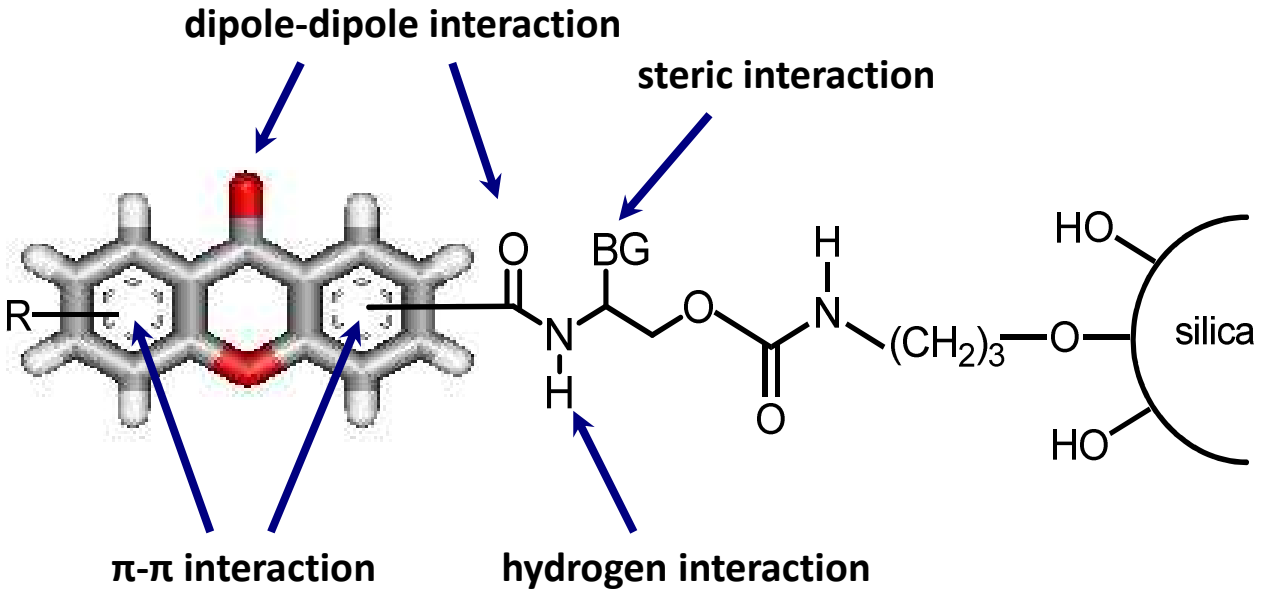
IV. DEVELOPMENT OF CSPS FOR LC

Why?



BG: Bulky group

XANTHONIC CHIRAL STATIONARY PHASE (XCSP)

New class of CSPs...

C. Fernandes, M.E. Tiritan, S, Cravo, Y. Phyo, A. Kijjoa, A.M.S. Silva, Q.B. Cass, M.M.M. Pinto, *Chirality*, **2017**, 29, 430–442.
M. Pinto, M.E. Tiritan, C. Fernandes, Q. Cass, Portuguese Patent nº 104679, in Boletim da Propriedade Industrial Nº 15/2011, 21-01-2011.

RESULTS AND DISCUSSION

IV. DEVELOPMENT OF CSPS FOR LC

XCSPs

LC columns → **Internal diameter** 4.6 and 2.1 mm

STRATEGY

CDX as selector

↓ $O=C=N(CH_2)_3Si(OCH_2CH_3)_3$

Silica-reactive derivative

↓ Silica

XCSP

↓ Packing →

LIQUID CHROMATOGRAPHY PERFORMANCE

Multimodal elution conditions

- Normal-phase
- Polar-organic
- Reversed-phase

Several racemates

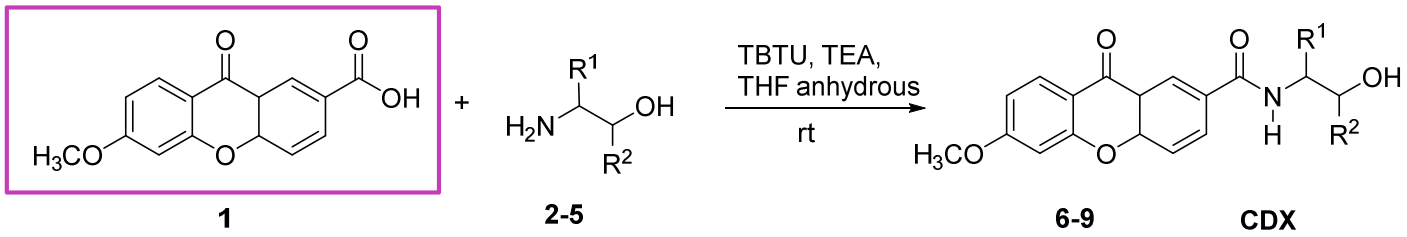
- Acid
- Neutral
- Basic
- Amino acids
- CDXs

C. Fernandes, M.E. Tiritan, S, Cravo, Y. Phyo, A. Kijjoa, A.M.S. Silva, Q.B. Cass, M.M.M. Pinto, *Chirality*, **2017**, 29, 430–442.
M. Pinto, M.E. Tiritan, C. Fernandes, Q. Cass, Portuguese Patent nº 104679, in Boletim da Propriedade Industrial Nº 15/2011, 21-01-2011.

RESULTS AND DISCUSSION

IV. DEVELOPMENT OF CSPS FOR LC

EXAMPLE:



XCSP 1 – XCSP 4

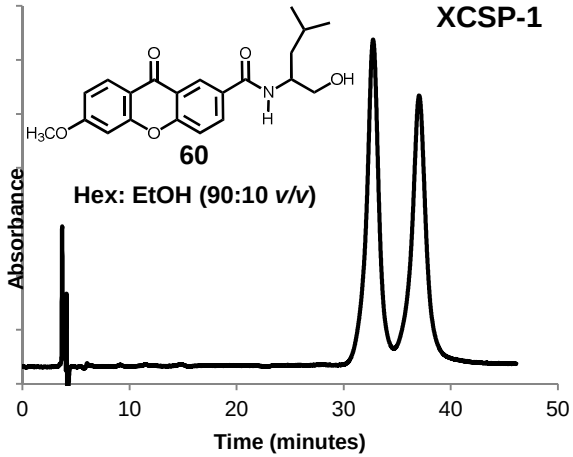
2,	**6**, **10**, **XCSP 1** (*S*)	R^1 = isopropyl	R^2 = H
3,	**7**, **11**, **XCSP 2** (*R*)	R^1 = phenyl	R^2 = H
4,	**8**, **12**, **XCSP 3** (*S*)	R^1 = phenyl	R^2 = H
5,	**9**, **13**, **XCSP 4** (*R,S*)	R^1 = phenyl	R^2 = phenyl

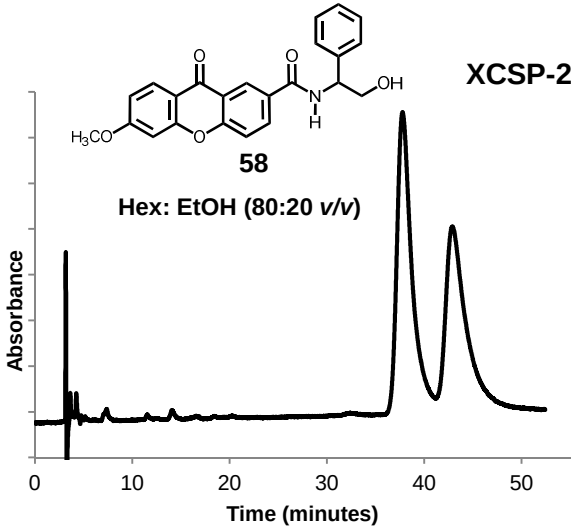
C. Fernandes, M.E. Tiritan, S, Cravo, Y. Phyo, A. Kijjoa, A.M.S. Silva, Q.B. Cass, M.M.M. Pinto, *Chirality*, **2017**, 29, 430–442.

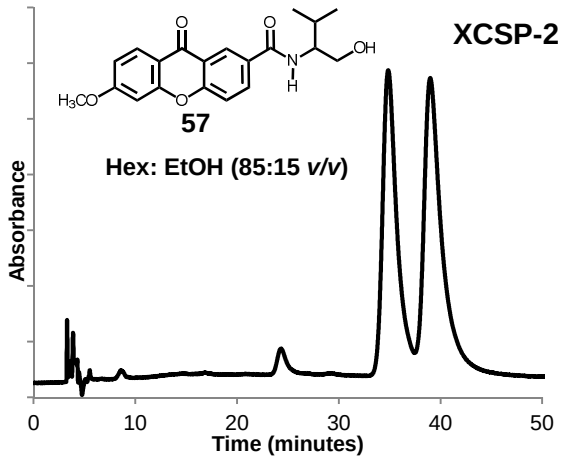
RESULTS AND DISCUSSION

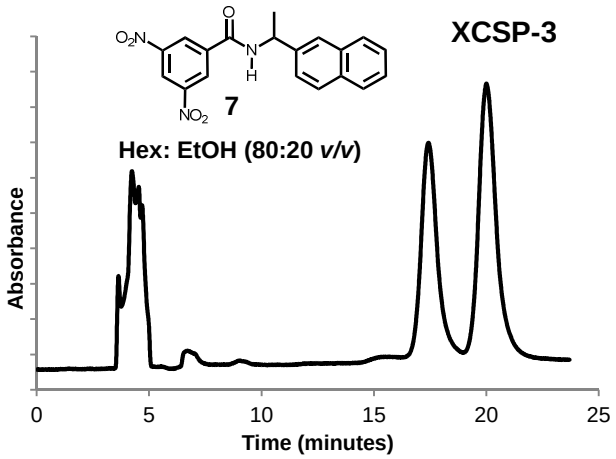
IV. DEVELOPMENT OF CSPS FOR LC

EXAMPLE OF CHROMATOGRAMS:









C. Fernandes, M.E. Tiritan, S, Cravo, Y. Phyo, A. Kijjoa, A.M.S. Silva, Q.B. Cass, M.M.M. Pinto, *Chirality*, **2017**, 29, 430–442.

IV. DEVELOPMENT OF CSPS FOR LC

New XCSPs

LC enantioselective capability

Reproducibility

Stability

Solvent versatility

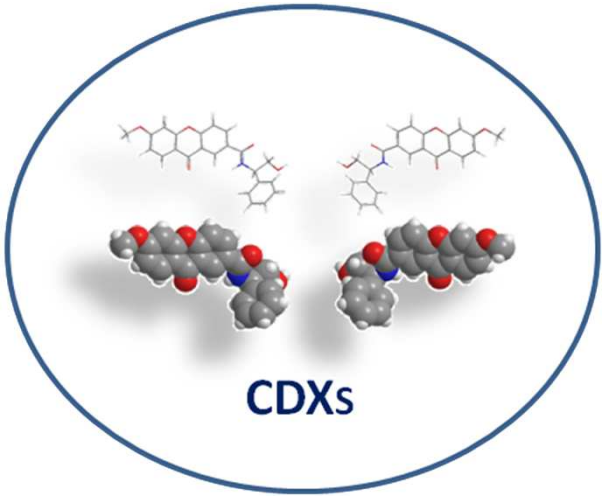
Proof of concept of reciprocity

Chiral self-recognition phenomenon

Inversion of elution order

CONCLUSIONS

THE SAME SMALL MOLECULES



BIOACTIVES

MEDICINAL CHEMISTRY

ANALYTIC TOOLS

LIQUID CHROMATOGRAPHY

ACKNOWLEDGMENTS

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