Journal of Chromatography A, 1241 (2012) 60–68

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Enantioseparation and chiral recognition mechanism of new chiral derivatives of xanthones on macrocyclic antibiotic stationary phases

Carla Fernandes[a,b], Maria Elizabeth Tiritan[a,c], Quezia Cass[d], Visvaldas Kairys[e], Miguel Xavier Fernandes[e], Madalena Pinto[a,b,*]

[a] Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[b] Laboratório de Química Orgânica e Farmacêutica, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[c] Centro de Investigação em Ciências da Saúde, Instituto Superior de Ciências da Saúde-Norte, (CICS-ISCS-N), Rua Central de Gandra 1317, 4585-116 Gandra PRD, Portugal
[d] Departamento de Química, Universidade Federal de São Carlos, SP, Rodovia Washington Luís (SP-310), km 235 – São Carlos, SP, Brasil
[e] Centro de Química da Madeira, Universidade da Madeira, Campus da Penteada, 9000-390 Funchal, Portugal

ARTICLE INFO

Article history:
Received 9 February 2012
Received in revised form 28 March 2012
Accepted 2 April 2012
Available online 11 April 2012

Keywords:
Macrocyclic antibiotic
Chiral stationary phases
Chiral derivatives of xanthones
Enantioselectivity
Enantiomeric purity
Chiral recognition

ABSTRACT

A chiral HPLC method using four macrocyclic antibiotic chiral stationary phases (CSPs) has been investigated for determination of the enantiomeric purity of fourteen new chiral derivatives of xanthones (CDXs). The separations were performed with the CSPs Chirobiotic T, Chirobiotic TAG, Chirobiotic V and Chirobiotic R under multimodal elution conditions (normal-phase, reversed-phase and polar ionic mode). The analyses were performed at room temperature in isocratic mode and UV and CD detection at a wavelength of 254 nm. The best enantioselectivity and resolution were achieved on Chirobiotic R and Chirobiotic T CSPs, under normal elution conditions, with R_S ranging from 1.25 to 2.50 and from 0.78 to 2.06, respectively. The optimized chromatographic conditions allowed the determination of the enantiomeric ratio of eight CDXs, always higher than 99%. In order to better understand the chromatographic behavior at a molecular level, and the structural features associated with the chiral recognition mechanism, computational studies by molecular docking were carried out using VDock. These studies shed light on the mechanisms involved in the enantioseparation for this important class of chiral compounds.

1. Introduction

The development of efficient methodologies to obtain both enantiomers with high enantiomeric purity is a very important task, especially in early stages of drug development. In this context, the use of HPLC with chiral stationary phases (CSPs) has proven to be the most helpful among the currently methods to achieve chiral separations and to measure the enantiomeric ratio [1,2]. On the other hand, macrocyclic antibiotic CSPs, introduced in 1994 by Armstrong et al. [3], became versatile and selective tools for the enantioresolution, evaluation of the enantiomeric purity and pharmacokinetic studies of different classes of chiral compounds [4–9]. The success of these CSPs can be associated with the diversity of their structures that contain a variety of functional groups, multiple stereogenic centers and inclusion cavities [10]. Furthermore, the possibility to perform at multimodal conditions, associated with their complementarily in selectivity profiles, increases their usefulness and applicability. So, nowadays the macrocyclic antibiotic CSPs are pointed out as one of the most versatile and robust CSPs [8].

In the past few years, several computational studies concerning chromatographic parameters have demonstrated to be very useful to understand the chiral recognition phenomenon of a variety of CSPs [11–18]. However, in spite of several studies regarding the structure of macrocyclic antibiotic CSPs [8,10,19–23], their physicochemical properties [8,10,20], applications [8,10,19,21], effect of the type of mobile phase [19,21,24] as well as thermodynamic investigations [25–27], relatively little is known in detail about their chiral recognition mechanisms at molecular level. It seems that the mechanism of chiral recognition is still not fully elucidated because of the structural complexity of the selectors which make investigations more complicated than with other selectors. Moreover, there are probably several kind of mechanisms that can contribute to the chiral recognition and subsequent retention, which depend not only on the nature of macrocyclic antibiotic CSP, but also of the structure of the analyte and on the elution chromatographic conditions [10]. Nonetheless, Bauvais et al. reported the elucidation of chiral recognition processes of nonsteroidal

* Corresponding author at: Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal. Tel.: +351 222078692; fax: +351 226093390.
E-mail addresses: madalena@ff.up.pt, madalenakijjoa@gmail.com (M. Pinto).

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2012.04.011

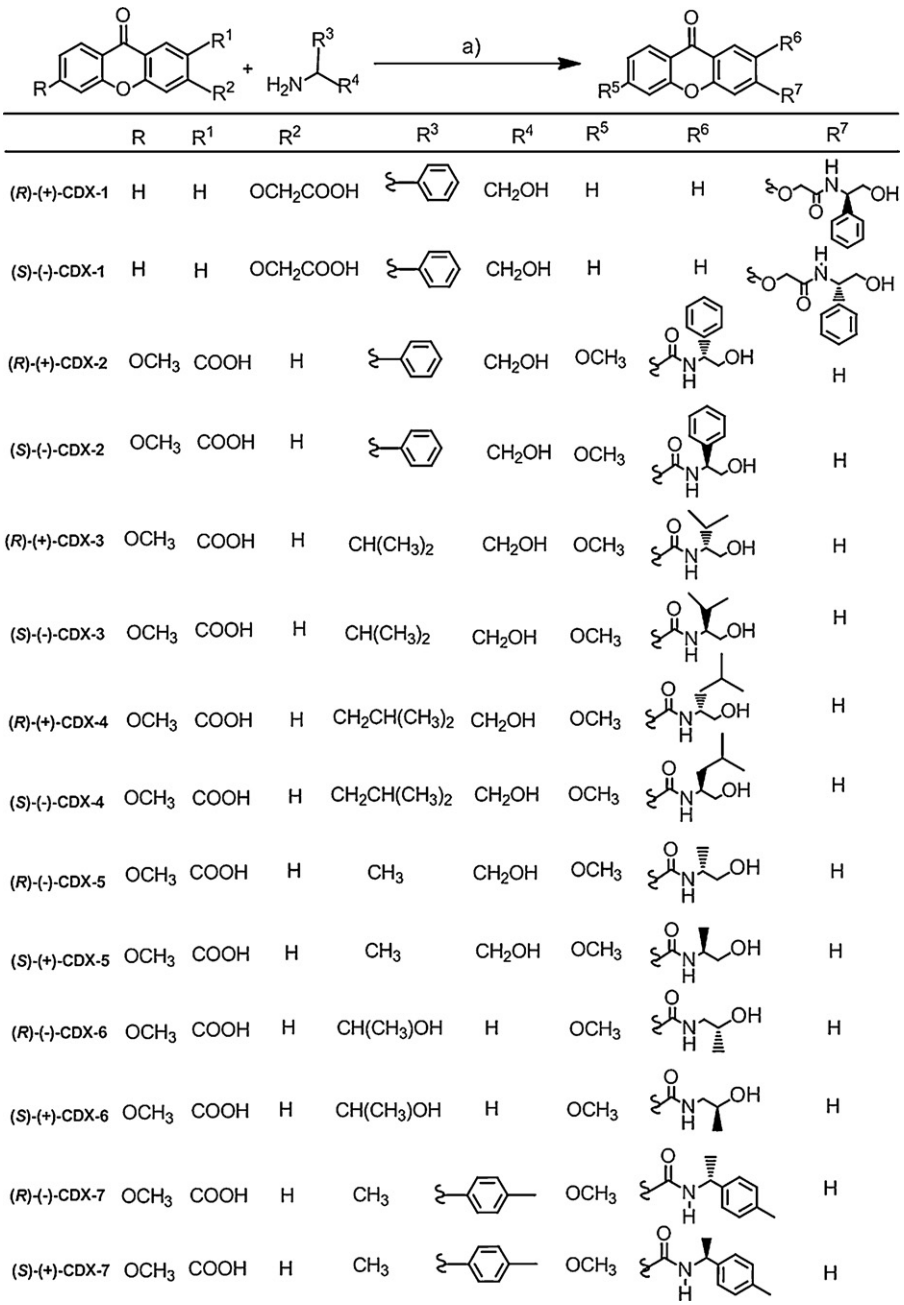

Fig. 1. Synthesis of (*R*)-(+) and (*S*)-(−)-CDX-1 (*N*-(2-hydroxy-1-phenylethyl)-2-((9-oxo-9*H*-xanthen-3-yl)oxy)acetamide); (*R*)-(+) and (*S*)-(−)-CDX-2 (*N*-(2-hydroxy-1-phenylethyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide); (*R*)-(+) and (*S*)-(−)-CDX-3 (*N*-(1-hydroxy-3-methylbutan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide); (*R*)-(+) and (*S*)-(−)-CDX-4 (*N*-(1-hydroxy-4-methylpentan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide); (*R*)-(−) and (*S*)-(+)-CDX-5 (*N*-(1-hydroxypropan-2-yl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide); (*R*)-(−) and (*S*)-(+)-CDX-6 (*N*-(2-hydroxypropyl)-6-methoxy-9-oxo-9*H*-xanthene-2-carboxamide); (*R*)-(−) and (*S*)-(+)-CDX-7 (6-methoxy-9-oxo-*N*-(1-(*p*-tolyl)ethyl)-9*H*-xanthene-2-carboxamide). Reagents and conditions: (a) TBTU, triethylamine, dry tetrahydrofuran, room temperature, 30 min to 3 h.

anti-inflammatory drugs, anti-neoplastic compounds and *N*-derivatized amino acids on vancomycin selector by docking studies and molecular dynamics simulations [28].

Due to their biological properties, the importance of xanthone (9*H*-xanthon-9-one) derivatives is well recognized in Medicinal Chemistry [29]. Considering that our group has a vast experience in synthesis and biological evaluation of xanthone derivatives [30–36]; some chiral members of this family are promising compounds for diverse biological/pharmacological activities [29,37–40]; and that the examples of synthetic chiral derivatives of xanthones (CDXs) described are scarce [37–40]; a small library of both enantiomers of new CDXs have been synthesized (Fig. 1) [41], their chiral enantioresolution evaluated in four macrocyclic antibiotic CSPs and the enantiomeric ratio measured.

The separations of the enantiomeric mixtures of CDXs were performed with the CSPs Chirobiotic T, Chirobiotic TAG, Chirobiotic V and Chirobiotic R (Fig. 2) under multimodal elution conditions (normal-phase, reversed-phase and polar ionic mode). The chromatographic conditions affording the best resolutions were used for the determination of the enantiomeric ratio of the new CDXs synthesized.

Additionally, in order to better understand the chromatographic parameters at a molecular level, and the structural features associated with the chiral recognition mechanism, computational