Journal of Pharmaceutical and Biomedical Analysis 89 (2014) 88–92

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Chromatographic separation and biological evaluation of benzimidazole derivative enantiomers as inhibitors of leukotriene biosynthesis

Roccaldo Sardella[a], Serkan Levent[b], Federica Ianni[a], Burcu Çalişkan[b], Jana Gerstmeier[c], Carlo Pergola[c], Oliver Werz[c], Erden Banoglu[b], Benedetto Natalini[a,*]

[a] Dipartimento di Chimica e Tecnologia del Farmaco, Università degli Studi di Perugia, Via del Liceo 1, 06123 Perugia, Italy
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Etiler, 06330 Yenimahalle, Ankara, Turkey
[c] Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, D-07743 Jena, Germany

ARTICLE INFO

Article history:
Received 5 August 2013
Accepted 23 October 2013
Available online 7 November 2013

Keywords:
Chromatographic enantioisolation
Polysaccharide-based stationary phases
5-Lipoxygenase-activating protein
FLAP
Benzimidazole

ABSTRACT

For an explicit analysis of the chirality on the effectiveness of a recently identified racemic benzimidazole derivative (BRP7) as inhibitor of leukotriene biosynthesis, we optimized a HPLC-based chiral chromatographic method enabling the quantitative isolation of its enantiomers in sufficient amount to carry out biological investigations. The use of a Lux Amylose-2 column revealed especially profitable to fulfil our task. Indeed, the employment of the amylose-based chiral stationary phase (CSP) in combination with a *n*-hexane/EtOH/DEA – 99/1/02 (v/v/v) mobile phase allowed getting the enantiomeric peaks fully resolved ($\alpha = 1.80$, $R_S = 2.39$). Four consecutive injections repeated at 1-min intervals produced overloaded peaks with a very limited level of isomeric contamination. This procedure allowed the isolation of ca. 20 mg of each enantiomer, with enantiomeric excess higher than 99% and 95% for the (*S*)- and the (*R*)-isomer, respectively. The enantiomeric elution order was established using synthetic reference compounds of lower enantiomeric excess values. The biological evaluation of the purified individual enantiomers revealed no significant difference in terms of their IC_{50} values with respect to the previously investigated racemic BRP7: 0.18 μM for the (*R*)-enantiomer ($R^2 = 0.999$) and 0.26 μM for the (*S*)-enantiomer ($R^2 = 0.986$).

1. Introduction

Leukotrienes (LTs) are potent lipid mediators with active roles in the initiation and amplification of the inflammatory response by regulating the recruitment and activation of leukocytes in inflamed tissues [1]. The formation of these important lipid mediators from arachidonic acid (AA) is catalyzed by a key enzyme, 5-lipoxygenase (5-LO), with a concomitant help from a complementary protein called 5-lipoxygenase-activating protein (FLAP). For LT biosynthesis in leukocytes, the substrate AA, which is released from membrane phospholipids by cytosolic phospholipase A_2 ($cPLA_2$), is cought up by FLAP, and then transferred by FLAP to 5-LO for biosynthesis of LTs [2]. Therefore, FLAP acts as a bridging protein for an efficient transfer of arachidonic acid to 5-LO active site at the nuclear membrane for a streamlined synthesis of LTs [3]. Although no FLAP inhibitor has yet reached the market, the recently developed FLAP inhibitors such as AM803 (Fig. 1A), AM643 (Fig. 1B), and AM103 (Fig. 1C) were shown to be efficacious in preclinical studies of inflammatory diseases as well as in clinical trials with patients suffering from asthma [4–8].

With the aim of discovering novel FLAP inhibitors, we recently reported the identification of a new chemotype bearing a benzimidazole core structure with an ibuprofen fingerprint (BRP7, Fig. 1D) as a potent inhibitor of LT biosynthesis in intact cells with a bioactivity in the submicromolar range [9]. Since the lead compound BRP7 was evaluated as a racemate, the aim of the current work is to investigate the effect of chirality on the BRP7 inhibitory potency.

In order to obtain BRP7 enantiomers, the "racemic approach" [10] was preferred over the traditional "chemical approach". With the former approach, the racemate is synthesized and then separated into its enantiomers through a suitable enantioselective chromatographic procedure, which is generally accepted to be the most rapid route to the preparation of small quantities of both, highly pure enantiomers [10]. Indeed, the racemate preparation is usually achieved by applying synthetic protocols with a much lower degree of complexity than those required for having access to the corresponding optically active forms. Even more

* Corresponding author. Tel.: +39 075 5855131; fax: +39 075 5855161.
E-mail address: natalini@chimfarm.unipg.it (B. Natalini).

0731-7085/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jpba.2013.10.039

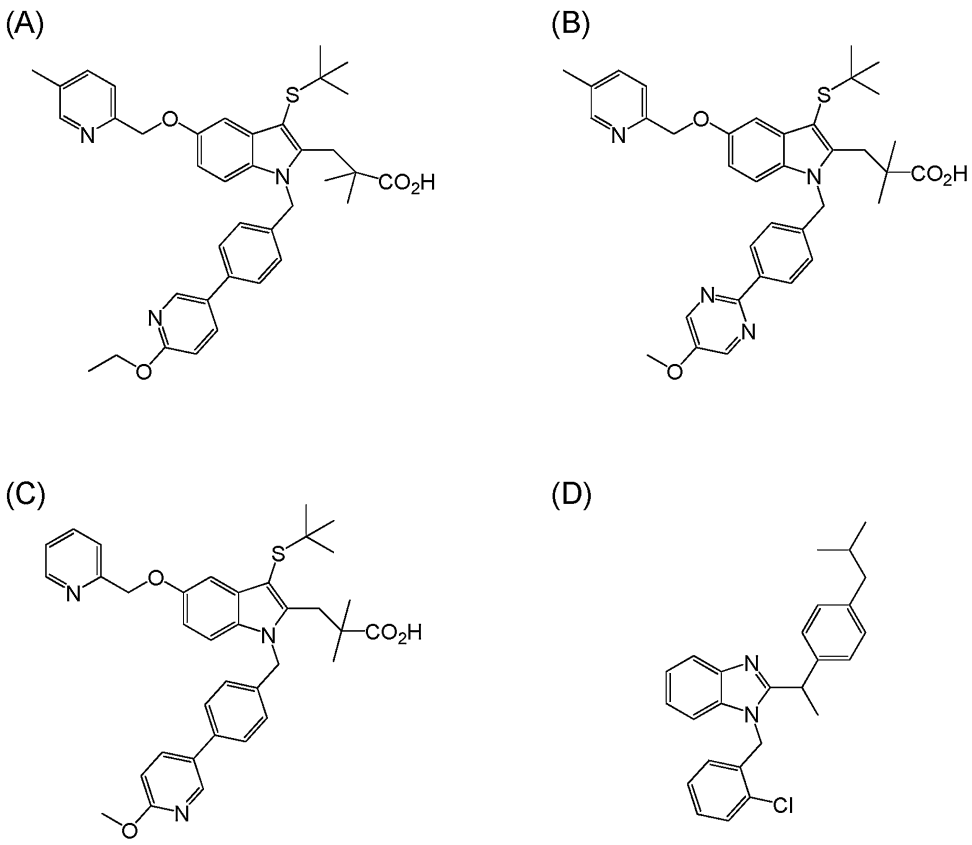

Fig. 1. Structure of (A) AM803, (B) AM643, (C) AM103 and (D) BRP7.

attractive is that suitable chromatographic approaches can provide both enantiomers at once, which is particularly convenient in the case of preliminary comparative biological assays. More to the point, small-scale preparative runs can be performed on analytical columns [11], when only very few milligrams of each enantiomer are needed. Usually, direct chromatographic enantioisolations are pursued after a careful selection of the best available chiral stationary phase (CSP) for the racemic compound of interest [10]. Indeed, apart from a very limited number of cases [12], user guides available for helping in the correct choice of the more appropriate CSP for a given application, are scarcely effective in the practice. The CSP selection step is then followed by the eluent composition optimization. All these aspects, along with results of the biological tests carried out with the individual BRP7 enantiomers, are the subject of the following sections.

2. Materials and methods

2.1. Chemicals and reagents

Racemic BRP7 was synthesized and characterized in our laboratory according to our reported procedure [9]. Analytical grade ethanol (EtOH), *n*-hexane, diethylamine (DEA), ethyl acetate (EtOAc), and 1,3,5-tri-*tert*-butylbenzene were purchased from Sigma–Aldrich (Milano, Italy). HPLC-grade water was obtained from a New Human Power I Scholar water purification system (Human Corporation, Seoul, Korea). All the employed mobile phases were degassed with 10 min sonication before use. Compounds to be injected were solubilized in the selected mobile phase and analyzed at the approximate concentration of 0.5–1.0 mg/mL.

2.2. Instrumentation

The enantioseparation analyses were carried out with the following three columns (Fig. 2): the columns Lux Amylose-2 (CSP 1) (250 mm × 4.6 mm I.D., containing amylose

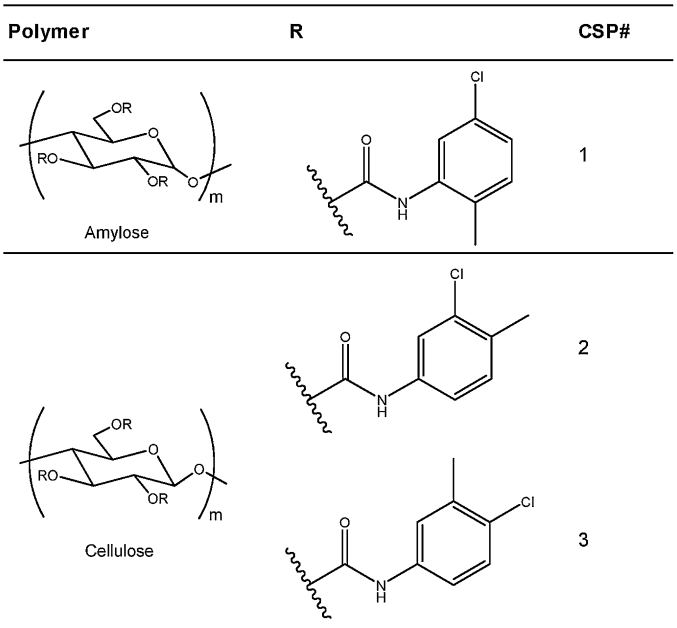

Fig. 2. Structure of the selected chloromethylated polysaccharide-based chiral selectors.