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Preparative enantiomeric separation of potent AMP-activated protein kinase activator by HPLC on amylose-based chiral stationary phase Determination of enantiomeric purity and assignment of absolute configuration

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Abstract

The development of high performance liquid chromatography method on amylose-based stationary phases (Chiralpak AD) has permitted to achieve the preparative enantioseparation of one benzimidazole derivative, potent-AMP-kinase (AMPK) activator with satisfactory yields. Analytical enantioseparation method was optimized and validated to determine the enantiomeric purity. Using the UV detection, repeatability, limits of detection (LD) and quantification (LQ) were determined. Single-crystal X-ray analysis was successful to determine the absolute configuration of the individual enantiomers. A relation between the retention order and the absolute configuration of the enantiomers was established.

Keywords: AMPK; Enantiomeric separation; Chiral stationary phases; Chiralpak AD; Validation; X-ray crystallography

1. Introduction

The AMP-activated protein kinase (AMPK) is the central component of a protein kinase cascade that plays a major role in energy sensing. AMPK itself plays a key role in the regulation of metabolism within the muscle cell and has been already identified as a potential target for type 2 diabetes mellitus and obesity [1–4]. We recently described the synthesis and biological evaluation of benzimidazole derivative as potent AMPK activators [5].

Compounds were designed starting from the lead compound **1** (S27847, Fig. 1): firstly, by modification of the cyclohexylphenyl moiety and secondly, by introducing diversity on the aromatic moiety of the benzimidazole ring to obtain potential therapeutic agents. Many of them present high *in vitro* activation of the AMP-kinase on fresh rat hepatocytes. Those compounds present a chiral center and pharmacological studies of each enantiomer are so required. Chiral high performance liquid chromatography (HPLC) is one of the most rapid and efficient methods to obtain directly both enantiomers in high optical purity in a single step [6,7].

The aim of this study was to achieve preparative enantioselective HPLC separation of one of the most active racemic compounds to test the enantiomerically pure molecules so as to

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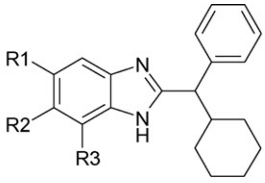
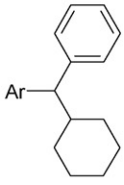


Compound	R1	R2	R3
1 (S27847)	H	H	H
2	CH_3	CH_3	H
3	H	Cl	H
4	H	CH_3	H
5	H	CH_3	CH_3
6	H	CF_3	H
7	H	F	H
8	OCH_3	OCH_3	H
9	H	OCH_3	H



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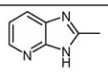
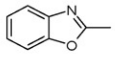
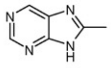
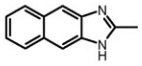
10	
11	
12	
13	
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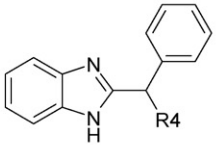

Compound	R4
15	CH_3
16	
17	

Fig. 1. Benzimidazoles derivatives.

enhance activity of benzimidazole derivatives. First, analytical methods were developed to determine the stationary and mobile phases which permitted the best enantiomeric separations: in literature benzimidazole derivatives chiral separations are mainly performed on Chiralpak AD as rabeprazole [8] or omeprazole [9–11]. Then, prior to the preparative scale, loading studies were performed to optimize the operational conditions. We then developed and validated analytical methods in order to quantify the enantiomeric purity. The absolute configuration of the individual enantiomers was established by X-ray analysis to obtain data for a mechanistic description of the chiral recognition in HPLC and of the ligand-receptor stereoselective interactions.

2. Experimental

2.1. Chiral liquid chromatography

Compounds **1**–**17** (Fig. 1) were prepared according to the synthetic pathway previously described [5], leading to a racemic mixture of enantiomers.