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Serendipitous discovery of a pH-dependant atropisomer bond rotation: Toward a write-protectable chiral molecular switch?☆

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Dedicated to Professor Wolfgang Lindner on the occasion of his 60th birthday.

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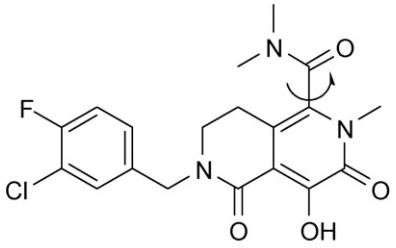
ABSTRACT

Owing to slow rotation of a sterically constrained dimethylamide substituent, two slowly interconverting enantiomers of a preclinical candidate for pharmaceutical development, 1, (6-(3-Chloro-4-fluoro-benzyl)-4-hydroxy-2-methyl-3,5-dioxo-2,3,5,6,7,8-hexahydro-[2,6]naphthyridine-1-carboxylic acid dimethylamide) are observed by chiral chromatography. Isolation of pure enantiomer by preparative chiral chromatography followed by enantiopurity analysis over time allowed for a study of the kinetics of enantiomer interconversion under a variety of conditions. Relatively slow racemization was observed in alcohol solvents, with a half life on the order of 5–10 h. A dramatic influence of aqueous buffer pH on racemization was noted, with higher pH leading to rapid racemization within a few minutes, and lower pH leading to essentially no racemization for periods up to a week. A hypothesis explaining this unusual effect of pH on carboxamide bond rotation is offered, and some suggestions for potential utility of such a system are considered.

1. Introduction

The chromatographic separation of slowly interconverting atropisomers is a popular subject within the field of chiral chromatography, with molecules from diverse structural classes having been studied using a variety of chromatographic techniques [1–14]. We herein report the serendipitous discovery of an unusual interconverting atropisomer system, **1**, where a strong influence of pH on carboxamide bond rotation is observed. Preliminary HPLC observations of compound **1** using chiral HPLC led to the initially surprising observation of peak splitting. Based on known atropisomeric systems containing hindered carboxamides, an explanation based on slow interconversion of enantiomeric carboxamide bond rotamers of **1** was proposed, and subsequent experiments were performed to more fully characterize the system (Scheme 1).



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Scheme 1. Restricted rotation about the carboxamide bond of 6-(3-chloro-4-fluoro-benzyl)-4-hydroxy-2-methyl-3,5-dioxo-2,3,5,6,7,8-hexahydro-[2,6]naphthyridine-1-carboxylic acid dimethylamide (compound **1**) gives rise to two slowly interconverting enantiomers which can be resolved using chiral chromatography.

2. Experimental

2.1. Materials

Compound **1,** (6-(3-chloro-4-fluoro-benzyl)-4-hydroxy-2-methyl-3,5-dioxo-2,3,5,6,7,8-hexahydro-[2,6]naphthyridine-1-carboxylic acid dimethylamide) is an investigational compound from these laboratories, the synthesis and pharmaceutical properties of which will be described in a forthcoming publication. Methanol, HPLC-grade solvent, was purchased from EMD Chemicals, Inc. (Gibbstown, NJ). Carbon dioxide (bone dry) was purchased from Airgas, Inc. (Radnor, PA). Chiralpak AD-H columns were purchased from Chiral Technologies (Exton, PA). Zorbax Extend C18 column was purchased from Agilent Technologies. Buffers were purchased from Fisher Scientific (Hampton, NH).

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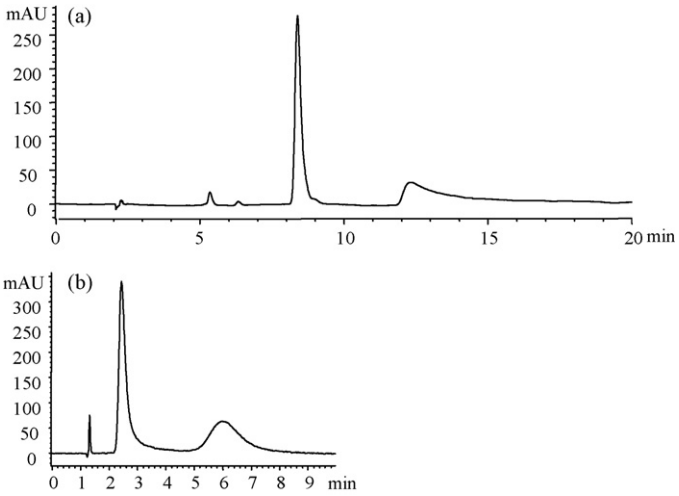

Fig. 1. Chromatographic separation of the enantiomers of interconverting atropisomer **1**: (a) chiral SFC method (Chiralpak AD-H; 4.6 × 250 mm; 20% MeOH/CO_2; 35 °C; 200 bar, 1.5 mL/min; UV 215 nm); (b) HPLC method (Chiralpak AD-H 4.6 × 150 mm, 100% MeOH; 1.5 mL/min; UV 254 nm).

2.2. Chiral SFC

Chiral SFC analysis was carried out using the Berger–Mettler Toledo analytical supercritical fluid chromatograph fitted with six position column selection valve and Agilent model 1100 diode array UV–vis detector. Column screening was carried out using a standard gradient approach described previously. [15] The optimized isocratic analytical chiral SFC method employed a Chiralpak AD-H column (250 × 4.6 mm), with an eluent of 20% methanol in carbon dioxide at 1.5 mL/min, 200 bar pressure, 35 °C oven temperature, 215 nm.

2.3. Circular dichroism (CD) studies

Circular dichroism (CD) studies were performed using a Jasco Model 810 CD spectrometer using ethanol as a solvent and operating at room temperature.

2.4. HPLC-CD

HPLC-CD analysis was performed using a model 1100 HPLC with DAD detector (Agilent, Palo Alto, CA), a model 1595 CD HPLC detector (Jasco, Easton, MD) operating at 225 nm. Chiral HPLC-CD analysis employed a Chiralpak AD-H column (4.6 × 150 mm) 100% MeOH; 1.5 mL/min; UV 254 nm). Achiral HPLC-CD employed a Zorbax Extend C18 (4.6 × 75 mm); 50% ACN/water; 1 mL/min; UV 254nm; CD 225 nm.

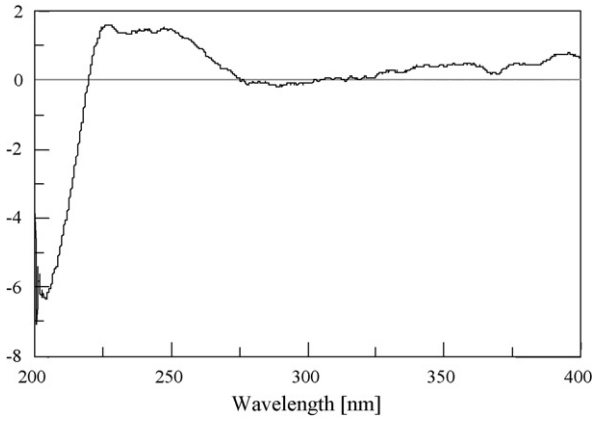

Fig. 2. CD spectrum in methanol of first eluted enantiomer from prep SFC purification of **1** on Chiralpak AD.

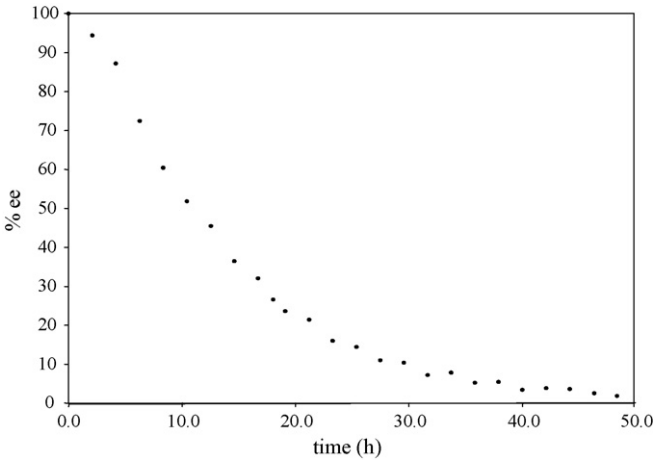

Fig. 3. Racemization of **1** in methanol as measured by chiral HPLC analysis on Chiralpak AD-H using method illustrated in Fig. 1c.

2.5. Semi-preparative SFC

Semi preparative separation of atropisomers of **1** was carried out using the Berger Multigram semi-preparative SFC system (Mettler–Toledo Autochem, Newark, DE) with a Chiralpak AD column 250 × 20 mm (Chiral Technologies) with a methanol/carbon dioxide eluent at a flow rate of 50 mL/min. Isolated fractions were quickly evaporated at room temperature by rotary evaporation and stored until use in a freezer. Initial attempts at rotary evaporation of isolated fractions using a heated water bath led to significant racemization during evaporation.

2.6. UV–vis spectroscopy

UV–vis spectra were recorded using a SpectraMax UV–vis Microplate Reader (Molecular Devices, Sunnyvale, CA) and a quartz 96-well microplate (Spike International, Wilmington, NC).

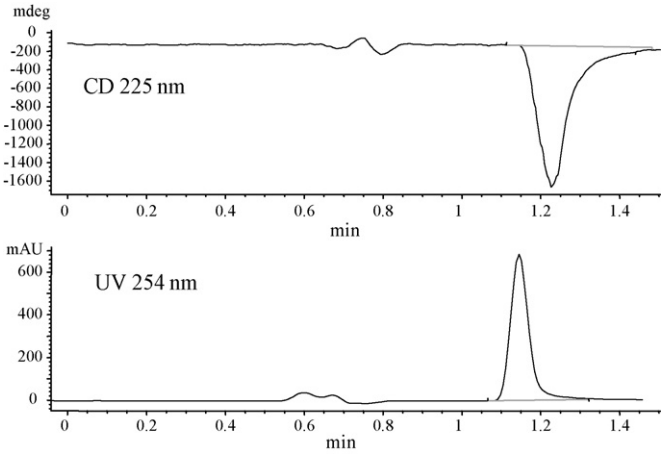

Fig. 4. Achiral HPLC with CD detection: extend C18; 4.6 × 75 mm; 50% ACN/water; 1 mL/min; UV 254 nm and CD 225 nm.