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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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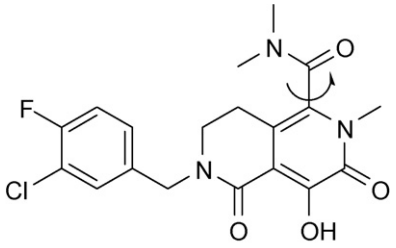
ABSTRACT

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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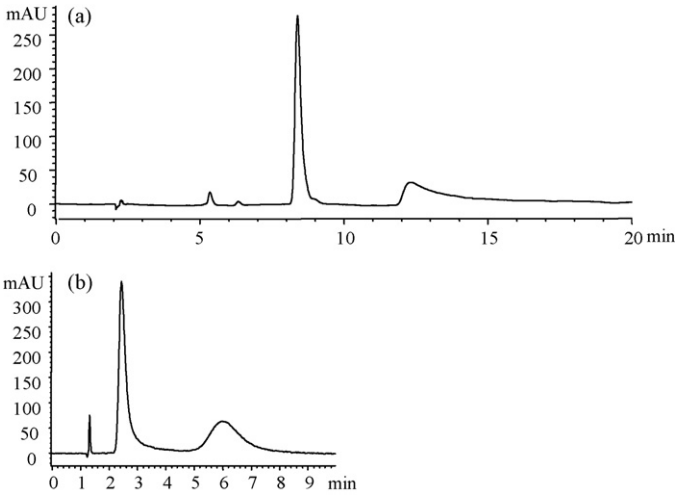

Fig. 1. Chromatographic separation of the enantiomers of interconverting atropisomer **1**: (a) chiral SFC method (Chiralpak AD-H; 4.6 × 250 mm; 20% MeOH/CO_2; 35 °C; 200 bar, 1.5 mL/min; UV 215 nm); (b) HPLC method (Chiralpak AD-H 4.6 × 150 mm, 100% MeOH; 1.5 mL/min; UV 254 nm).

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.

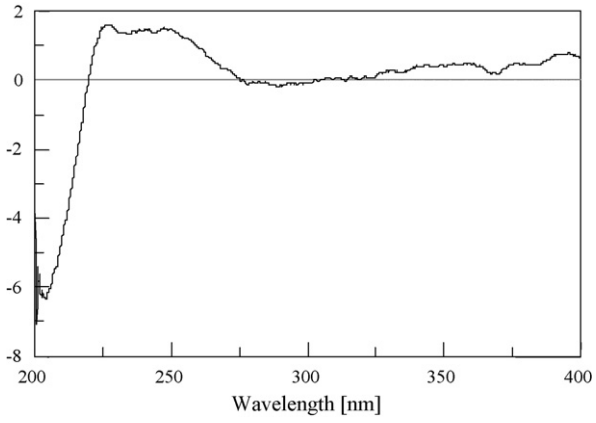

Fig. 2. CD spectrum in methanol of first eluted enantiomer from prep SFC purification of **1** on Chiralpak AD.

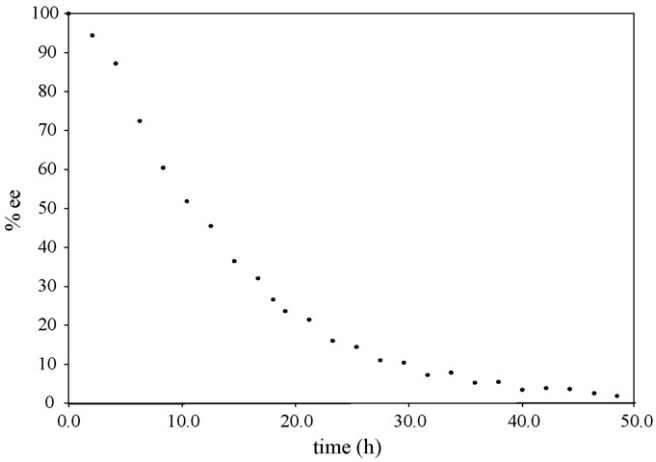

Fig. 3. Racemization of **1** in methanol as measured by chiral HPLC analysis on Chiralpak AD-H using method illustrated in Fig. 1c.

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).

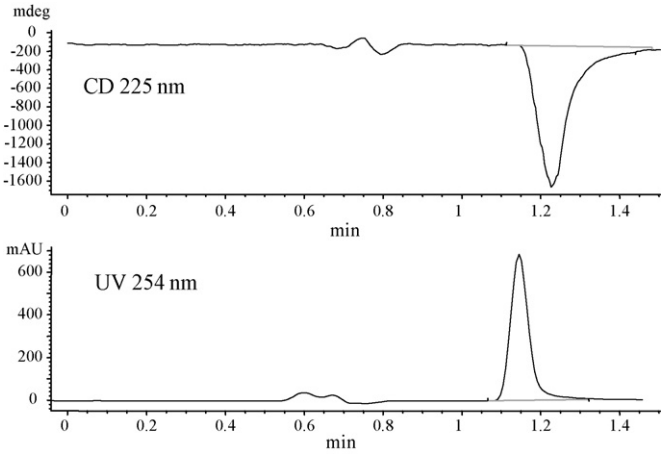

Fig. 4. Achiral HPLC with CD detection: extend C18; 4.6 × 75 mm; 50% ACN/water; 1 mL/min; UV 254 nm and CD 225 nm.

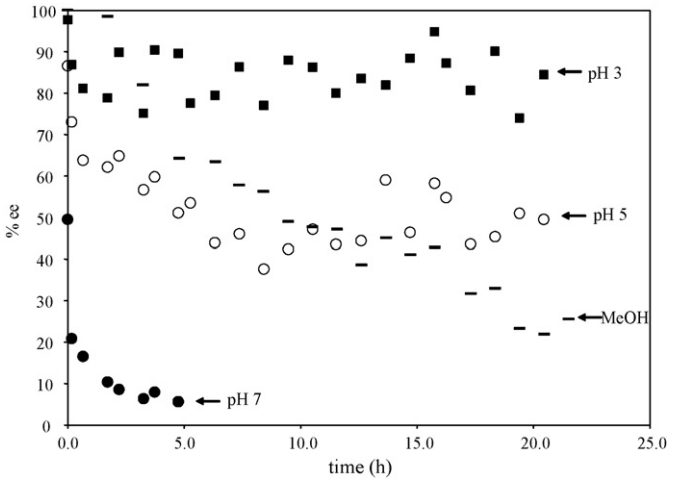

Fig. 5. Effect of buffer pH on racemization of **1**. Samples prepared by addition of 100 μL 1 mg/mL methanol solution of isomer 1 from preparative SFC separation to 400 μL methanol, with addition of 500 μL of aqueous buffer solution. Samples analyzed using the achiral HPLC with CD detection method illustrated in Fig. 4.

3. Results and discussion

Following initial observations of peak doubling of **1** *via* chiral HPLC, chiral SFC method development screening [16] was undertaken, leading to identification of Chiralpak AD-H as a suitable column for baseline SFC resolution (Fig. 1a). An HPLC method based on this initial lead was developed to provide baseline resolution of enantiomers within 8 min (Fig. 1b).

Semi-preparative enantiomer separation of 50 mg of racemate (Chiralpak AD (20 μm); 21.1 × 250 mm; 40% MeOH; CO_2; 35 °C) afforded access to enantioenriched material for further study. Analysis of isolated enantiomer by circular dichroism revealed an appropriate observation wavelength for HPLC-CD experiments of 225 nm (Fig. 2). An overnight CD timecourse investigation showed that in methanol solution, racemization occurs relatively quickly at room temperature, with a half life of only a few hours.

A follow up timecourse investigation using chiral HPLC analysis (Chiralpak AD-H; 4.6 × 50 mm; 100% MeOH; 1.5 mL/min) showed a half-life of racemization at room temperature of about 8.4 h (Fig. 3) corresponding to an energy barrier for bond rotation of about 24 kcal/mol.

In order to support an investigation of the effect of aqueous pH on enantiomer interconversion, an achiral HPLC method with CD detection was developed, following the observation that direct injection of aqueous samples onto Chiralpak AD-H led to column destruction. A representative chromatogram (Fig. 4) showing injection of the second eluted enantiomer from Chiralpak AD-H illustrates the rapid 1.5 min analysis time available with this method.

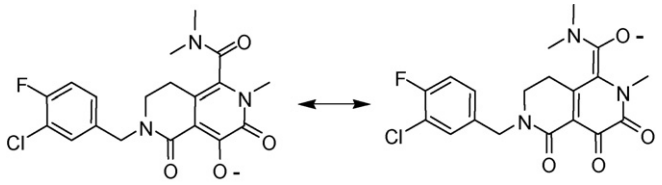

Fig. 6. Deprotonation of **1** may lead to tautomeric form in which planar carboxamide structure is stabilized, thereby aiding racemization.

Using this fast assay, a dramatic influence of pH on racemization was noted. As illustrated in Fig. 5, the rate of racemization in 50% MeOH/pH 5 buffer is similar to the rate in pure methanol. However, racemization at pH 7 and above was found to be exceedingly rapid, while racemization at pH 3 and below was quite slow, with samples still retaining considerable enantiopurity for more than one week. It should be noted that in this instance, the use of achiral HPLC with CD detection provides only an approximate measure of enantiopurity, as evidenced by the considerable scatter observed in the data. Nevertheless, a clear trend can be observed that is indicative of the strong dependence of enantiomer interconversion on pH.

A dramatic influence of pH on the racemization of a chiral ketone, ester or similar compound that racemizes *via* a deprotonation pathway is to be expected. However, the influence of pH on the racemization of **1** is rather surprising, as racemization in this instance involves only a simple bond rotation. The structure illustrated in Fig. 6 may help to explain this pH dependence, with the delocalized anionic tautomer helping to stabilize the planar transition state required for bond rotation.

An investigation of the effect of pH on the UV–vis absorbance spectra of 1 revealed a dramatic shift to higher wavelength at high pH, consistent with the extended conjugation proposed in the foregoing hypothesis (Fig. 7).

A number of potential applications for chiral molecular switches [17] based on atropisomer interconversion have been suggested, including photochromic triggers for liquid crystal data storage [18] and structural elements for molecular motors. [19,20] We previously reported the use of immobilized amide atropisomers as imprintable media for carrying out chromatographic separations. [21] In this approach, deracemization of the stationary phase is accomplished by prolonged exposure to a single enantiomer of a guest analyte that is bound with high affinity and high enantioselectivity. A serious drawback of this approach stems from the

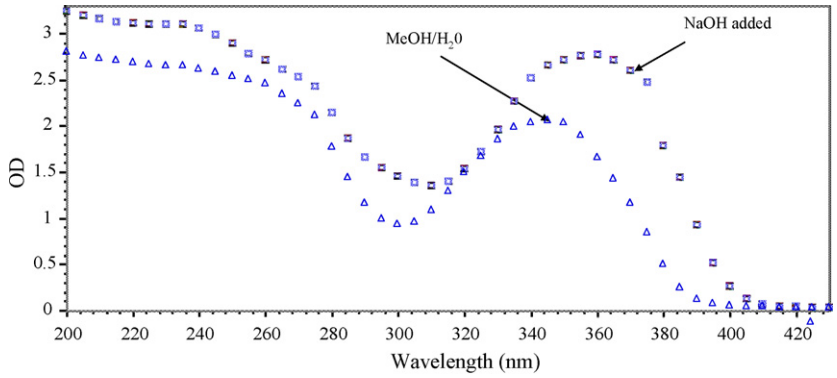

Fig. 7. Deprotonation of **1** leads to an increased absorbance at higher wavelength in the UV–vis spectrum.

fact that the imprinted stationary phase soon 'fades', returning to the equilibrium racemic mixture of immobilized atropisomers. Longer operational lifetime of the stationary phase can be obtained by selecting atropisomers with a higher barrier to racemization, but slow racemization also means that longer time is required for imprinting *via* deracemization.

In the present system, the property of atropisomer interconversion at high pH, and the ability to halt switching at low pH may be a useful combination that could be of general utility, offering, in essence, a write-protectable chiral molecular switch. Simplified analogs of the core of **1** may be expected to share these pH-dependent racemization properties, and may prove useful as chiral molecular switch components.

4. Conclusion

Serendipitous discovery of a slow rotation of a sterically constrained dimethylamide substituent within a preclinical candidate for pharmaceutical development led to the observation of two slowly interconverting enantiomers by chiral chromatography. Subsequent investigation revealed a dramatic influence of aqueous buffer pH on racemization, 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 potential utility of the system as a write-protectable chiral molecular switch is considered.

Acknowledgements

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