Investigation of the Mechanism of Racemization of Litronesib in Aqueous Solution: Unexpected Base-Catalyzed Inversion of a Fully Substituted Carbon Chiral Center

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ABSTRACT: Mitosis inhibitor (*R*)-litronesib (LY2523355) is a 1,3,4-thiadiazoline, bearing phenyl and *N*-(2-ethylamino)ethanesulfonamidomethyl substituents on tetrahedral C5. Chiral instability has been observed at pH 6 and above with the rate of racemization increasing with pH. A positively charged trigonal intermediate is inferred from the fact that a *p*-methoxy substituent on the phenyl accelerated racemization, whereas a *p*-trifluoromethyl substituent had the opposite effect. Racemization is proposed to occur through a relay mechanism involving intramolecular deprotonation of the sulfonamide by the side chain amino group and attack of the sulfonamide anion on C5, cleaving the C5–S bond, to form an aziridine; heterolytic dissociation of the aziridine yields an ylide. This pathway is supported by (1) a crystal structure providing evidence for a hydrogen bond between the sulfonamide NH and the amino group, (2) effects of substituents on the rate of racemization, and (3) computational studies. This racemization mechanism results from neighboring group effects in this densely functionalized molecule. Of particular novelty is the involvement of the side-chain secondary amino group, which overcomes the weak acidity of the sulfonamide by anchimeric assistance. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2797–2808, 2014

Keywords: racemization; chiral inversion; mechanism; neighboring group; relay ionization; ylide

INTRODUCTION

Chiral integrity is essential to the biological function of many drugs. The maintenance of chiral integrity during manufacture, formulation, storage, and distribution is of paramount importance and is a critical aspect of the development of safe and effective drugs.[1–3] This paper describes an unexpected racemization reaction observed with a novel drug candidate.

In recent years, the molecular motors known as kinesins have emerged as potential targets for cancer chemotherapy. Extensive screening has led to the identification of a number of cell-permeable small molecules that inhibit mitosis by blocking the function of essential spindle proteins other than tubulin. In particular, Eg5 plays a key role in mitosis including chromosome positioning, separating the centrosome, and establishing a bipolar spindle. One such Eg5 inhibitor is the 1,3,4-thiadiazoline (*R*)-litronesib (LY2523355, **1**), which has undergone clinical evaluation for therapeutic treatment of human malignancies.

During the development of a liquid formulation intended for intravenous administration, the observation was made that the chiral purity of (*R*)-litronesib fell during storage of aqueous solutions. Racemization requires the tetrahedral carbon to be transiently converted to a trigonal carbon by rupture of the bond to one of its substituents. The chiral stability of (*R*)-litronesib was excellent under acidic conditions but base-catalyzed racemization was observed at pH values greater than 6. This observation was unexpected because reasonable acid-catalyzed racemization pathways could be proposed, for example, cleavage of the C5–S bond by protonation of N3 to give carbocation (**2**), which would be stabilized by delocalization into the attached nitrogen atom and phenyl ring (Scheme 1). On the contrary, base-catalyzed processes that could be expected to occur even at neutrality were more difficult to envision. As a consequence, a mechanistic investigation was undertaken to elucidate the operative pathway.

EXPERIMENTAL

Materials

Litronesib was obtained from Eli Lilly & Company. Other reagents were obtained from Sigma–Aldrich Chemical Company and used as received.

Nuclear Magnetic Resonance

The nuclear magnetic resonance (NMR) data were acquired on a Varian VNMRS 400 spectrometer equipped with an automated triple band probe. For proton spectra, the typical acquisition parameters included eight scans, a 4.5 s acquisition time, a 45° pulse width, and a 4629.5 Hz sweep width. The FIDs were zero-filled to 32K data points and multiplied by a matched filter exponential window function prior to Fourier transformation.

Abbreviations used: DEA, diethyl amine; DMEA, dimethylethyl amine; IPA, isopropyl alcohol; MP, mobile phase.

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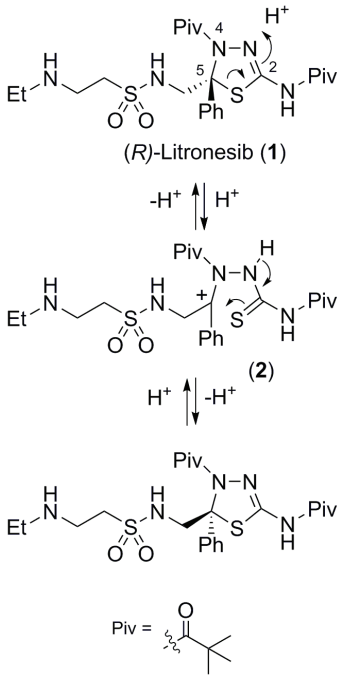

Scheme 1. Postulated acid-catalyzed racemization of (*R*)-litronesib. This pathway was excluded by the effect of pH on the rate of racemization.

The resulting spectra were baseline corrected and referenced to the solvent signal (2.50 ppm). For carbon spectra, the typical acquisition parameters included a 2.6 s acquisition time, a 45° pulse width, and a 25,000 Hz sweep width. The number of scans varied according to the concentration of the sample. The FIDs were zero-filled to 64K data points and multiplied by exponential window function (2 Hz) prior to Fourier transformation. The resulting spectra were baseline corrected and referenced to the solvent signal (39.5 ppm).

Mass Spectrometry

High-resolution positive ion electrospray (ES^+) spectra were obtained by one of the two systems: (1) LC/MS using an Agilent 6230 time-of-flight (TOF) spectrometer coupled to an Agilent 1200 LC system. The system was controlled and data manipulated with Agilent MassHunter software; (2) an LTQ Orbitrap Discovery (ThermoScientific). Nominal mass ES^+ spectra were obtained using a Waters single quadrupole detector mass spectrometer with Waters Empower software.

HPLC Methods

Achiral HPLC analyses were performed on a Waters Acquity UPLC system with photodiode array and electrospray MS detection. The column, a 2.1 × 100 mm BEH-C18, 1.7 μm (Waters), was operated at 45°C. The flow rate was maintained at 0.6 mL/min. Mobile phase (MP) A consisted of 95/5 (v/v) 17 mM acetic acid titrated to pH 5.6 with ammonium hydroxide/acetonitrile, and MP B was acetonitrile. MP B was increased linearly from 0% to 90% at 18% per minute for 5 min and held at 90% for 1 min. Chromatograms were extracted at 240 nm.

Chiral supercritical fluid HPLC analyses were performed on an Agilent 1100 series HPLC modified to pump carbon dioxide and equipped with an Aurora SFC Systems Fusion A5 module. Two different columns were used for the enantiomer analyses; a 4.6 × 150 mm Daicel Chiralpak IA, 5 μm for the *N*-methyl and *N*-acetyl derivatives of litronesib and a 4.6 × 150 mm Daicel Chiracel OD-H, 5 μm for **1**, and the *p*-trifluoromethyl and *p*-methoxy analogs of **1**. The columns were operated at 40°C. The flow rate was maintained at 5.0 mL/min. MP A was liquid carbon dioxide and MP B was methanol containing 0.2% (v/v) triethylamine. MP B was increased linearly from 5% to 15% at 1.25% per minute for 8 min. Chromatograms were extracted at 290 nm.

Preparative Chiral Separations

The preparative chiral separation of the racemic *N*-methyl derivative of litronesib was carried out on an 8 × 35 cm Chiralpak AD column, eluting with 15:85:0.2 ethanol–heptane–DMEA (dimethylethyl amine) (400 mL/min, 290 nm UV detection). The resulting enantiomers were analyzed on a 0.46 × 15 cm Chiralpak AD-H column, eluting with 15:85:0.2 EtOH–heptane–DMEA at 0.6 mL/min (290 nm UV detection).

The racemic *p*-methoxy intermediate (**18a**) was separated on an 8 × 35 cm Chiralpak AD column by eluting with 60/40 heptane–isopropyl alcohol (IPA) (400 mL/min, 310 nm UV detection). The resulting enantiomers were analyzed on a 0.46 × 15 cm Chiralpak AD-H column, eluting with 60/40 heptane–IPA at 0.6 mL/min (280 nm UV detection).

The racemic *p*-trifluoromethyl intermediate (**18b**) was separated by preparative SFC using a Chiralpak AD-H column, with CO_2 and methanol containing 0.1% diethyl amine (DEA) as cosolvent at a wavelength between 214 and 359 nm. The resulting enantiomers were analyzed on a 0.46 × 15 cm Chiralpak AD-H column, eluting with a gradient mixture of MP A: 0.2% DEA in hexane and MP B: 0.2% DEA in EtOH at 0.8 mL/min (5%–15% B after 7 min, held for 8 min, lowered back to 5% B in 1 min and then maintained for a further 9 min, 25 min total run time, 285 nm UV detection).

Ionization Constants

Titrations were performed using Sirius Analytical T3 (T310020) with SiriusT3Control software and SiriusT3Refine Version: 1.1.0.10. Three titrations were performed from pH 2 to 12, in a single vial at varying ratios of cosolvent MeOH–water mixture; conversion to 100% aqueous p*K*a values was accomplished by extrapolation to 0% cosolvent via a Yasuda–Shedlovsky plot. Percentages of cosolvent used for FastpKaUV were approximately 25–34–46; for potentiometric, 30–40–50. Computational p*K*a predictions were made using MarvinSketch™ 5.2.6 (2009) (ChemAxon, Ltd.).

X-Ray Crystallography

A high-quality single crystal of litronesib was grown by recrystallization from 88% acetone and 12% isopropyl ether. The crystal was mounted on a fiber at 100(2) K. Data were collected using a CuKα radiation source (λ = 1.54178 Å) and a Bruker D8 based 3-circle goniometer diffractometer equipped with a SMART 6000CCD area detector (Bruker-AXS, Madison, Wisconsin). Cell refinement and data reduction were performed using the *SAINT* program V7.68a.[4] The unit cell was indexed, having triclinic parameters of $a = 6.2219(3)$ Å, $b = 9.8401(5)$ Å, $c = 11.3183(6)$ Å, and $\alpha = 96.676(3)^\circ$, $\beta = 98.394(3)^\circ$, $\gamma = 103.872(3)^\circ$. The cell volume of crystal structure was 657.23(6) Å^3. The density was calculated to be 1.293 g/cm^3 at 100 K. The