Factors Influencing the Interconversion of a New Class of Dibenzodiazepine Sulfonamide Atropisomers

CHRISTOPHER J. WELCH,* XIAOYI GONG, WES SCHAFER, HARRY CHOBANIAN, LINUS LIN, MIRLINDA BIBA, PING LIU, YAN GUO, AND ADAM BEARD

Merck Research Laboratories, Merck & Co., Inc., Rahway, New Jersey

Contribution to the Special Thematic Project "Advances in Chiroptical Methods"

ABSTRACT A novel family of atropisomers based on a conformationally constrained seven membered ring system is investigated using a combination of preparative chiral chromatography, circular dichroism, and other analytical techniques. The influence of structure on the rate of atropisomer interconversion was explored with a series of analogs showing a range of interconversion rates ranging from very fast (undetectable on the HPLC timescale) to very slow (half life of many days). *Chirality 21:E105–E109, 2009.* © 2009 Wiley-Liss, Inc.

KEY WORDS: atrpoisomer; dibenzodiazepine; enantiomerization; circular dichroism; chiroptical switch

INTRODUCTION

In the intense exploration of chemical space that is modern pharmaceutical discovery chemistry, new and interesting compound classes that display atropisomerism (isomerism based on restricted rotation about a single bond) are occasionally encountered.[1] When pharmaceutical lead molecules display atropisomerism, important questions concerning the rate of interconversion of the two isomeric forms of the molecule must be addressed. If the interconversion is extremely rapid—on the order of a few minutes or less—the existence of the two isomeric forms can largely be ignored, because a single molecule of the drug would typically interconvert between the two forms a number of times within the body of a patient.

At the other extreme, atropisomers with a very low rate of interconversion—on the order of many hours or days—can generally be assumed to exist in only one isomeric form during the transit through the body of the patient and can be treated in the same manner as other drugs that can exist in more than one isomeric form (e.g. chiral drugs). This generally means that the pharmacological activity and metabolic fate of each isomer must be studied and understood—a process that generally leads to the selection of one of the two isomers of the drug—which is then synthesized and manufactured in pure form.

For atropisomers with intermediate interconversion barriers, with half lives on the order of an hour or less, the situation is more complex. Making and storing such compounds in isomerically pure form can be a considerable challenge, and substantial isomerization in vivo is to be expected during the transit of the drug through the body. The situation is further complicated by the fact that pH or enzyme facilitated isomerization sometimes occurs, introducing the possibility of different rates of isomerization for different patient groups. Consequently, when atropisomerism is discovered within a lead compound class in a medicinal chemistry program, it is important to quickly come to an understanding of the factors influencing the rate of isomer interconversion.

We herein present a study in which an unexpected atropisomerism found among members of a class of sulfonamide-substituted dibenzodiazepines (Fig. 1) was characterized using preparative chiral chromatography in combination with time-based circular dichroism (CD) and other analytical techniques. Understanding gained from an investigation of the influence of structure on the rate of atropisomer interconversion has led to the ability to tune between very fast (minutes) and very slow (days) interconversion rates.

EXPERIMENTAL SECTION

Chemicals

All solvents used were HPLC grade or better. Methanol, ethanol, acetonitrile, hexanes, water, acetic acid, and triethylamine were purchased from Sigma–Aldrich (St. Louis, MO). The benzodiazepine sulfonamide compounds 1–7 used in this study were synthesized in house. The individual atropisomers of these compounds were isolated through chiral preparative separations with the conditions listed in Table 1. The synthesis, characterization, and biological activity of the benzodiazepine sulfonamide compounds will be described in a forthcoming publication.

Columns

Chiralcel OD-RH analytical column (150 mm × 4.6 mm, particle size 5 μm), Chiralcel OD and Chiralpak AS semi-

*Correspondence to: Christopher J. Welch, Merck Research Laboratories, RY 801 C101, Merck & Co., Inc., Rahway, New Jersey.
E-mail: christopher_welch@merck.com
Received for publication 21 May 2009; Accepted 21 July 2009
DOI: 10.1002/chir.20785
Published online 2 November 2009 in Wiley InterScience (www.interscience.wiley.com).

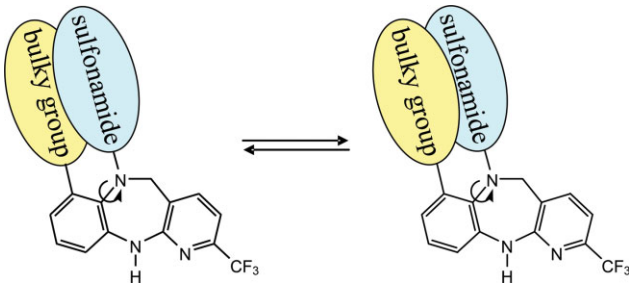


Fig. 1. Atropisomerism discovered in sulfonamide-substituted dibenzodiazepines resulting from restricted rotation about C—N bond. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

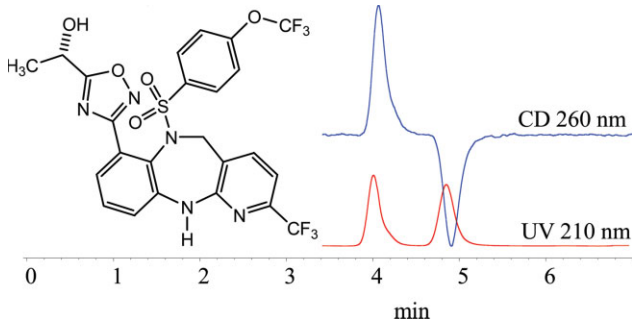


Fig. 2. Chromatographic separation of slowly interconverting atropisomers of sulfonamide-derived dibenzodiazepine, 1. The two observed peaks are related to each other as diastereomers, owing to the presence of the (*S*)-carbinol substituent. Conditions: Chiralcel OD-RH (150 mm × 4.6 mm, 5 μm), 42% phosphoric acid, 0.1% water/58% acetonitrile, 1.2 ml/min. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

preparative columns (250 mm × 20 mm, particle size 10 μm) were obtained from Chiral Technologies, (West Chester, PA).

Instrumentation

Time-based CD measurements were made using a Jasco model J-810 CD spectropolarimeter. The temperature controlled quartz cuvette used has a path length of 10 mm and was obtained from Hellma GmbH (Müllheim, Germany). A Julabo model F32 water bath (Julabo, Sealbach/Black Forest, Germany) was used to circulate heated water through the cuvette for temperature control. The actual in-cell temperature was measured and calibrated using a thermocouple probe.

HPLC analysis was carried out using an Agilent model 1100 HPLC system with a quaternary pump and diode array UV-visible detector (Agilent Technologies, Palo Alto, CA). Preparative HPLC purification of atropisomers was carried out using an Agilent model 1100 preparative HPLC system.

RESULTS AND DISCUSSION

Our initial observations of atropisomerism within the dibenzodiazepine sulfonamide class of compounds came about through the fortuitous observation of a pair of peaks of equal area in the chiral HPLC analysis of what was thought to be an enantiopure carbinol, 1 (Fig. 2). Although this initial observation was somewhat surprising and unexpected, closer investigation soon led to the suspicion that the two peaks corresponded to atropisomers and not enantiomers of the carbinol. This finding was confirmed by the appearance of similar pairs of peaks of equal area for related compounds in which the carbinol stereocenter is replaced with an achiral substituent. Examination of molecular models reveals the nature of the atropisomerism, with bond rotation about the C—N being nearly impossible for compounds with a combination of a large sulfonamide substituent and a large bulky group (Fig. 1).

Restricted rotation leading to atropisomerism has been previously reported for hindered seven membered ring systems, with perhaps the best known cases being the well known benzodiazepine class of molecules such as diazepam (Valium), where rapid equilibration between two enantiomeric conformations can be studied via the preferential binding of one of the enantiomers to serum proteins, leading to a detectable induced CD signal (Fig. 3).[2] In the present case, the rate of atropisomer interconversion for the somewhat related dibenzodiazepine sulfonamides is

TABLE 1. The preparative separation conditions of the benzodiazepine sulfonamides

Compound	Preparative separation conditions
1	Chiralcel OD (250 mm × 20 mm) with 15% ethanol/hexanes
2	Chiralcel OD (250 mm × 20 mm) with 15% ethanol/hexanes
3	Chiralcel OD (250 mm × 20 mm) with 15% ethanol/hexanes
4	Chiralcel OD (250 mm × 20 mm) with 10% ethanol/hexanes
5	Chiralcel OD (250 mm × 20 mm) with 15% ethanol/hexanes
6	Chiralcel OD (250 mm × 20 mm) with 15% ethanol/hexanes
7	Chiralpak AS (250 mm × 20 mm) with 10% ethanol/hexanes

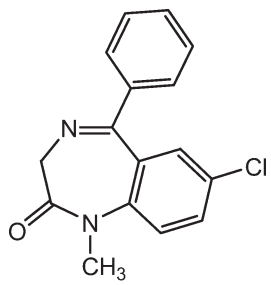


Fig. 3. Atropisomerism is well known in the related benzodiazepine family of compounds, where interconversion of enantiomeric conformers is relatively rapid.

much slower, affording isomers that interconvert slowly on the "HPLC timescale."[3]

Chromatographically separable atropisomers that show no evidence of on-column interconversion (typically manifested by a "plateau" between the two peaks of interest) can often be preparatively resolved using chiral chromatography.[4–6] Accordingly, we set out to resolve the isomers of these compounds, and to monitor the reversion of a single isomer back to the equilibrium mixture of atropisomers. Preparative chiral separation of the atropisomers of 1 was readily accomplished using a semipreparative Chiralcel OD column (250 mm × 20 mm, particle size 10 μm) at an isocratic condition of 15% ethanol and 85% hexanes, and the individual isomers were isolated for study.

CD spectroscopy is a generally useful tool for monitoring atropisomer equilibration,[7] especially for compounds like 1 where the isomers of interest contain chromophores held at fixed but opposite angles. A timecourse CD spectrum of one of the isolated atropisomers of compound 1 at room temperature shows a gradual decrease in signal, indicating that atropisomer interconversion is occurring. Repeating this experiment at elevated temperature (60°C) affords the results as shown in Figure 4a. Periodic scans collected over a 3 day period clearly show the decay of the CD signal over time. A plot of CD signal at both 260 nm and 330 nm shows decay over time to a baseline value of 0 mdeg (Fig. 4b), confirming that the (*S*)-carbinol stereocenter of 1 makes essentially no contribution to the observed CD signal at these higher wavelengths (because of the presence of this carbinol stereocenter, the two atropisomers of 1 are diastereomers, not enantiomers). At any given time point, the diastereomeric excess (de) of 1 can be calculated by comparing the measured CD signal to the CD signal of the pure atropisomer of 1 measured at the initial time point. A plot of the logarithm of the calculated de values vs. time (Fig. 4c) affords a straight line, which suggests a first order kinetics of interconversion for the atropisomers of 1.[8–10] The rate constant for atropisomer interconversion can be readily derived using the slope of the linear plot (1.23×10^{-5} sec^{-1}), which is more conveniently expressed as a half life (7.8 h).

In general, interconversion of atropisomers does not depend greatly upon pH or additives; however, in the past, we have occasionally encountered some unusual exceptions to this rule.[11] In the present case, we found no effect of additives (triethylamine and acetic acid) or aqueous buffer of different pH (3, 7, and 10) on the rate of the interconversion of the atropisomers of compound 1.

With this understanding of the nature of the dibenzodiazepine sulfonamide atropisomerism for compound 1 in hand, we next turned to the analysis of structurally related molecules to better understand the factors influencing atropisomer interconversion. The results of these studies are summarized in Figure 5.

A number of structural analogs showed no sign of atropisomerism by chiral HPLC or SFC, for example, when the R- substituent is either H or F, or when the sulfonamide substituent is replaced with amides derived from carboxylic acids. Presumably, these compounds are interconverting between the two atropisomeric forms too rapidly to be visualized on the "HPLC timescale."

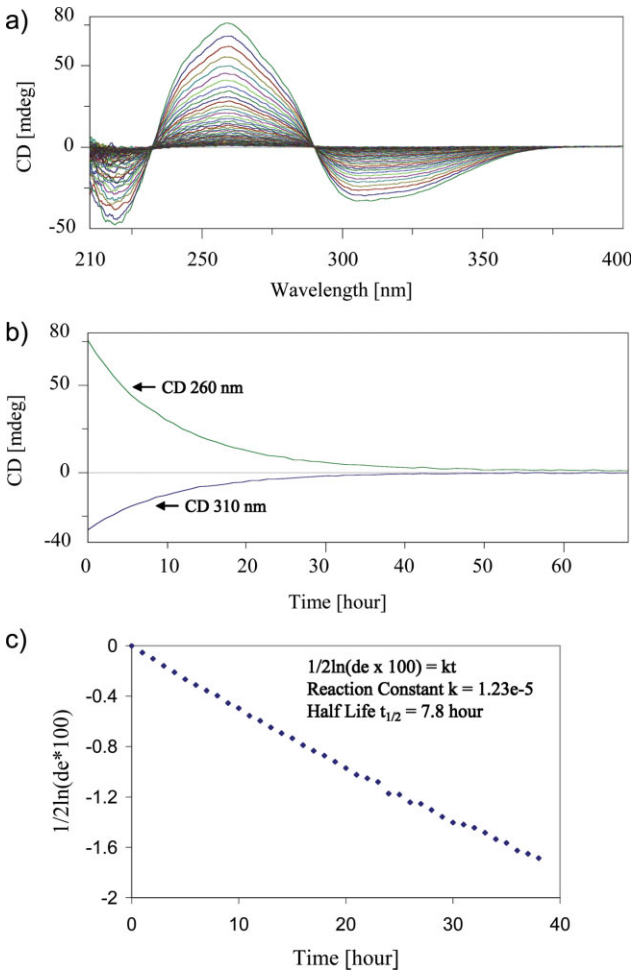
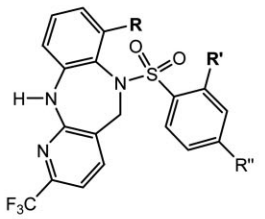


Fig. 4. Timecourse circular dichroism studies allow study of the kinetics of interconversion for the atropisomers of 1 at 60°C in ethanol. (**a**) Periodic CD scans collected over a 3 day period. (**b**) Exponential decay of CD signals at both 260 nm and 330 nm. (**c**) Plotting logarithm of calculated de values vs. time allows calculation of rate constant (1.23×10^{-5} sec^{-1}) and half life of atropisomer interconversion (7.8 h). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Compound 2 in which the heterocyclic aromatic substituent of 1 has been replaced by a quaternary sp^3 substituent shows much slower interconversion ($t_{1/2}$ of 29.3 h vs. 7.8 h), reflecting the greater steric bulk and resulting increased barrier to interconversion of the quaternary sp^3 substituent. Compound 3 in which an additional methyl group has been added to the substituent at the R position and in which the R″ substituent has been changed from CF_3 to OCF_3 shows faster interconversion ($t_{1/2}$ = 2.2 h), a result that is somewhat difficult to interpret, as neither the electronic change from CF_3 to OCF_3 in the sulfonamide substituent nor the additional methyl group in the R substituent would, a priori, be expected to have a dramatic influence on the barrier to atropisomer interconversion.



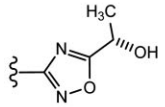
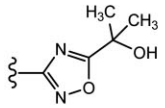
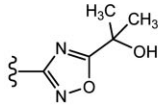
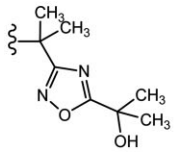
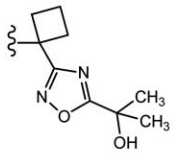
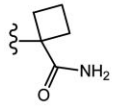
cmpd.	R-	R'-	R''-	$t_{1/2}$ (EtOH, 60°C)
1		H	CF_3	7.8 h
2		H	CF_3	89.3 h
3		H	OCF_3	2.2 h
4		CH_3	OCF_3	1.1 h
5		H	OCF_3	>250h
6		H	OCF_3	>250h
7		H	OCF_3	>250h

Fig. 5. Effect of structure of dibenzodiazepine sulfonamides on rate of atropisomer interconversion at 60°C in ethanol (~0.04 mg/ml), as determined by timecourse CD analysis at 260 nm.

Comparison of compounds 3 and 4 was also somewhat surprising. The two compounds are identical, but for the presence of an ortho methyl substituent on the aryl sulfonamide in compound 4. Somewhat surprisingly, the compound with the additional ortho methyl group exhibits faster interconversion ($t_{1/2}$ = 1.1 h vs. 2.2 h), Perhaps, rather than simply increasing steric bulk, this ortho methyl substituent is playing an electronic role, engaging in hydrogen bonding, or simply biasing the molecule toward a preferred conformation that is nearer the transition state for atropisomer interconversion.

Compounds 5, 6, and 7, in which a quaternary sp^3 center has been inserted adjacent to the R group position, all show essentially no atropisomer interconversion even after days of heating at 60°C. This difference is highlighted in Figure 6, where atropisomer interconversion for compounds 3 and 5 at 60°C are compared.

This initial survey of the effect of structure on atropisomer interconversion for the dibenzodiazepine sulfonamides clearly shows that through appropriate substitution the rates of atropisomer interconversion can be tuned from very fast (undetectable on HPLC timescale) to very

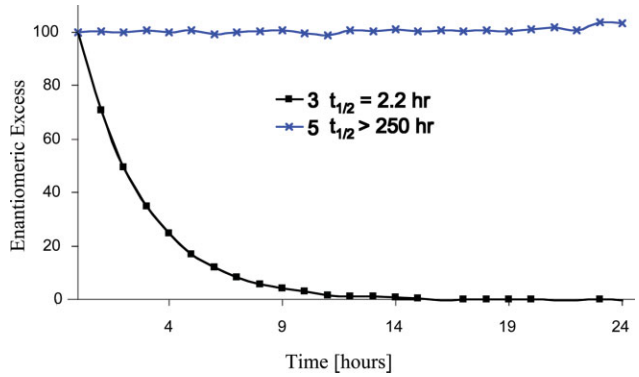


Fig. 6. Effect of a quaternary sp^3 center adjacent to the R group on the rate of atropisomer interconversion at 60°C in ethanol, as determined by timecourse CD analysis at 260 nm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

slow (days or longer). Clearly, additional work will be needed to more completely characterize this new family of atropisomers; however, the ability to tune interconversion rate, combined with the intense CD signal that these compounds display, suggests that this compound class could be of some use in the design of chiroptical sensors or switches, an area of increasing recent interest.[12–14]

CONCLUSION

In summary, these studies describe some of the factors influencing interconversion of a novel family of benzodiazepine sulfonamide atropisomers. A combination of preparative chromatography and time-based CD was used to characterize the rate of interconversion for a variety of structural analogs, leading to an understanding of the structural features responsible for either fast or slow atlropisomer interconversion.

LITERATURE CITED

1. Clayden J. Atropisomerism (preface to tetrahedron symposium in print). Tetrahedron 2004;60:4335.
2. Fitos I, Visy J, Zsila F, Mady G, Simonyi M. Conformation selectivity in the binding of diazepam and analogues to a1-acid glycoprotein. Biorg Med Chem 2007;15:4857–4862.
3. Mannschreck A, Zinner H, Pustet N. The significance of the HPLC time scale: an example of interconvertible enantiomers. Chimia 1989; 43:165–166.
4. Pirkle WH, Welch CJ, Zych AJ. Chromatographic investigation of the slowly interconverting atropisomers of hindered naphthamides. J Chromatogr 1993;648:101–109.
5. Welch CJ, Kress MH, Beconi M, Mathre DJ. Studies on the racemization of a stereolabile 5-aryl-thiazolidinedione. Chirality 2003;15:143–147.
6. Reist M, Testa B, Carrupt PA. The racemization of enantiopure drugs: helping medicinal chemists to approach the problem. Enantiomer 1997;2:147–155.
7. Harada N, Ono H, Uda H, Parveen M, Ud Din KhanNizam, Achari B, Dutta PK. Atropisomerism in natural products. Absolute stereochemistry of biflavone, (-)-4′,4‴,7,7″-tetra-O-methylcupressuflavone, as determined by the theoretical calculation of CD spectra. J Am Chem Soc 1992;114:7687–7692.
8. Moore WJ. Physical Chemistry, 4th ed. Englewood Cliffs, NJ: Prentice-Hall; 1972.
9. Buerkle W, Karfunkel H, Schurig V. Dynamic phenomena during enantiomer resolution during complexation gas chromatography: a kinetic study of enantiomerization. J Chromatogr 1984;288:1–14.
10. Trapp O, Schoetz G, Schurig V. Determination of enantiomerization barriers by dynamic and stopped-flow chromatographic methods—review article. Chirality 2001;13:403–414.
11. Welch CJ, Biba M, Pye P, Angelaud R, Egbertson M. Serendipitous discovery of a pH-dependant atropisomer bond rotation: toward a write-protectable chiral molecular switch? J Chromatogr B 2008;875: 118–121.
12. Feringa B. Molecular Switches. Weinheim: Wiley-VCH; 2001.
13. Redic R, Schuster GB. Chirochromic triggers: 3,3′-disubstituted-2,2′-binaphthalene-1,1′-diols and 2-methylindoline derived phosphoramidates. J Photochem Photobiol A: Chem 2006;179:66–74.
14. Klamroth T, Kroener D. Stereoselective isomerization of an ensemble of adsorbed molecules with multiple orientations: stochastic laser pulse optimization for selective switching between achiral and chiral atropisomers. J Chem Phys 2008;129:1–10.