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Determination of Kinetic Parameters of Enantiomerization of Benzothiadiazines by DCXplorer

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ABSTRACT Benzothiadiazines differently substituted at the sulfonamidic nitrogen atom, at the stereogenic carbon atom and at the anilinic nitrogen atom have been synthesized and fully characterized. Enantioseparation of these compounds has revealed rapid on-column enantiomerization. The recently developed software DCXplorer has been successfully applied to calculate enantiomerization kinetic parameters. Enantiomerization barriers of 3-phenyl substituted benzothiadiazines, calculated in this work, have indicated a higher enantiomerization rate suggesting that the aromatic substituent exerts a strong effect on the enantiomerization process. Methyl substitution on N^2 position led to higher free energy barriers of enantiomerization, suggesting negative influence of methyl in the N^2 position on enantiomerization kinetics. However, methylation on N^4 position increases the enantiomerization rates significantly. The results obtained have been employed to postulate an enantiomerization mechanism for chiral benzothiadiazine type compounds. *Chirality 22:789–797, 2010.* © 2010 Wiley-Liss, Inc.

KEY WORDS: benzothiadiazines; HPLC; enantiomerization; enantioseparation

INTRODUCTION

The U.S. Food and Drug Administration requires that "the stability protocol for enantiomeric drug substances and drug products should include a method or methods capable of assessing the stereochemical integrity of the drug substance and drug product."[1] Recently chiral benzothiadiazines type compounds have attracted particular attention as AMPA receptor positive allosteric modulators.[2–9] As there is experimental evidence that indicate the stereospecific pharmacological action of benzothiadiazine type compounds, it gains important to evaluate the stereochemical stability of their active enantiomers.

It is possible to study the enantiomerization of chiral labile compounds by different techniques depending on their interconversion free energy barriers. Among the most commonly used methods, classical chromatographic off-column techniques consisting of an examination of batchwise racemization kinetics of a single enantiomer after enantioselective separation.

If enantiomerization takes place in the same time-scale of the chromatographic separation process, a characteristic plateau appears between the two peaks of the interconverting enantiomers.[10–13] From the resulting peak shapes obtained by enantioselective dynamic HPLC (DHPLC), it is possible to extract the necessary information to calculate racemization kinetic parameters by several methods such as the theoretical plate model, stochastic model, continuous flow model, peak deconvolution method, approximation function, and the unified equation.[14–19]

DHPLC is a very attractive technique, because it requires only minute amounts of the racemic or enantiomer-enriched mixture.

Recently, Trapp et al. have developed the software program DCXplorer that permits the calculation of reaction rate constants of enantiomerization employing the unified chromatography equation.[19–28]

As we have demonstrated that some chiral 3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide type compounds undergo rapid enantiomerization during enantioseparation in reverse phase conditions,[29,30] DCXplorer has been applied to calculate enantiomerization rate constants of synthesized benzothiadiazines **1–12** here (Fig. 1). Different substituents were placed in the thiadiazine ring to understand the influence of these substituents on enantiomerization activation barrier.

The data obtained by dynamic HPLC were used to gain further insight in understanding the enantiomerization

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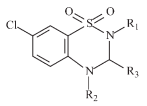


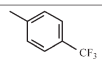
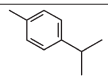
Compounds	R_1	R_2	R_3
1	-H	-H	
2	-H	-H	
3	-H	-H	
4	-H	-H	
5	-H	-H	
6	-H	-H	
7	-H	-H	
8	$-CH_3$	-H	
9	$-CH_3$	-H	
10	-H	$-CH_3$	
11	-H	-H	
12	-H	-H	

Fig. 1. Compounds investigated.

mechanism of chiral 3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide type compounds.

EXPERIMENTAL

Instrumentation

The chromatographic apparatus consisted of a Jasco Pu-2080 Plus pump, a Rheodyne 7725 manual injector equipped with a 20 μl sample loop and a Jasco UV-2075 Plus detector. Chromatograms were recorded with Jasco J-700 program. Column temperature regulation was achieved with a Jasco CO-2067 column oven.

The column used was Chiralcel OD-RH [cellulose tris(3,5-dimethylphenylcarbamate); 150 mm × 4.6 mm ID; 5 μm] purchased from Daicel. Melting points were determined with an electrothermal apparatus and are uncorrected. The ^{1}H and the ^{13}C NMR spectra were recorded on a Bruker Avance 400 apparatus (400.13 and 100.62 MHz, for ^{1}H and ^{13}C, respectively) with $CDCl_3$ and DMSO as solvents and TMS as internal standard (δ = 7.24 for ^{1}H spectra; δ = 77.0 for ^{13}C spectra). Chemical shifts (δ) are in part per million and coupling constant (*J*) in hertz. Multiplicities are abbreviated as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet.

The IR spectra were recorded with a Digilab Scimitar Series FTS 2000 FT-IR spectrophotometer. GC-MS analyses were performed with an Agilent Technologies 6850 series II gas chromatograph (with a 5% phenyl-polymethylsiloxane capillary column, 30 m, 0.25 mm ID), and a 5973 Network mass selective detector operating at 70 eV. The electrospray ionization (HR-ESI-MS) experiments were carried out in a hybrid QqTOF mass spectrometer (PE SCIEX-QSTAR) equipped with an ion spray ionization source. MS (+) spectra were acquired by direct infusion (5 ml/min) of a solution containing the appropriate sample (10 pmol/ml), dissolved in a solution 0.1% acetic acid, methanol:water 50:50 at the optimum ion voltage of 4800 V.

All pH measurements were made with an Orion Research EA940 pH-meter.

Synthesis

General procedure for the synthesis of derivatives 1–12. A mixture of the appropriate 2-amino-5-chlorobenzensulfonamide (4 mmol) and a suitable aldehyde (40 mmol) in 2-propanol (40 ml) supplemented with 2 ml of ethyl acetate saturated with dry HCl was refluxed and stirred for 2 h at 50°C. After cooling, the resulting suspension was concentrated to dryness under reduced pressure. The solid residue was purified by column chromatography on silica gel (elution solvent: ethyl acetate:petroleum ether (40–60°C), 1:1 (v/v) for compounds **2–6** and **11–12**; 6:4 (v/v) for compounds **7** and **10;** and 7:3 (v/v) for compounds **8** and **9**).

An alternative pathway was also used for the synthesis of compound **9**. A solution of (±)7-chloro-3-(2-chlorophenyl)-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide ((±)-**3**) (1.0 mmol) in tetrahydrofuran (10 ml) was supplemented dropwise at 0°C with a solution of lithium diisopropylamide (LDA) (1.0 mmol) in tetrahydrofuran. Methyl iodide (3.0 mmol) was then added and the mixture was stirred for 1 h at room temperature. The reaction mixture was quenched with water (50 ml) and the insoluble material was extracted three times with diethyl ether (75 ml). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure. The solid residue was purified by column chromatography on silica gel [elution solvent: ethyl acetate:petroleum ether (40–60°C), 7:3 (v/v)] (Yield 85%).

4-(7-Chloro-1,1-dioxido-3,4-dihydro-2*H*-1,2,4-benzothiadiazin-3-yl)phenol ((±)-1). The title compound was synthesized as previously reported.[4]

(±)7-Chloro-3-(2-methoxyphenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-2). Yield 71%, mp 172–173.2°C. ^{1}H NMR ($CDCl_3$) δ = 3.85 (s, 3H), δ = 6.15 (d, 1H, *J* = 12.2 Hz), δ = 6.92 (d, 1H, *J* = 8.9 Hz), δ = 7.06 (t, 1H, *J* = 7.5 Hz), δ = 7.12 (d, 1H,

J = 8.3 Hz), δ = 7.36 (dd, 1H, J = 2.5 Hz, J = 8.9 Hz), δ = 7.43 (t, 1H, J = 8.3 Hz), δ = 7.47 (s, 1H, broad, exchange with D_2O), δ = 7.55 (d, 1H, J = 2.5 Hz), δ = 7.71 (d, 1H, J = 7.5 Hz), δ = 7.95 (d, 1H, J = 12.2 Hz, exchange with D_2O). ^{13}C NMR ($CDCl_3$) δ 55.7, 61.7, 111.3, 118.2, 119.8, 120.4, 122.1, 122.9, 124.5, 128.0, 130.5, 132.8, 142.9, 156.1. FTIR (KBr) 3440, 3330, 3020, 1484, 1215, 1167 cm^{-1}. GC-MS (70 eV) *m/z* 324(82) [M^+], 259(20), 206 (38), 126 (95), 119 (100), 91 (50). HRMS-ESI: calcd. for $C_{14}H_{14}Cl N_2O_3S$ [M+H]$^+$ 325.0415; found: 325.0412.

(±)7-Chloro-3-(2-chlorophenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-3). Yield 76%, mp 211.2–212.3°C. ^1H NMR ($CDCl_3$) δ = 6.18 (s, 1H), δ = 6.95 (d, 1H, J = 8.9 Hz), δ = 7.40 (dd, 1H, J = 2.2 Hz, J = 8.9 Hz), δ = 7.47–7.59 (m, 4H), δ = 7.68 (s, 1H), δ = 7.87–7.89 (m, 1H), δ = 8.16 (s, 1H, broad). ^{13}C NMR ($CDCl_3$) δ 64.8, 118.4, 120.4, 122.4, 122.9, 127.6, 129.1, 129.5, 130.9, 132.3, 133.0, 133.9, 142.7. FTIR (KBr) 3369, 3201, 3320, 2925, 2828, 1607, 1485, 1318, 1155 cm^{-1}. GC-MS (70 eV) *m/z* 328 (50) [M^+] 247 (55), 207 (100), 126 (75). HRMS-ESI: calcd. for $C_{13}H_{11}Cl_2 N_2O_2S$ [M+H]$^+$ 328.9920; found: 328.9922.

(±)7-Chloro-3-(4-chlorophenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-4). Yield 64%, mp 227.3–228.5°C. ^1H NMR ($CDCl_3$) δ = 5.82 (s, 1H), δ = 6.93 (d, 1H, J = 8.9 Hz), δ = 7.38 (d, 1H, J = 8.9 Hz), δ = 7.53–7.55 (m, 3H), δ = 7.62 (s, 1H), δ = 7.69 (d, 2H, J = 8.2 Hz), δ = 8.1 (s, 1H, broad, exchange with D_2O). ^{13}C NMR ($CDCl_3$) δ 67.5, 118.4, 120.2, 122.3, 122.8, 128.5, 129.4, 132.9, 133.8, 135.8, 142.6. FTIR (KBr) 3387, 3252, 3020, 2924, 2854, 1606, 1487, 1325, 1159 cm^{-1}. GC-MS (70 eV) *m/z* 328 (40) [M^+] 281 (27), 247 (25), 207 (100), 126 (42). HRMS-ESI: calcd. for $C_{13}H_{11}Cl_2 N_2O_2S$ [M+H]$^+$ 328.9920; found: 328.9921.

(±)7-Chloro-3-(3-chlorophenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-5). Yield 72%, mp 201.6–202.8°C. ^1H NMR ($CDCl_3$) δ = 5.83 (s, 1Hz), δ = 6.94 (d, 1H, J = 8.9 Hz), δ = 7.39 (dd, 1H, J = 2.5 Hz, J = 8.9 Hz), δ = 7.50–7.65 (m, 5Hz), δ = 7.78 (s, 1H, broad, exchange with D_2O), δ = 8.10 (s, 1H, broad, exchange with D_2O). ^{13}C NMR ($CDCl_3$) δ 67.5, 118.4, 120.3, 122.3, 122.9, 126.4, 127.4, 129.1, 130.4, 132.9, 133.1, 139.1, 142.6. FTIR (KBr) 3381, 3210, 3064, 2924, 2869, 1605, 1498, 1318, 1162 cm^{-1}. GC-MS (70 eV) *m/z* 328 (95) [M^+] 247 (49), 142 (52), 126 (100). HRMS-ESI: calcd. for $C_{13}H_{11}Cl_2N_2O_2S$ [M+H]$^+$ 328.9920; found: 328.9922.

(±)7-Chloro-3-[4-(trifluoromethyl)phenyl]-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-6). Yield 83%, mp 205.3–206.8°C. ^1H NMR ($CDCl_3$) δ = 5.93 (s, 1Hz), δ = 6.94 (d, 1H, J = 8.9 Hz), δ = 7.39 (dd, 1H, J = 2.5 Hz, J = 8.9 Hz), δ = 7.56 (d, 1H, J = 2.5 Hz), δ = 7.68 (s, 1H, broad, exchange with D_2O), δ = 7.90 (d, 2H, J = 8.5 Hz), δ = 8.20 (s, 1H, broad, exchange with D_2O), δ = 8.85 (d, 2H, J = 8.5 Hz). ^{13}C NMR ($CDCl_3$) δ 67.6, 73.8, 118.4, 120.3, 122.4, 122.8, 125.3, 125.4, 128.5, 132.9, 141.2, 142.6. FTIR (KBr) 3390, 3216, 3050, 2950, 2854, 1606, 1491, 1326, 1160 cm^{-1}. GC-MS (70 eV) *m/z* 362 (100) [M^+] 281 (75), 173 (25), 142 (30), 126 (80). HRMS-ESI: calcd. for $C_{14}H_{11} ClF_3N_2O_2S$ [M+H]$^+$ 363.0183; found: 363.0181.

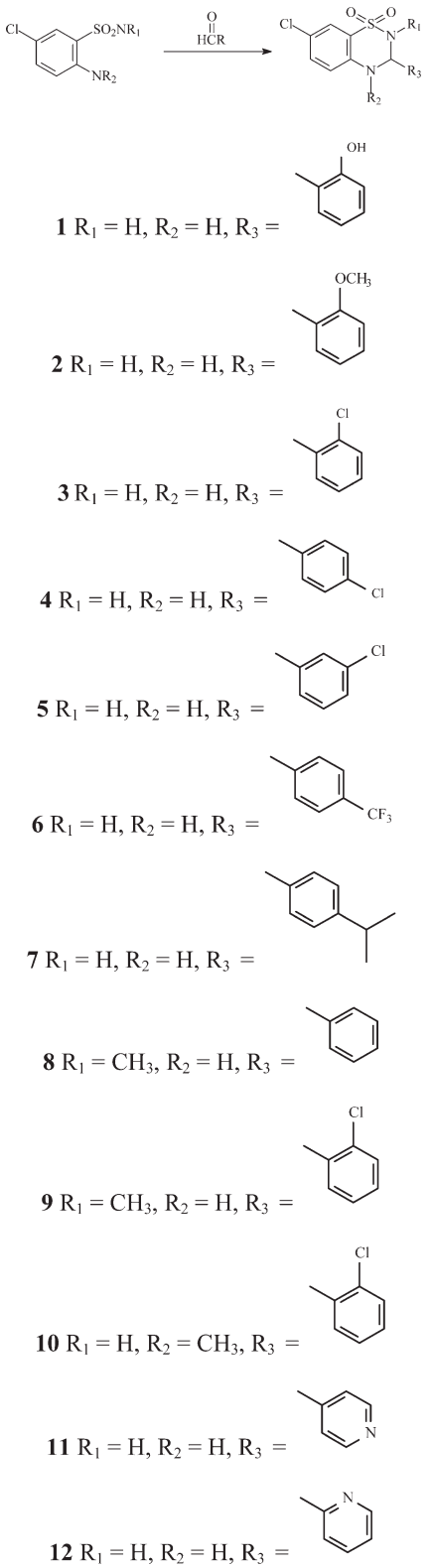


Fig. 2. General synthesis procedure for compounds **1–12.**

TABLE 1. Chromatographic enantioseparation of racemic benzothiadiazine derivatives 1–12

	(±)-**1**	(±)-**2**	(±)-**3**	(±)-**4**	(±)-**5**	(±)-**6**	(±)-**7**	(±)-**8**	(±)-**9**	(±)-**10**	(±)-**11**	(±)-**12**
k_1	2.38	5.75	10.26	8.94	9.07	7.07	12.58	10.57	11.6	10.47[a]	1.69	2.84
k_2	3.52	5.75	14.20	11.64	12.84	9.88	21.01	11.38	13.23	11.10[a]	2.34	3.76
α	1.48	1.00	1.38	1.30	1.41	1.39	1.67	1.07	1.14	1.06[a]	1.77	1.77

Column: Chiralcel OD-RH; mobile phase: water:acetonitrile 50:50 (v/v); $T = 25°C$; flow: 0.5 ml/min.
[a]Analysis temperature 4°C.

(±)7-Chloro-3-(4-isopropylphenyl)-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-7). Yield 68%, mp 204–205.6°C. ^{1}H NMR ($CDCl_3$) δ = 1.22 (d, 6H, J = 6.9 Hz), δ = 2.92 (septet, 1H, J = 6.9 Hz), δ = 5.74 (d, 1H, J = 10.9 Hz), δ = 6.93 (d, 1H, J = 9.0 Hz), δ = 7.33–7.38 (m, 3H), δ = 7.54–7.59 (m, 4H), δ = 7.99 (d, 1H, J = 10.9 Hz). ^{13}C NMR ($CDCl_3$) δ 23.8, 33.3, 68.1, 118.3, 119.9, 122.1, 122.8, 126.4, 127.5, 132.8, 134.4, 142.7, 149.6. FTIR (KBr) 3393, 3228, 3051, 2957, 1608, 1496, 1320, 1150 cm^{-1}. GC-MS (70 eV) *m/z* 336 (100) [M^+],255 (40), 147 (41), 127 (95). HRMS-ESI: calcd. for $C_{16}H_{18}Cl\ N_2O_2S$ $[M+H]^+$ 337.0779; found: 337.0777.

(±)7-Chloro-2-methyl-3-phenyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-8).[31] Yield 80%, mp 208–210°C ^{1}H NMR (DMSO-d_6) δ = 2.30 (s, 3H), δ = 6.29 (s, 1H), δ = 7.13 (d, 1H, J = 9.0 Hz), δ = 7.46 (m, 3H), δ = 7.57(m, 4H), δ = 7.77(s, 1H). FTIR (KBr) 3378, 3276, 3053, 2952, 1605, 1318,1156 cm^{-1}.

HRMS-ESI: calcd. for $C_{14}H_{14}ClN_2O_2S$ $[M+H]^+$ 309.0466; found: 309.0466.

(±)7-Chloro-3-(2-chlorophenyl)-2-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-9). Yield 91%, mp 179.8–180.9°C. ^{1}H NMR ($CDCl_3$) δ = 2.34 (s, 3H), δ = 6.61 (s, 1H), δ = 7.10 (d, 1H, J = 9.0 Hz), δ = 7.47 (dd, 1H, J = 2.4 Hz, J = 9.0 Hz), δ = 7.51–7.54 (m, 2H), δ = 7.58–7.62 (m, 2H), δ = 7.78–7.81 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 29.1, 68.3, 118.8, 119.2, 121.1, 124.0, 127.1, 129.7, 130.2, 131.2, 131.6, 133.3, 133.4, 142.1. FTIR (KBr) 3379, 3067, 2999, 2933, 1605, 1487, 1320, 1152 cm^{-1}. GC-MS (70 eV) *m/z* 342 (9) [M^+], 249 (100), 231 (21), 214(62), 177 (24), 152 (27). HRMS-ESI: calcd. for $C_{14}H_{13}Cl_2N_2O_2S$ $[M+H]^+$ 343.0076; found: 343.0075.

(±)7-Chloro-3-(2-chlorophenyl)-4-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-10). Yield 97%, mp 189.3–190.5°C. ^{1}H NMR (DMSO-d_6) δ = 2.76 (s, 3H), δ = 6.11 (s, 1H), δ = 7.08 (d, 1H, J = 9.0 Hz), δ = 7.37–7.45 (m, 3H), δ = 7.54–7.63 (m, 3H), δ = 8.70 (s, 1H, broad, exchange with D_2O). ^{13}C NMR ($CDCl_3$) δ 36.1, 71.4, 116.8, 120.4, 122.7, 124.5, 127.3, 129.0, 129.8, 130.4, 132.5, 133.3, 133.6, 143.5. FTIR (KBr) 3232, 3060, 2923, 2854, 1598, 1486, 1319, 1161 cm^{-1}. GC-MS (70 eV) *m/z* 342 (28) [M^+], 203 (45), 140 (100), 111(18). HRMS-ESI: calcd. for $C_{14}H_{13}Cl_2N_2O_2S$ $[M+H]^+$ 343.0076; found: 343.0074.

(±)7-Chloro-3-pyridin-4-yl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-11). Yield 97%, mp 206.2–207.9°C. ^{1}H NMR ($CDCl_3$) δ = 6.21 (d, 1H, J = 10.6 Hz), δ = 7.09 (d, 1H, J = 9.0 Hz), δ = 7.44 (dd, 1H, J = 2.4 Hz, J = 9.0 Hz), δ = 7.56 (d, 1H, J = 2.4 Hz), δ = 8.21 (d, 3H, J = 6.1 Hz), δ = 8.63 (d, 2H, J = 10.6 Hz). ^{13}C NMR ($CDCl_3$) δ 66.2, 118.7, 120.7, 122.4, 122.7, 125.2, 133.3, 142.2, 142.9, 154.9. FTIR (KBr) 3267, 3047, 2828, 1602, 1486, 1324, 1174 cm^{-1}. GC-MS (70 eV) *m/z* 293 (80) [M^+] 189 (75), 125 (100), 105 (46). HRMS-ESI: calcd. for $C_{12}H_{11}ClN_3O_2S$ $[M+H]^+$ 295.0262; found: 296.0261.

(±)7-Chloro-3-pyridin-2-yl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide ((±)-12). Yield 98%, mp 179.6–180.8°C. ^{1}H NMR ($CDCl_3$) δ = 6.08 (d, 1H, J = 10.3 Hz), δ = 7.12 (d, 1H, J = 8.9 Hz), δ = 7.42 (dd, 1H, J = 2.4 Hz, J = 8.9 Hz), δ = 7.50 (s, 1H, broad, exchange with D_2O), δ = 7.54 (d, 1H, J = 2.4 Hz), δ = 7.70 (dd, 1H, J = 5.7 Hz, J = 7.1 Hz), δ = 7.91 (d, 1H, J = 8.0 Hz), δ = 8.20 (t, 1H, J = 8.0 Hz), δ = 8.47 (d, 1H, J = 10.3 Hz), δ = 8.75 (d, 1H, J = 5.7 Hz). ^{13}C NMR ($CDCl_3$) δ 67.2, 118.8, 120.4, 122.2, 122.7, 123.1, 125.2, 133.1, 140.6, 142.1, 146.5, 153.8. FTIR (KBr) 3212, 3051, 2990, 2918, 2830, 1612, 1506, 1475, 1458, 1331, 1160 cm^{-1}. GC-MS (70 eV) *m/z* 295 (15) [M^+] 230 (18), 215 (100), 203 (14), 126 (18), 79 (22). HRMS-ESI: calcd. for $C_{12}H_{11}ClN_3O_2S$ $[M+H]^+$ 296.0262; found: 296.0260.

Enantioselecive dynamic HPLC

Separation of enantiomers of (±)-**1**-(±)-**12** were carried out isocratically at different temperatures (4–40°C) on Chiralcel OD-RH column. The mobile phase consisted of water:acetonitrile 50:50 (v/v). The compounds were dissolved in acetonitrile (for compounds **1–10**) or ethanol (for compounds **11** and **12**) and subsequently diluted 1:100 (v/v) with mobile phase to a final concentration of 10 μg/ml. The injection volume was 20 μl. The detector was set at 254 nm.

The enantiomerization kinetic parameters of benzothiadiazine derivatives **1–12** have been investigated by dynamic chromatography experiments (DHPLC) by using the DCXplorer software developed by Trapp.[19] The program employs the unified chromatography equation to directly evaluate elution profiles in a graphical user interface.[19–28] Chromatographic row data in ASCII have been opened with the DCxplorer software and the elution profiles have been evaluated by zooming in on the area of the interconverting peaks. All chromatographic parameters have been directly determined by integration and have been used to calculate reaction rate constants.[19–28]

Evaluation of Activation Parameters $\Delta G^{\#}$, $\Delta H^{\#}$, *and* $\Delta S^{\#}$

The Gibbs free activation energy $\Delta G^{\#}(T)$ has been calculated from the kinetic rate constants by fitting the data to the Eyring equation:

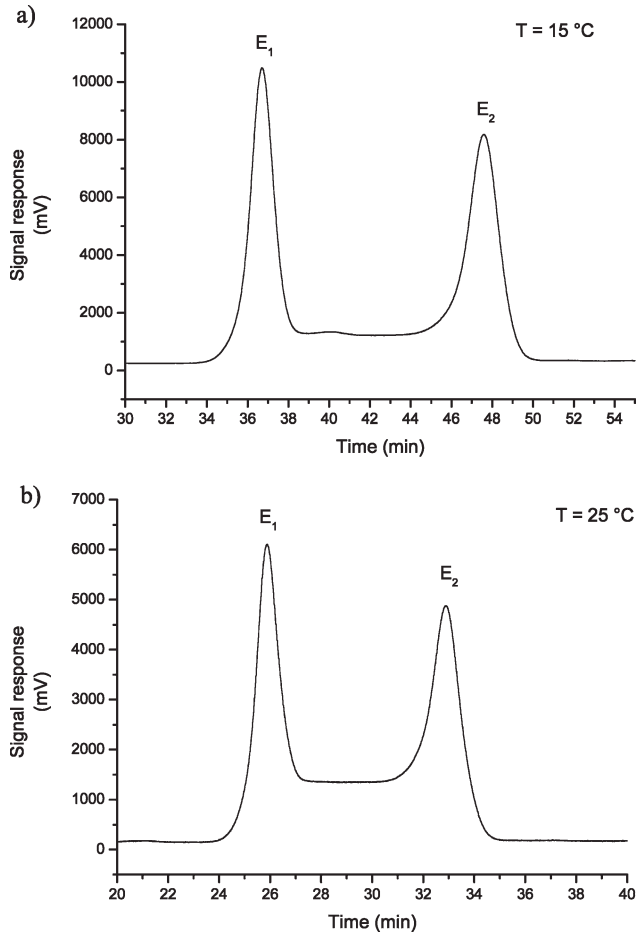


Fig. 3. Enantioresolution of (±)-**4**. Column: Chiralcel OD-RH (15 × 0.46 I.D., 5 μm); mobile phase: water:acetonitrile 50:50 (v/v); temperature: (a) 15°C, (b) 25°C.

$$\Delta G^{\#}(T) = -\mathrm{RT}\ \ln\left(\frac{kh}{\kappa k_{\mathrm{B}} T}\right) \qquad (1)$$

where k is the kinetic rate constant, k_{B} the Boltzmann constant ($k_{\mathrm{B}} = 1{,}380{,}662 \times 10^{-23}$ J K^{-1}), h Planck's constant ($h = 6{,}626{,}176 \times 10^{-34}$ J s), R the universal gas constant ($R = 831{,}441$ J K mol^{-1}), κ the transmission coefficient (κ = 0,5 for the reversible microscopic interconversion), and T the temperature (K).

As the experiments on compounds **1–12** were performed at different temperatures (4–40°C), it was possible to evaluate the enantiomerization activation enthalpy $\Delta H^{\#}$ from the slope and the enantiomerization activation entropy $\Delta S^{\#}$ from the intercept of the Eyring plot [$\ln(k_1/T$ versus T^{-1})].

RESULTS AND DISCUSSION

Chemistry

The synthesis of compounds **1–12** was achieved by closure of the appropriate 2-amino-5-chlorobenzensulfonamide and suitable aldehyde in the presence of hydrochloric acid as the catalyst (Fig. 2). An alternative pathway was used for the synthesis of compound **9**. In previous work, sele-

TABLE 2. Rate constants of enantiomerization of racemic benzothiadiazine derivatives 1–12 determined in DHPLC by DCXplorer at different temperatures

	(±)-**1**	(±)-**2**	(±)-**3**	(±)-**4**	(±)-**5**	(±)-**6**	(±)-**7**	(±)-**8**	(±)-**9**	(±)-**10**	(±)-**11**	(±)-**12**
4°C	3.06 ± 0.10 × 10^{-4}	[a]	0.85 ± 0.34 × 10^{-4}	0.52 ± 0.09 × 10^{-4}	0.38 ± 0.45 × 10^{-4}	0.44 ± 0.81 × 10^{-4}	0.36 ± 0.20 × 10^{-4}	[b]	[b]	[a]	1.15 ± 0.10 × 10^{-4}	2.33 ± 1.32 × 10^{-4}
15°C	9.35 ± 0.21 × 10^{-4}	[a]	1.37 ± 0.08 × 10^{-4}	1.45 ± 0.05 × 10^{-4}	1.11 ± 0.04 × 10^{-4}	1.14 ± 0.02 × 10^{-4}	1.76 ± 0.08 × 10^{-4}	[b]	[b]	[a]	3.38 ± 0.09 × 10^{-4}	5.17 ± 0.37 × 10^{-4}
25°C	22.23 ± 0.05 × 10^{-4}	[a]	3.62 ± 0.01 × 10^{-4}	3.70 ± 0.01 × 10^{-4}	2.89 ± 0.06 × 10^{-4}	3.24 ± 0.02 × 10^{-4}	2.51 ± 0.09 × 10^{-4}	[b]	[b]	[a]	7.25 ± 0.21 × 10^{-4}	7.20 ± 0.18 × 10^{-4}
37°C	[a]	[a]	10.02 ± 0.15 × 10^{-4}	9.80 ± 0.10 × 10^{-4}	8.36 ± 0.08 × 10^{-4}	8.76 ± 0.14 × 10^{-4}	9.25 ± 0.17 × 10^{-4}	[b]	[b]	[a]	17.3 ± 0.04 × 10^{-4}	[a]

Column: Chiralcel OD-RH; eluent: water:acetonitrile 50:50 (v/v).
[a]Enantiomerization too fast to be calculated.
[b]Any enantiomerization occurred during enantioseparation time-scale.

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TABLE 3. Free energy barrier of enantiomerization of racemic benzothiadiazine detivatives 1–12 determined in DHPLC by DCXplorer at different temperatures

	(±)-**1**	(±)-**2**	(±)-**3**	(±)-**4**	(±)-**5**	(±)-**6**	(±)-**7**	(±)-**8**	(±)-**9**	(±)-**10**	(±)-**11**	(±)-**12**
4°C	85.97 ± 0.03	[a]	89.19 ± 0.97	90.16 ± 0.04	90.87 ± 0.28	90.55 ± 0.43	90.98 ± 0.13	[b]	[b]	[a]	88.32 ± 0.20	86.84 ± 1.78
15°C	86.76 ± 0.05	[a]	91.44 ± 0.15	91.28 ± 0.09	91.93 ± 0.08	91.86 ± 0.03	90.82 ± 0.08	[b]	[b]	[a]	89.23 ± 0.07	88.20 ± 0.18
25°C	87.64 ± 0.06	[a]	92.22 ± 0.01	92.14 ± 0.01	92.76 ± 0.05	92.47 ± 0.01	93.12 ± 0.09	[b]	[b]	[a]	90.45 ± 0.07	90.08 ± 0.05
37°C	[a]	[a]	92.23 ± 0.04	92.25 ± 0.01	92.70 ± 0.02	92.58 ± 0.04	92.44 ± 0.05	[b]	[b]	[a]	90.82 ± 0.06	[a]

Column: Chiralcel OD-RH; eluent: water:acetonitrile 50:50 (v/v).
[a]Enantiomerization too fast to be calculated.
[b]Any enantiomerization occurred during enantioseparation time-scale.

TABLE 4. Activation parameters of benzothiadiazines investigated

	(±)-**1**	(±)-**2**	(±)-**3**	(±)-**4**	(±)-**5**	(±)-**6**	(±)-**7**	(±)-**8**	(±)-**9**	(±)-**10**	(±)-**11**	(±)-**12**
$\Delta H^{\#}$ (kJ/mol)	11.61 ± 1.63	[a]	7.07 ± 3.02	10.73 ± 0.77	11.74 ± 1.05	11.27 ± 1.01	11.47 ± 4.36	[b]	[b]	[a]	9.23 ± 1.02	0.07 ± 3.29
$\Delta S^{\#}$ (J/molK)	7.01 ± 0.47	[a]	6.16 ± 0.88	−7.27 ± 0.22	−7.64 ± 0.31	−7.48 ± 0.29	−7.53 ± 1.28	[b]	[b]	[a]	−6.62 ± 0.30	−3.88 ± 0.95
r	0.9975	[a]	0.9803	0.9990	0.9983	0.9984	0.9724	[b]	[b]	[a]	0.9979	0.9714
s_y	0.09	[a]	0.25	0.06	0.09	0.09	0.37	[b]	[b]	[a]	0.09	0.18

r denotes the correlation coefficient and s_y is the residual deviation of the linear regression of the Eyring plots.
[a]Enantiomerization too fast to be calculated.
[b]Any enantiomerization occurred during enantiomeriztion time-scale.

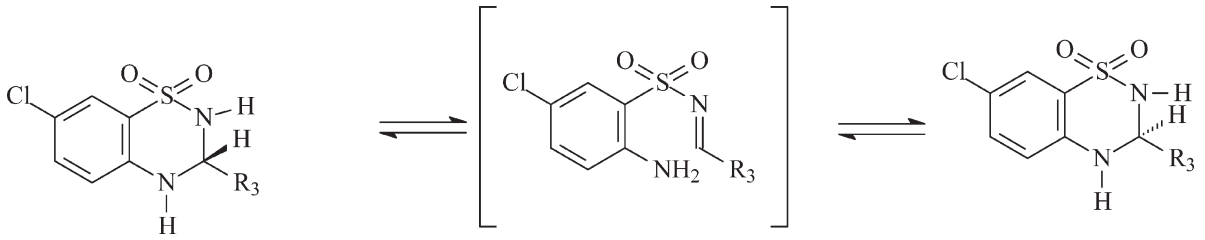

Fig. 4. Enantiomerization mechanism postulated.

ctive alkylation of heterocyclic ring at N^2 of benzothiadiazine ring was obtained with potassium carbonate as base in refluxing acetonitrile.[3,5] (±)7-Chloro-3-(2-chlorophenyl)-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide ((±)-**3**) was selectively alkylated with LDA and methyl iodide to give compound **9**. Selective methylation occurred in the 2-position of (±)7-Chloro-3-(2-chlorophenyl)-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide ((±)-**3**). This second method proved to be an effective alternative to the potassium carbonate procedure and may be adopted for further synthesis of 2-alkylbenzothiadiazines.

The obtained benzothiadiazines were fully characterized by ^1H and ^{13}C NMR spectroscopy, FTIR, and GC-MS.

Chiral Separation

Among different commercial available chiral stationary phases (CSP), cellulose 3,5-dimethylphenylcarbamate (Chiracel OD-RH) was chosen, because it was previously employed successfully for the enantioseparation of chiral benzothiadiazine-type compounds.[4,29,30,32–34]

The enantioseparation of chiral benzothiadiazines **1–12** was performed in reversed phase conditions using a Chiracel OD-RH column with mobile phases of water:acetonitrile at different percentages. Among the mobile phases used, water:acetonitrile 50:50 (v/v) was chosen, as it gave the higher enantioresolution with acceptable analysis times (Table 1). Although good enantioseparation at 25°C was obtained for all compounds with the exception of **2** and **10**, baseline enantiomeric resolution was achieved only for **8** and **9** (Table 1). A pronounced plateau was observed between the peaks corresponding to the two enantiomers, indicating that rapid enantiomerization of compounds **1**, **3–7**, and **10–12** occurred in water:acetonitrile during enantioseparation at 25°C (Fig. 3). Conversely, any plateau observed in the chromatograms corresponding to enantioseparation of compounds **8** and **9** at temperatures between 4 and 37°C using water:acetonitrile 50:50 (v/v) as mobile phase indicated that any racemization occurs in the experimental conditions used (Table 1). At 25°C, enantioseparation was achieved for compounds **2** and **10** using mobile phases with different percentages of acetonitrile. Compound **10** was enantioseparated at 4°C with a separation factor of 1.06. Compound **2** has shown a partial enantioseparation only at −10°C. We were unable to ascribe the poor enantioseparation of **2** and **10** to a fast on-column enantiomerization or to weak stereoselectivity of the CSP.

Enantiomerization

In this work DCXplorer was employed to calculate kinetic enantiomerization parameters of compounds **1**, **3–7**, and **10–12** at temperatures between 4 and 37°C.

The results obtained are reported in Table 2. The enantiomerization rate constants obtained were used to calculate free energy barriers of enantiomerization ($\Delta G^{\#}$) applying the Eyring equation and the results are reported in Table 3. The activation enthalpies $\Delta H^{\#}$ and activation entropies $\Delta S^{\#}$ of the tested compounds were obtained by plotting $\ln(k_1/T)$ as a function of T^{-1} according to the Eyring equation and are summarized in Table 4.

It was possible to calculate enantiomerization rate constants of compound **1** at 4, 15, and 25°C but not at 37°C because of peak coalescence. As shown in Table 3, the free energy barrier of **1** was lower than those of the other benzothiadiazines tested except for **10** indicating that the *o*-hydroxyphenyl substituent at C^3 position dramatically increases the enantiomerization rate.

The enantiomerization rate constants of compound **5** are slightly lower than those of compounds **3** and **4**, indicating that *m*-chloro phenyl substituent possesses an inhibitory effect on enantiomerization rate in comparison with *o*- and *p*-chloro phenyl substituents.

Compounds **6** and **7,** bearing respectively a *p*-trifluoromethylphenyl and a *p*-isopropylphenyl substituent at C^3 position, show quite similar enantiomerization rate constants indicating that the two substituents have similar influences on enantiomerization kinetics. With the aim of evaluating the influence of methyl substituents on N^2- and N^4 positions, enantiomerization rate constants were calculated for compounds **8**, **9**, and **10**.

No enantiomerization occurred for compounds **8** and **9** between 4 and 37°C, indicating that N^2-methyl substituent dramatically increases free energy barrier of enantiomerization.

As the rapid thermal enantiomerization of compound **10** results in peak coalescence between 15 and 37°C, it was not possible to calculate enantiomerization rate constants.

Plateau formation was observed only at 4°C, indicating that rapid enantiomerization takes place during chromatographic separation. This data suggests that a methyl substituent at N^4 considerably increases enantiomerization rates. Compound **11** has a free energy barrier of enantiomerization slightly greater than for compound **12** indicating that a 2-pyridine substituent at C^3 increases enantiomerization rate.

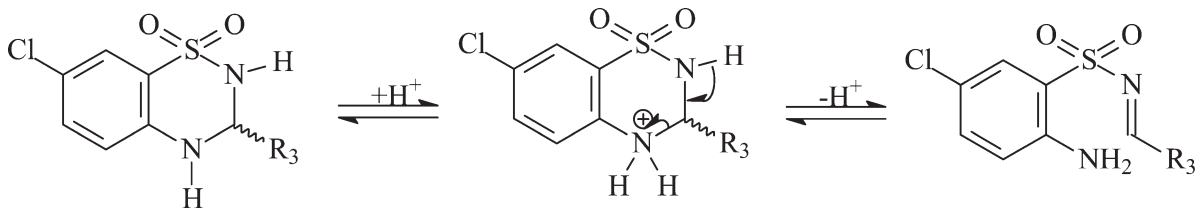

Fig. 5. Pathway example of ring opening.

Previous studies regarding enantiomerization of 3-alkyl substituted benzothiadiazines suggested an enantiomerization barrier of about 94 kJ/mol under similar experimental conditions.[32–35] Enantiomerization barriers of 3-phenyl substituted benzothiadiazines, calculated in the present work, indicated a higher enantiomerization rates suggesting that aromatic substituents exert a strong effect on the enantiomerization process. The methyl substitution on N^2 position (compounds **8** and **9**) led to higher free energy barriers of enantiomerization, suggesting a negative influence of this methyl group on enantiomerization kinetics. However, methylation on N^4 significantly increases enantiomerization rates.

The resultant free energy barriers of enantiomerization of compounds with aromatic substituents at C^3 are consistent with an interconversion mechanism that involves an imine intermediate with the double bond between C^3 and N^2 (Fig. 4). Because aromatic imines are more stable than aliphatic imines, aromatic substituents at the stereogenic carbon atom decrease free energy barriers of enantiomerization. The formation of imine intermediates can occur via several pathways and one example is given in (Fig. 5). As the observed negative entropy of enantiomerization suggests, a bimolecular reaction mechanism can occur, probably protonation of N^4, followed by deprotonation of N^2 and simultaneous imine bond formation between N^2 and C^3 (Fig. 5). This pathway has been proposed to account for the observed enantiomerization behavior of the benzothiadiazine-type compounds tested. The electron donating effect of a methyl substituent at N^4 (**10**) accelerates enantiomerization presumably as a consequence of a more favorable protonation of nitrogen. However, the methyl substituent at N^2 in **8** and **9** accounts for their higher energy enantiomerization barriers, because the replacement of the acidic proton suppresses ring opening. This enantiomerization mechanism proposed is supported by previous studies. Mollica et al. suggested that hydrolysis of benzothiadiazine compounds (e.g. hydrochlorthiazide) can occur via an imine intermediate that undergoes attack by water to form a carbinolamine, which subsequently decomposes.[36] Moreover, we have recently disclosed the rapid enantiomerization and reversible hydrolysis in acidic conditions of (±)-7-chloro-3-methyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (IDRA21) to 2-amino-5-chlorobenzensulfonamide and acetaldehyde by a mechanism similar to that observed for hydrolysis of imine compounds.[30] Ghelardoni et al. have reported the action of KBH_4 on the 3-alkyl or 3-aryl substituted 3,4-dihydro-1,2,4-benzothiadiazines 1,1-dioxyde in water-ethanol as solvent.[37] Compounds with the structure of 2-aminobenzensulfon-N-alkylamides or 2-aminobenzensulfon-N-benzylamides were obtained, suggesting the formation and subsequently reduction of an imine intermediate.[37]

Therefore, all the observed effect of substituents at N^2, C^3, and N^4 on enantiomerization rates, the negative enantiomerization entropy, and other evidence cited supported the postulated enantiomerization mechanism.

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