Semipreparative HPLC Enantioseparation, Chiroptical Properties, and Absolute Configuration of Two Novel Cyclooxygenase-2 Inhibitors

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ABSTRACT A direct semipreparative HPLC enantioseparation of two chiral thiazolidinone derivatives having cyclooxygenase-2 inhibition activity was performed on the Chiralpak IA chiral stationary phase. Semipreparative amounts of enantiopure forms were collected using acetonitrile-ethanol-trifluoroacetic acid mixtures as mobile phase. The absolute configuration of both compounds was unequivocally established by single-crystal X-ray diffraction method and correlated to the chiroptical properties of isolated enantiomers. *Chirality 22:56–62, 2010.*

KEY WORDS: HPLC; chiral stationary phase; chiralpak IA; absolute configuration; chiroptical properties; anti-inflammatory agents; sulfonamide derivatives; thiazolidinone derivatives; COX-2-inhibitors

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) exhibit their biological action by inhibiting cyclooxygenase (COX) enzymes that are required for prostaglandin synthesis. Three COX isozymes have been indentified so far: COX-1, COX-2, and COX-3.[1] The third physiological form of COX (COX-3) has recently been supposed to be a COX-2 variant appearing 48 h after the start of the inflammatory process, which may activate the biosynthesis of endogenous mediators involved in the resolution of inflammation. COX-2 is almost undetectable under physiological conditions, but it is expressed in inflammatory cells, whereas COX-1 is involved in cytoprotective physiological mechanisms and expressed in many tissues such as the stomach and the kidney. After this evidence there has been a growing interest in the search for new COX-2 selective inhibitors with anti-inflammatory activity and without the side effects related to COX-1 inhibition such as ulcers and bleeding. At the present time, the Coxib family is considered to be the most promising class of COX-2 selective inhibitors.[2–4] Recently, a novel series of chiral thiazolidin-4-one derivatives showing a good inhibitory activity towards COX-2 and structurally related to Celecoxib and Rofecoxib (see Figure 1), has been presented by some authors of this work.[5] In this article, we focus on the HPLC enantioseparation of two of them (compound **1** and **2**, Fig. 1) using the amylose-based Chiralpak IA as a chiral stationary phase (CSP). The Chiralpak IA CSP is the first of a commercially available series of polysaccharide-based selectors immobilized onto macroporous silica.[6,7] The stability of the immobilized-type CSPs is not conditioned by polarity or nature of mobile phases, unlike the coated-type CSPs which are compatible with a limited range of solvents (typically hydrocarbon/alcohol mixtures).[8,9] It has been demonstrated that 3,5-dimethylphenylcarbamate of amylose fixed to the chromatographic matrix (Chiralpak IA) has enantiomer resolving ability for a very broad range of chiral analytes in normal-phase, polar organic and reversed-phase modes.[10–13] Its high efficiency and versatility makes IA packing material especially attractive for the production of pure enantiomers from a racemate at the semipreparative scale.[14–18]

The development of single-enantiomer drugs requires sophisticated methodologies and materials to overcome the additional problems relating to chirality, such as the production and analysis of enantiomerically pure molecules and the determination of their absolute configuration. Approximately 10–50 mg of single enantiomers are usually necessary for exhaustive biological and structural characterizations of a chiral bioactive compound. Consequently, the first part of this report is devoted to the development of a suitable IA CSP/eluent system for the isola-

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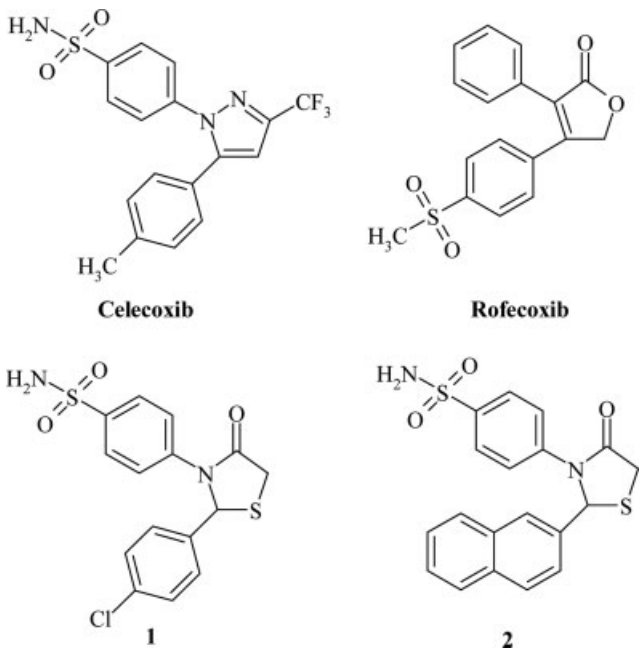

Fig. 1. Chemical structure of COX-2 inhibitors.

tion of enantiomerically pure forms of **1** and **2** at the semipreparative scale. The composition of the eluent was systematically investigated taking into account several parameters that affect the scaling-up procedure such as resolution, sample solubility and retention time.

Finally, the absolute configuration and chiroptical properties of collected enantiomers were exhaustively determined.

MATERIALS AND METHODS

The chemicals, solvents for synthesis and spectral grade solvents were purchased from Aldrich (Italy) and used without further purification. Melting points (uncorrected) were determined automatically on a FP62 apparatus (Mettler-Toledo). ^{1}H-NMR spectra were recorded on a Bruker AMX (300 MHz). Chemical shifts are expressed as δ units (parts per millions) using TMS as an internal standard. Coupling constants *J* are valued in Hertz (Hz). Elemental analysis for C, H, and N were performed on a Perkin-Elmer 240 B microanalyzer and the analytical results were within ±0.4% of the theoretical values for all compounds. All reactions were monitored by TLC performed on silica gel plates 0.2-mm thick (60 F254 Merck); spots were visualized by UV light.

HPLC enantioseparations were performed using stainless-steel Chiralpak IA (250 × 4.6 mm I.D. and 250 × 10 mm I.D.) columns (Chiral Technologies Europe, Illkirch, France). HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy). The HPLC apparatus consisted of a Perkin-Elmer (Norwalk, CT) 200 lc pump equipped with a Rheodyne (Cotati, CA) injector, a 50-μl sample loop, a HPLC Dionex TCC-100 oven (Sunnyvale, CA) and a Perkin-Elmer polarimeter model 241 equipped with Hg/Na lamps and a 40-μl flow-cell. The signal was acquired and processed by Clarity software (DataApex, Prague, Czech Republic).

The mobile phases were filtered and degassed by sonication just before use. In analytical enantioseparations, standard solutions were prepared by dissolving about 2 mg of sample into 10 ml of ethanol or methanol. The injection volume was 20–50 μl. In semipreparative enantioseparation a 1000 μl sample loop was used. After semipreparative separation, the collected fractions were analyzed by chiral analytical columns to determine their enantiomeric excess (e.e.).

The column hold-up time (t_0 = 3.0 min for 250 × 4.6 mm I.D. column) was determined from the elution of an unretained marker (toluene), using ethanol as eluent, delivered at a flow-rate of 1.0 mL/min.

Specific rotations of stereoisomers of **1** and **2**, dissolved in acetonitrile, were measured at 589 nm by a Perkin-Elmer polarimeter model 241 equipped with a Na lamp. The volume of the cell was 1 ml and the optical path 10 cm. The system was set at a temperature of 20°C using a Neslab RTE 740 cryostat. The circular dichroism (CD) spectra of enantiomers of **1** and **2**, dissolved in acetonitrile (concentration 0.2 mg/ml) in a quartz cell (0.1 cm-path length) at 20°C were measured using a Jasco Model J-700 spectropolarimeter. The spectra were average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg).

General Procedure for the Preparation of 4-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl) benzenesulfonamide, 1, and 4-(2-(naphthalen-2-yl)-4-oxothiazolidin-3-yl)benzenesulfonamide, 2

Benzenesulfonamide (4.00 g, 0.0232 mmol), 4-chlorobenzaldehyde or 2-naphthaldehyde (0.0232 mmol), and mercapthoacetic acid (3.3 mL, 0.0464 mmol), dissolved in 120 mL of toluene, were reacted under stirring at reflux in a Dean-Stark apparatus for 48 h. The precipitated crude solid was washed with ether and column chromatographed on silica gel, using $CHCl_3$/methanol as eluent. The separated white solid was crystallized from ethanol.

1. Yield 45%; mp: 201–203°C. ^{1}H-NMR (DMSO) δ 4.05 (d, 1H, CH_2, *J* = 16.0), 4.18 (d, 1H, CH_2, *J* = 15.9), 6.73 (s, 1H, CH), 7.43 (s, 2H, NH_2, D_2O-exch.), 7.47 (d, 2H, Ar, *J* = 8.4); 7.57 (d, 2H, Ar, *J* = 8.4); 7.67 (d, 2H, 4-Ar, *J* = 8.7), 7.86 (d, 2H, Ar, *J* = 8.7). Anal. Calcd for $C_{15}H_{13}ClN_2O_3S_2$: C, 48.84; H, 3.55; N, 7.59. Found: C, 48.86; H, 3.56; N, 7.60.
2. Yield 52%; mp: 213–215°C. ^{1}H-NMR (DMSO) δ 4.09 (d, 1H, CH_2 *J* = 16.2); 4.24 (d, 1H, CH_2 *J* = 16.2); 6.91 (s,1H, CH); 7.33 (s, 2H, NH_2, D_2O-exch.), 7.59-7.63 (m, 2H, naft.); 7.68–7.73 (m, 3H, naft. and phenyl); 7.82 (d, 2H, phenyl, *J* = 8.7); 7.96-8.01 (m, 4H, naft). Anal. Calcd for $C_{19}H_{16}N_2O_3S_2$: C, 59.36; H, 4.19; N, 7.29. Found: C, 59.36; H, 4.18; N, 7.30.

X-ray Crystal Structure Analysis

X-ray diffraction analysis of (−)-1 and (+)-2. In both cases Cu/Kα radiation (40mA/-40KV), monochromated by an Oxford Diffraction Enhance ULTRA assembly and an Oxford Diffraction Excalibur PX Ultra CCD were used for cell parameters determination and data collection.

The integrated intensities, measured using the ω scan mode, were corrected for Lorentz and polarization effects.[19]

Direct methods of SIR2004[20] were used to resolve the structure and it was refined using the full-matrix least squares on F^2 provided by SHELXL97.[21]

Multi-scan symmetry-related measurement was used as the experimental absorption correction type.

Compound (−)-1. $C_{15}H_{13}N_2O_3S_2Cl$, cell content $C_{32}H_{29}N_5O_6S_4Cl_2$, PM = 778.74, Triclinic, space group P 1, a = 8.053(1), b = 8.286(1), c = 13.737(1)Å, α = 92.451(3) β = 102.290(3) γ = 97.267(4), V = 886.1(2) Å^3, Z = 1 D_c = 1.459, μ = 4.282 mm^{-1}, F(000) = 402.

Colorless, prismatic shaped crystals suitable for collection were obtained from acetonitrile/ethanol and RX-analysis was performed with a Goniometer Oxford Diffraction KM4 Xcalibur2 at room temperature.

Six thousand seven hundred and six reflections were collected with a 5.40<θ<61.91 range with a 96.9% completeness to theta; 4024 were independent, the parameters were 460 and the final R index was 0.0361 for reflections having I>2σI, and 0.0492 for all data. Absolute Flack structure parameter 0.0388 with esd 0.0138.

As it can be noticed from the molecular weight, the asymmetric unit contains an acetonitrile molecule cocrystallized with two molecules of compound (−)-**1**.

The non-hydrogen atoms were refined anisotropically, whereas the hydrogen atoms were refined as isotropic. Hydrogens were assigned in calculated positions except for those on sulfonamidic nitrogens that were located in the Fourier difference map.

An important observation on the crystal packing is the presence of two intramolecular and three intermolecular hydrogen bonds.

Compound (+)-2. $C_{19}H_{16}N_2O_3S_2$, cell content $C_{38}H_{32}N_4O_6S_4$, PM = 768.92, Monoclinic, space group P 2_1, a = 10.252(1), b = 10.204(1), c = 17.259(1) Å, β = 97.449(1), V = 1790.3(3) Å^3, Z = 2 D_c = 1.426, μ = 2.886 mm^{-1}, F(000) = 800.

Colorless, needle shaped crystals were obtained from acetonitrile/ethanol and RX-analysis was performed with a Goniometer Oxford Diffraction KM4 Xcalibur2 at low temperature (100 K).

Six thousand eight hundred and sixty eight reflections were collected with a 4.35<θ<70.58 range with a 93.8% completeness to theta; 4691 were independent, the parameters were 485 and the final R index was 0.0414 for reflections having I>2σI, and 0.0478 for all data. Absolute Flack structure parameter 0.0109 with esd 0.0180.

As it can be noticed from the molecular weight, the asymmetric unit contains two molecules of compound (+)-**2** but there is no symmetry plane (and a space group P 2_1/m), due to the fact that we have a pure enantiomer and not a racemic mixture.

TABLE 1. Retention factor (k_1) for the first eluting enantiomer, enantioseparation (α), and resolution (*Rs*) factors of 1 and 2 in polar organic conditions

Compound	Mobile phase (v:v)	k_1	α	*Rs*
1	Methanol[a]	0.37 (*S*)-(−)[d]	3.86	8.08
	Ethanol[b]	0.51 (*S*)-(−)	4.43	13.47
	2-Propanol[c]	0.73 (*S*)-(−)	1.38	2.72
	1-Propanol[c]	0.57 (*S*)-(−)	2.19	6.58
	Acetonitrile[a]	0.27 (*S*)-(−)	2.37	3.92
	Ethyl acetate[a]	0.31 (*S*)-(−)	1.29	1.22
	Ethanol-Acetonitrile-TFA 95:5:0.1[b]	0.46 (*S*)-(−)	3.93	13.92
	Ethanol-Acetonitrile-TFA 90:10:0.1[b]	0.34 (*S*)-(−)	3.87	11.86
	Ethanol-Acetonitrile-TFA 75:25:0.1[b]	0.21 (*S*)-(−)	3.14	7.19
	Ethanol-Acetonitrile-TFA 50:50:0.1[a]	0.14 (*S*)-(−)	2.92	6.04
2	Methanol	0.44 (*S*)-(−)	2.00	4.48
	Ethanol	0.67 (*S*)-(−)	1.87	5.98
	2-Propanol	1.02	1.00	–
	1-Propanol	0.82 (*S*)-(−)	1.13	1.10
	Acetonitrile	0.34 (*S*)-(−)	1.23	1.08
	Ethyl acetate	0.33	1.00	–
	Ethanol-Acetonitrile-TFA 95:5:0.1	0.61 (*S*)-(−)	1.61	4.51
	Ethanol-Acetonitrile-TFA 90:10:0.1	0.55 (*S*)-(−)	1.40	3.01

Column, Chiralpak IA (250 × 4.6 mm I.D.); flow rate, temperature, 25°C; detection, UV at 254 and 280 (for ethyl acetate) nm and polarimetric at 365 nm.
[a]1 ml min^{-1}.
[b]0.5 ml min^{-1}.
[c]0.35 ml min^{-1}.
[d]Absolute configuration and on-line optical rotation of the first-eluted enantiomer.

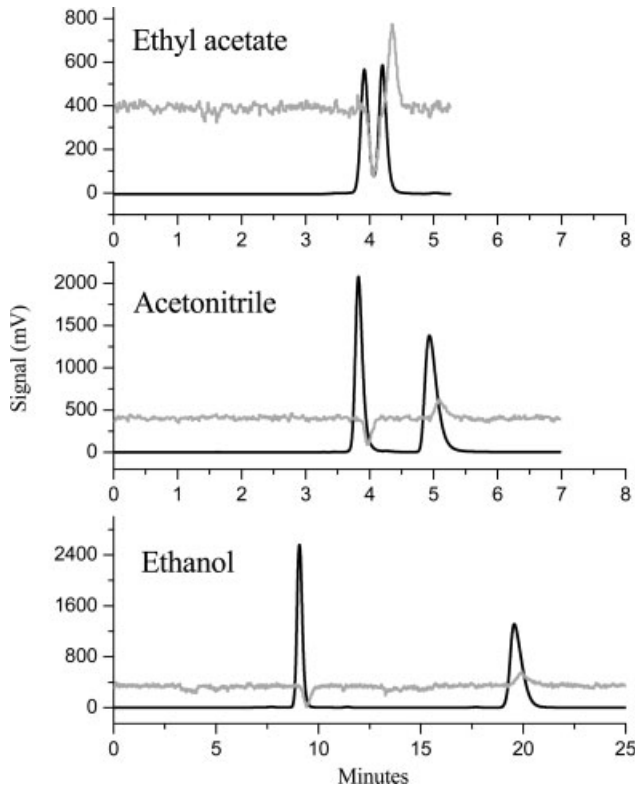

Fig. 2. Chromatograms of **1** obtained by simultaneous on-line polarimetric (gray line) and UV (black line) detection. Column, Chiralpak IA (250 × 4.6 mm I.D.); eluent, from top to bottom: ethyl acetate, acetonitrile and ethanol; see Table 1 for other conditions.

An important observation on the crystal packing is the presence of four intermolecular hydrogen bonds.

The non-hydrogen atoms were refined anisotropically whereas hydrogen atoms were refined as isotropic; they were assigned in calculated positions.

The X-ray CIF file for these structures have been deposited at the Cambridge Crystallographic Data Centre and allocated with deposition numbers CCDC 710777 (Compound **1**) and CCDC 710778 (Compound **2**). (Copies of the crystallographic data for this article can be obtained, free of charge, from CCDC, 12 Union Road, Cambridge, CB2 1EZ UK [e-mail: deposit@ccdc.cam.ac.uk; internet:// www.ccdc.cam.ac.uk]).

RESULTS AND DISCUSSION

The polar organic mode is an attractive condition for semipreparative HPLC enantioseparation of analytes with medium polarity and poor solubility or insolubility in mixtures containing hydrocarbon.[23] This is the case of compounds **1** and **2** which are highly soluble in acetonitrile, sparingly soluble in alcohol and ethyl acetate and insoluble in n-hexane. Successful enantioseparations using Chiralpak IA CSP in combination with polar organic eluents have been previously reported.[11,24] Thus, two analytes were initially screened on Chiralpak IA using net methanol, ethanol, 2-propanol, acetonitrile and ethyl acetate as a mobile phase.

The obtained chromatographic results are summarized in Table 1. Among alcohol-based mobile phases, 2-propanol and 1-propanol gave the lowest selectivity. In practical experience, these two alcohols are rarely used as a mobile phase for semipreparative enantioseparation, because of their high viscosity and difficulty of evaporation from collected fractions.[25]

The best chiral discrimination for compound **1** was obtained with ethanol, as demonstrated in Figure 2. Marginal (compound **1**) or null (compound **2**) enantioselectivity was observed with ethyl acetate.

In the case of compound **2**, enantioselectivity differences between methanol and ethanol were not large. However, ethanol yielded a higher resolution than methanol due to better efficiency. Although from the analysis of chromatographic data net ethanol proved to be the best performing mobile phase, it was not considered convenient for scaling-up enantioseparation at the semipreparative level. This for two valid reasons: (i) the sample solubility of **1** and **2** in ethanol was not high enough to allow appreciable amounts of racemate to be separated for single injection and (ii) retention times were too long. In practice, owing to the high viscosity of ethanol and in accordance with the column back-pressure limit indicated for the Chiralpak IA column (about 1500 psi), the retention times cannot be appreciably shortened by increasing the flow rate.

It can be seen from the results resumed in Table 1, the progressive addition of acetonitrile to ethanol, in the presence of a constant amount (0.1%) of trifluoroacetic acid, lead to a reduction in retention, enantioselectivity and resolution. The effects on enantioselectivity and resolution were more pronounced for compound **2** which was only

TABLE 2. Chromatographic and polarimetric analysis of the pooled fractions containing the first (F1) and second (F2) eluted enantiomers of 1 and 2

			F1[b]		F2[b]	
Compound	Mobile phase	A[a]	e.e.(%)	$[\alpha]_D^{20}$	e.e.(%)	$[\alpha]_D^{20}$
1	Ethanol/acetonitrile/TFA 90:10:0.1 (v/v/v)	20	>99.0	−136 (c = 0.1, CH_3CN)	>99.0	+140 (c = 0.1, CH_3CN)
2	Ethanol/acetonitrile/TFA 95:5:0.1 (v/v/v)	10	>99.0	−98 (c = 0.1, CH_3CN)	>99.0	+97 (c = 0.1, CH_3CN)

Column: Chiralpak IA (250 × 10 mm I.D.); flow-rate, 3.0 ml min^{-1}; detector, UV at 280 nm; temperature, 25°C.
[a]Amount of sample (in mg) resolved in a single semipreparative run.
[b]Enantiomeric purity and polarimetric data for the pooled fractions containing the first (F1) and second (F2) eluted enantiomers.

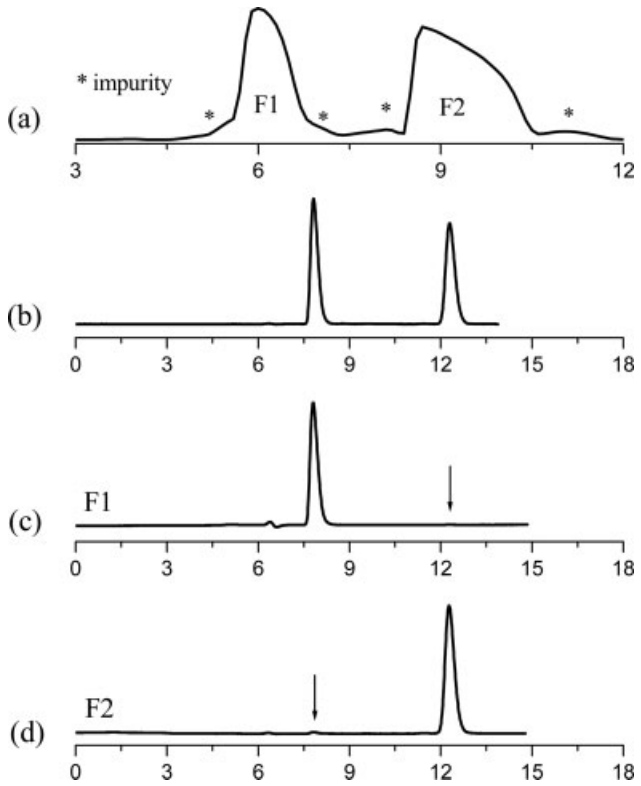

Fig. 3. (**a**): Semipreparative resolution of 20 mg of **1**; (**b**) Analytical HPLC enantioseparation of **1**; (**c**) and (**d**): Chromatographic analysis of isolated enantiomers. Column, (a): Chiralpak IA (250 × 10 mm I.D.), (b), (c), and (d): Chiralpak IA (250 × 4.6 mm I.D); eluent, ethanol-acetonitrile-TFA 90-10-0.1 (v/v/v); flow-rate, (a): 3.0 ml min^{-1}, (b), (c), and (d): 0.5 ml min^{-1}; detector, UV at 280 (a) and 254 nm (b, c and d); temperature, 25°C.

weakly resolved with net acetonitrile. However, the addition of 10% of the non-alcoholic cosolvent produced a reduction in the elution time of the more retained enantiomer of **1** by about 40% without significatively impairing enantioselectivity or resolution (the enantioselectivity and resolution factor values changed from 4.43 and 13.47 to 3.87 and 11.86, respectively).

As was expected from the acid nature of the analytes, the presence of 0.1% of trifluoroacetic acid caused an enhancement in peak symmetry. A further benefit which was particularly useful for semipreparative applications consisted in improved sample solubility.

So, based on the chromatographic results of analytical screening, the acetonitrile-ethanol-TFA 90-10-0.1 (v/v/v) and acetonitrile-ethanol-TFA 95-5-0.1 (v/v/v) mixtures were identified, respectively, as the most suitable eluents for the enantioseparation of **1** and **2** on the 1-cm I.D. IA column.

Table 2 shows the enantiomeric excess and rotation specific for pooled fractions containing the first eluted (F1) and second eluted (F2) enantiomer, after injection of 20 mg of **1** and 10 mg of **2**. The racemic forms were dissolved in 1000 μL of the acetonitrile-ethanol-TFA 50-50-0.1 (v/v/v) mixture. As shown in Figure 3, the isolation of enantiopure forms of **1** was performed in short also in presence of unknown impurities.

On-line (Table 1) and off-line (Table 2) polarimetric analysis indicated that the first eluting enantiomer of both compounds was levorotatory under all investigated conditions.

At the best of our knowledge, no stereochemical characterization for molecules structurally related to **1** or **2** has been reported. We therefore used single-crystal X-ray diffraction to unambiguously assign[26] the absolute configuration to the collected enantiomers. As illustrated in Figure 4, (−)-**1** and (+)-**2** were shown to have (*S*) and (*R*) absolute configuration at the stereogenic center located on the thiazolidinone ring.

HPLC analysis of non racemic samples enriched by enantiomers of known chirality made it possible to assign the enantiomer elution order on the Chiralpak IA CSP: the (*S*)-(−)-enantiomers of both compounds **1** and **2** were eluted before their (*R*)-(+) counterparts (Table 1).

The CD spectra of the enantiomers of **1** and **2** are shown in Figure 5. Solutions of (*R*)-**1** exhibited two strong Cotton effects of opposite sign at 209 and 229 nm followed by two weaker CD bands at 250 (negative) and 280 nm (positive). It should be noted that, in the case of the (*R*)-**2** enantiomer, the intense negative CD band located at a

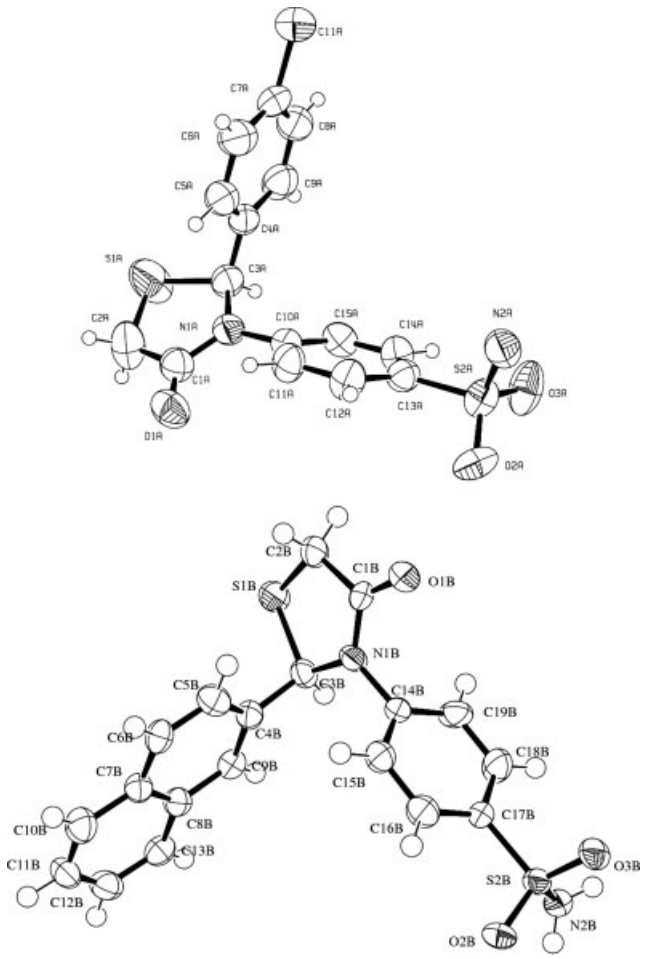

Fig. 4. An ORTEP view of the molecular structure of the (*S*)-(−)-**1** enantiomer (top) and (*R*)-(+)-**2** enantiomer (bottom).

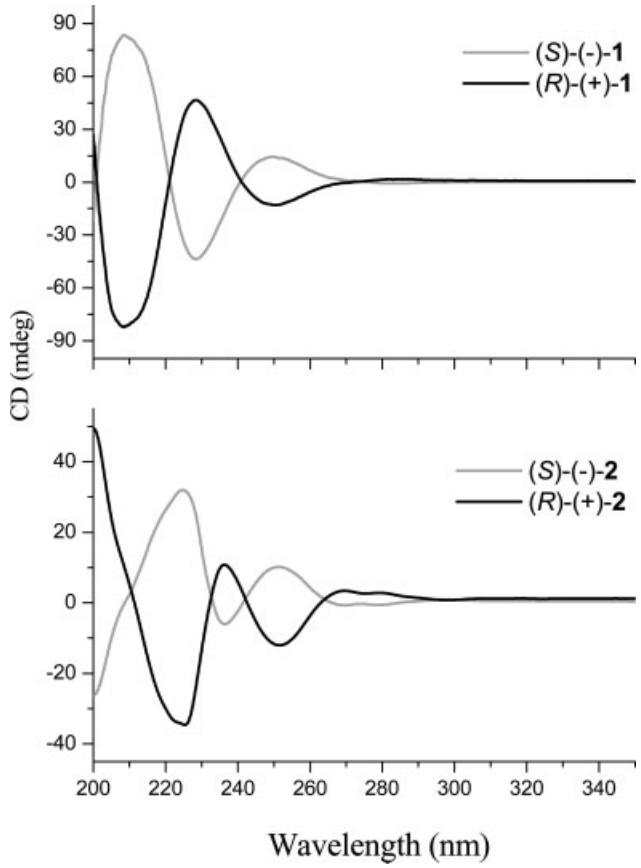

Fig. 5. CD spectra of enantiomers of **1** and **2** in acetonitrile.

shorter wavelength was red shifted at 227 nm. The slight differences in CD properties reiterate the difference between the chromophores (2-naphtyl and 4-chlorophenyl) linked to stereogenic center.

To sum up, the enantioselective HPLC method based on Chiralpak IA CSP in the presence of acetonitrile-ethanol-TFA mixtures provided rapid access to mg amounts of enantiopure forms of compounds **1** and **2**. The application of the optimized CSP/eluent system supports a first comparative biological evaluation of the influence of stereochemistry on cyclooxygenase-2 inhibition activity. Further, the crystallographic and CD data spectra presented in this study may be used to empirically assign the absolute configuration of compounds structurally related to **1** and **2**.

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