Configurational Assignments of the Diastereomers of 3,3'-(1,2-Ethanediyl)bis[2-(3-fluorophenyl)-5-methyl-4-thiazolidinone] Derivative with Four Stereogenic Centers.

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Diastereomers of antiinflammatory/analgesic and antihistaminic 3,3'[(1,2-ethanediyl)bis(2-aryl-4-thiazolidinone)] derivatives possessing two stereogenic centers (indicated as BIS 2*C) have been widely investigated in recent years. The 5,5'-dimethyl analogues (BIS 4*C), now reported, have been synthesized by reaction of (±) α-mercaptopropionic acid and *N*,*N*'-di(3-fluorobenzyliden)ethylenediamine. Because the 2 and 2'carbons bear the same groups and similarly the 5 and 5' carbons, and the latter groups are different from the former, four enantiomeric pairs and two *meso* forms exist in this situation. These diastereomers were identified by the concerted use of nmr spectroscopy and hplc on chiral stationary phase.

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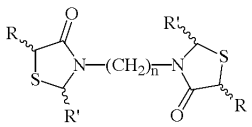
Several 3,3'-bis[2-substituted-4-thiazolidinone] chiral derivatives display interesting stereoselective anti-inflammatory, analgesic and antihistaminic profiles [1-5]. Many series of bisthiazolidinones with 2 and 2' stereogenic centers (BIS 2*C with n = 0, 2; R = H; R' = Alk, Ar, Het.) were widely investigated and several structure/activity relationships were established. In particular it was pointed out that: a*)* all series exhibit minor acute toxicity and gastric damage than established NSAIDs as indomethacin and phenylbutazone; b*)* the more interesting derivatives prove to be those with n = 2, R' = Ar or Het and 2*R*2'*S*-*meso* configuration; c*)* when R' = Ar, substitution at the *meta* position is in general the most beneficial with F, Cl, CH_3O being the favorable substituents; d*)* the corresponding 1,1'-disulfones display increased antiinflammatory activity, especially when R' = Het.



n = 0,2;
R = H [BIS 2*C], CH_3 [BIS 4*C]
R' = Alk, Ar, Het

The particular stereochemistry of all these bisthiazolidinones seemed to us very intriguing and prompted several investigations in recent years [1,2,6,8]. In particular conformational analysis and molecular modeling studies were carried out on the 2*R*2'*R*,5*S*5'*S* and 2*R*2'*S*,5*S*5'*R* isomers of 3,3'-(1,2-ethanediyl)bis[2-(3-fluorophenyl)-4-thiazolidinone], the configurations being assessed by X-ray diffractometry [1].

In pursuing this research, we investigated the effect of the introduction of methyl groups at the 5 and 5' positions that generate compounds with four stereogenic centers (BIS 4*C). Since the 2 and 2' carbons bear the same substituent (Ar) and 5 and 5' also bear the same substituent (CH_3), the number of possible stereoisomers is reduced from sixteen to ten, namely four enantiomeric pairs $dl_{1\text{-}4}$ and two *meso* forms $meso_{1\text{-}2}$. They can display *trans/trans*, *trans/cis* or *cis/cis* geometries with respect to the Ar/CH_3 disposition of each thiazolidinone ring (Table 1).

The object of this note is configurational assignments by nmr experiments of the diastereoisomers of 3,3'-(1,2-ethanediyl)bis[2-(3-fluorophenyl)-5-methyl-4-thiazolidinone] whose hplc diastereo- and enantioseparation was already reported [7].

These assignments will allow us to correlate the stereochemistry of these 5,5'-dimethyl substituted bisthiazolidinones with the interesting antiinflammatory profile relative to their BIS 2*C analogues.

Results and Discussion

The configurational assignment of the 3,3'-(1,2-ethanediyl)bis[2-(3-fluorophenyl)-5-methyl-4-thiazolidinone] stereoisomers, whose physical and analytical data are reported in Table 2, has been achieved by means of ^{1}H nmr supported by nOe experiments, while the analogy with the BIS 2*C analogues has greatly helped for the assignment.

In the latter compounds, in fact, the relative configuration of 2,2' carbons affects both ethylene fragment and 2H, 2'H resonances. Thus the CH_2-CH_2 protons resonate as AA'XX' systems in 2*R*,2'*R* (or 2*S*,2'*S*) isomers, while they resonate as AA'BB' in 2*R*,2'*S* (or 2*S*,2'*R*) isomers. In addition the 2H, 2'H resonance of 2*R*,2'*R* (or 2*S*,2'*S*) is always shifted down-field (0.4-0.3 ppm) with respect to that of 2*R*,2'*S* (or 2*S*,2'*R*).

Moreover, inspection of the vicinal coupling constants allowed us to investigate conformational equilibria in solution [1]. It was established that 2*R*,2'*R* and 2*S*,2'*S* isomers greatly prefer the *gauche* conformation, while 2*R*,2'*S*-*meso* isomer exists as a rapid interconverting mixture of three rotamers (2 *gauche* and 1 *trans*) so that the average of these magnetic environments is observed.

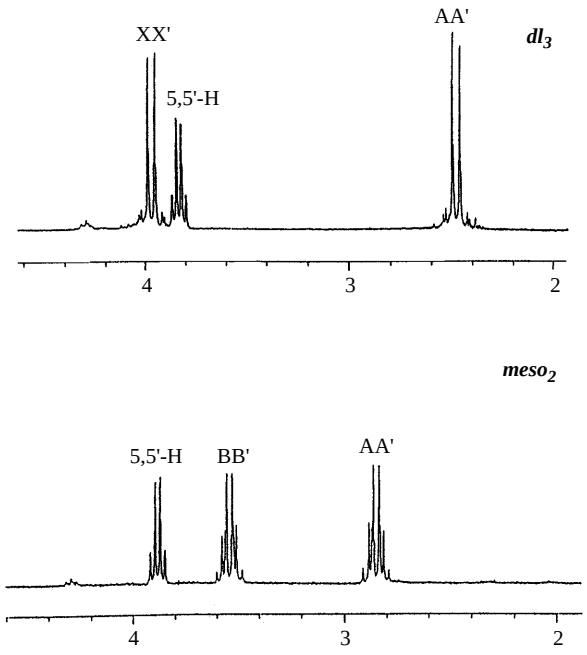


Figure 1. H[1] nmr Spectra (deuteriochloroform, 300 MHz) of ***dl*$_3$** and ***meso*$_2$** selected as representative diastereomers of type A and B respectively.

In consequence the preliminary inspection of the ^{1}H nmr spectra of 3,3'-(1,2-ethanediyl)bis[2-(3-fluorophenyl)-5-methyl-4-thiazolidinone] diastereomers (Table 3) reveals that type A and type B fractions are different in their AA'XX' systems in that Δν values between the AA' and XX' resonances are greater in A than in B products (~ 400 Hz *versus* ~ 200 Hz). Thus, taking into account the BIS2*C acquisitions, it can be first assessed that all the 2*R*,2'*R* and 2*S*,2'*S* isomers are contained in the A fractions, while those with the 2*R*,2'*S* and 2*S*,2'*R* configurations are confined in B fractions (Figure 1).

In particular, among type A isomers, ^{1}H nmr spectra of ***dl*$_1$** and ***dl*$_3$** show single sets of signals, due to the magnetically equivalent protons of the 5-membered heterocycle rings. In consequence A_1 and A_2 fractions should contain *dl*$_1$, *dl*$_2$, *dl*$_3$ enantiomeric pairs, whereas B_1 and B_2 fractions should contain ***dl*$_4$**, ***meso*$_1$** and ***meso*$_2$** isomers (see Table 1).

Instead ***dl*$_2$** shows two sets of similarly intense signals, as expected by a *trans/cis* geometry. Thus 2*R*5*R*, 2'*R*5'*S* and 2*S*5*S*, 2'*S*5'*R* configurations can be assigned to this enantiomeric pair.

In order to correctly assign all the signals, spin decoupling experiments were carried out on *dl*$_2$. In particular the relationships between δ 1.65 doublet and δ 3.91 signal on one hand and between δ 1.60 doublet and δ 4.00 quartet on the other hand were established (Table 3).

All experimental evidence indicates that the substituted thiazolidin-4-one rings prefer twisted solution conformations with 1-S and 5-C out of the plane defined by 2-C, 3-N and 4-C [8,9,10]: in this situation the equatorial substituent on 5-C resonates at low-field owing to deshielding effect of the adjacent nearly coplanar carbonyl group (Figure 2).

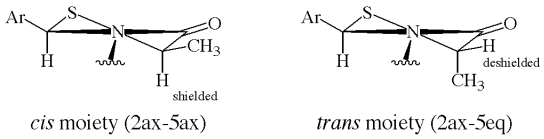


Figure 2. Schematic drawing of the preferred conformations of *cis*- and *trans*-2,5-disubstituted thiazolidinones.

In the *cis* moiety, in fact, the steric interaction prompts the phenyl ring and the methyl group to occupy pseudo-equatorial orientations. This results in the location of the CH_3 in the carbonyl deshielding zone producing, in turn, the up-field shift of 5-CH resonance in the *cis*-2,5-disubstituted moiety. Furthermore an evident long-range coupling (J = 0.8 Hz) between the quartet at δ 3.91 and 2-CH (δ 5.93) signal confirms the *cis* 1,3-pseudodiaxial geometry for 2-H/5-H. On the contrary in the *trans* moiety of the molecule the 5'-CH is instead in the deshielding zone of the carbonyl group thus resonating at lower field (4.00 ppm) than 5-CH.

In the ***dl*$_3$** derivative that exhibits one set of resonances, irradiation of 2-CH induces a comparable nOe on both 5-CH (6%) and the *ortho* aromatic protons (8%), suggesting a *cis/cis* geometry. Moreover the observed long-range coupling (J = 1.5 Hz) through S atom confirms the 2-H/5-H *cis* 1,3-pseudodiaxial orientation. In addition, irradiation of the 2-CH signal (H_a, H_b) resulted in a signal enhancement for the XX' part (3.97 ppm, H_1, H_4) of the AA'XX' system (6%) suggesting that they are in very close proximity and XX' are in the deshielding region of the carbonyl group (Figure 3). Such results can be rationalized by assuming that *dl*$_3$ enantiomeric pair prefers the *gauche* solution conformation, as assessed by computer-simulated spectra. In fact ethylene chain protons show $J_{1,2} = J_{3,4} = -14.3$, $J_{1,3} = J_{3,4} = 3.97$, $J_{1,4} = 12.5$ and $J_{2,3} = 2.65$ Hz values, the difference between $J_{1,4}$ and $J_{2,3}$ excluding the presence of any solution equilibrium.

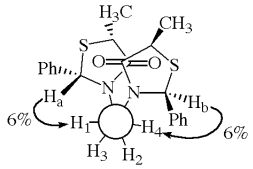


Figure 3. nOe Experiments on ***dl*$_3$** (*cis*/*cis*) in the preferred *gauche* disposition.

Table 1

Possible Diastereoisomers of 3,3'-(1,2-Ethanediyl) bis[2-(3-flourophenyl)-5-methyl-4-thiazolidinone].

Isomer [a]	Ar/CH_3 Disposition	Configuration C-2	C-2'	C-5	C-5'	Fraction
		R	R	R	R	
***dl*$_1$**	*trans/trans*					
		S	S	S	S	
						A_1
		R	R	R	S	
***dl*$_2$**	*trans/cis*					
		S	S	S	R	
		R	R	S	S	
***dl*$_3$**	*cis/cis*					A_2
		S	S	R	R	
		R	S	R	R	
***dl*$_4$**	*trans/cis*					
		S	R	S	S	B_1
***meso*$_1$**	*trans/trans*	R	S	R	S	
***meso*$_2$**	*cis/cis*	R	S	S	R	B_2

[a] Tlc fractions, as discussed in the synthesis section.

In conclusion ***dl*$_3$** has 2*R*5*R*,2'*S*5'*S* and 2*S*5*S*,2'*R*5'*R* enantiomeric configurations.

Finally the *trans/trans* geometry of ***dl*$_1$** is supported by the higher chemical shifts of 5,5'-protons (4.05 *versus* 3.83 ppm of ***dl*$_3$**) indicating greater deshielding effects by carbonyl groups. In fact the *trans* geometry in these rings prompts methyl substituents to assume pseudo-axial orientation. Moreover, irradiation of 2,2'-CH has no effect on the intensity of the 5,5'-CH protons suggesting they are not topologically close together. Instead a relevant nOe enhancement (7 %) is observed on the XX' part (4.11 ppm) of the AA'XX' system.

Type B stereoisomers that bear opposite configurations at 2C and 2'C stereogenic centers, can also exist as *trans/trans*, *trans/cis* and *cis*/*cis* compounds (Table 1). Also, the ^{1}H-nmr spectrum of B_2 fraction, that chiral-phase hplc showed to be a *meso* form [7], clearly reveals the isochronism of the corresponding protons on thiazolidinone rings. The irradiation of the 2-CH induces a nOe effect (8%) on 5-CH. Such an effect, as well as the clear long-range coupling (J = 1.3 Hz) between 2H/5H resonances (Table 3), supports the *cis* geometry of these protons in both rings. Thus we are dealing with 2*R*5*S*,2'*S*5'*R* isomer (***meso*$_2$**) and again, as expected in a 2,5-*cis* moiety, the 5,5'-CH protons are not in the deshielding zone of the carbonyl groups.

The mixture B_1 on chiral stationary phase hplc exhibited three broad peaks, two of which are of nearly equal area [7]. Despite extended efforts, however, a milligram-scale separation was unsuccessful, thus the configurational assignments were made based on the ^{1}H nmr spectrum of the mixture. The presence of multiple sets of signals with different intensities and the analogy with the magnetic behavior of compounds A with corresponding geometry, allow the chemical shifts of each isomer to be exactly assigned (Table 3). In fact, the *trans*/*cis* relationship of ***dl*$_4$** is supported by two sets of 2,2'-CH (5.51, 5.56 ppm) and 5,5'-CH (3.92, 4.04 ppm) signals as already explained for enantiomeric pair ***dl*$_2$** of type A with the same *trans*/*cis* relationship. However, the mixture shows additional single signals for 2,2'-CH (5.53 ppm) and 5,5'-CH (4.02 ppm) attributable to isomer with the *trans*/*trans* geometry (see discussion for ***dl*$_1$**). Thus we can deduce that B_1 mixture is formed by ***meso*$_1$** with configuration 2*R*5*R*,2'*S*5'*S* and ***dl*$_4$** enantiomeric pair with configurations 2*R*5*R*,2'*S*5'*R* and 2*S*5*S*,2'*R*5'*R*. In addition the ^{1}H nmr spectrum allows ***dl*$_4$**:***meso*$_1$** 60:40 ratio to be measured.

In conclusion the complete assignment of 3,3'-(1,2-ethanediyl)bis[2-(3-fluorophenyl)-5-methyl-4-thiazolidinone] diastereomers has been achieved. Not all isomers, however, are available in quantities that allow comparative *in vitro* pharmacological evaluation, and that remains our final objective.

Table 2

Physical and Analytical Data of 3,3'-(1,2-Ethanediyl) bis[2-(3-fluorophenyl)-5-methyl-4-thiazolidinone] Diastereomers

Fraction	Diastereomer	Ir (cm^{-1}) ν C=O [a]	mp [b] °C	Rf [c]	Analysis % [d] C	H	N
	dl$_1$	1682	118-120				
A$_1$ [e]				0.93	58.72	5.06	6.14
	dl$_2$	1680	121-123				
A$_2$	*dl*$_3$	1678	120-123	0.90	58.69	4.82	6.41
	dl$_4$						
B$_1$ [e]		1664	164-166	0.74	58.86	5.02	6.50
	meso$_1$						
B$_2$	*meso*$_2$	1660	193-195	0.67	59.05	4.74	6.38

[a] KBr pellet; strong bands with many shoulders; [b] Recrystallization solvent: methanol; [c] Eluent chloroform/diethyl ether 9:1; [d] Molecular formula $C_{22}H_{22}F_2N_2O_2S_2$ Calcd: C 58.93; H 4.91; N 6.25. [e] Mixture ***dl*$_2$**:***dl*$_1$** 93:7, mixture ***dl*$_4$**:***meso*$_1$** 60:40 determined by means of ^{1}H nmr spectra.

Table 3
^{1}H nmr (deuteriochloform, 300 MHz) Parameters of 3,3'-(1,2-Ethanediyl)-bis[2-(3-fluorophenyl)-5-methyl-4-thiazolidinone] Diastereomers.

Fraction	Diastereomer	CH_2CH_2 [a]	2, 2'-CH [b]	5, 5'-CH [c]	CH_3 [d]	Aromatic protons [e]
	dl$_1$	2.57, 4.11	5.83	4.05 (J = 6.9)	1.62 (J = 6.9)	7.00 - 7.36
A_1						
	dl$_2$	2.50, 4.02	5.91,	3.91 (dq, J = 6.9, 0.8)	1.65 (J = 6.9)	7.00 - 7.36
			5.93 (d, J = 0.8)	4.00 (J = 6.9) [f]	1.60 (J = 6.9)	
A_2	*dl*$_3$	2.47, 3.97	5.92 (d, J = 1.5)	3.83 (dq, J = 6.9, 1.5)	1.62 (J = 6.9)	7.03 - 7.34
	dl$_4$ [g]	2.88, 3.56	5.51, 5.56	3.92 (J = 6.9)	1.61 (J = 6.9)	7.13 - 7.40
B_1				4.04 (J = 6.9)		
	meso$_1$ [g]	2.79, 3.75	5.53	4.02 (J = 6.9)	1.61 (J = 6.9)	7.13 - 7.40
B_2	*meso*$_2$	2.85, 3.54	5.55 (d, J = 1.3)	3.88 (dq, J = 6.9, 1.3)	1.61 (J = 6.9)	7.01 - 7.37

J expressed in Hz. [a] AA'XX' systems . [b] Singlet. [c] Quartet. [d] Doublet. [e] 4H, multiplet. [f] Overlapped by XX' protons and assigned by NOE experiments. [g] Assigned from the mixture.

EXPERIMENTAL

Melting points were determined with a Kofler-Reichert hot-stage apparatus and are uncorrected. Precoated silica gel plates (Merck F 254) were used for analytical controls using diethyl ether/petroleum ether 40-60 °C as eluent in variable proportions ranging from 7:3 to 9:1. Chromatographic separations were performed on columns packed with silica gel (Merck 60/70-230 mesh) using as eluent diethyl ether/petroleum ether 40-60 °C 9:1. Radial tlc was carried out by a Chromatotron apparatus (Harrison Research, Model 7924T) with Merck silica gel 60P F_{254} gypsum-containing plates, by using petroleum ether (40-60 °C)/diethyl ether 9:1 as eluent.

The hplc system consisted of a Shimadzu Model Class VP 5 chromatograph. The mobile phase was hplc-grade *n*-hexane/2-propanol (9:1). As stationary phase an Ultrasphere silica gel column from Beckman (15 cm x 2 mm I.D.) was used. The separation was carried out at 25 °C.

Elemental analyses (C,H,N), which have been determined by means of a C. Erba mod. 1106 Elem. Analyzer, were within ± 0.4 % of theoretical values. Ir spectra were registered (KBr pellet) on a Perkin Elmer mod. 1720 Spectrophotometer. ^{1}H-nmr spectra were recorded in deuteriochloroform solutions on a Varian 300 MHz Spectrometer. Chemical shifts are expressed in δ units (ppm) relative to tetramethylsilane as an internal standard; coupling constants (J) are expressed in Hz. The spectra were analysed by HyperNMR program [11]. Nuclear Overhauser effect (nOe) measurements were performed by FT difference method after a preliminary rough evaluation of the longitudinal relaxation time of the protons.

3,3'-(1,2-Ethanediyl)bis[2-(3-fluorophenyl)-5-methyl-4-thiazolidinone].

The mixture of all diastereomers was obtained by the reaction of (±) α-mercaptopropionic acid (0.03 mole, 3.18 g) and *N*,*N*'-di-3-fluorobenzylidenethylenediamine (0.01 mole, 2.72 g) in refluxing anhydrous toluene (50 ml) for 6 hours. Removal of the solvent *in vacuo* gives an oily residue, which was dissolved in chloroform and repeatedly washed with an aqueous solution of sodium carbonate (20%), then dried with sodium sulfate. Yield 88%. A preliminary tlc control (chloroform:diethyl ether 9:1) of the crude mixture resulted in two pairs of close spots indicated for convenience with A_1 and A_2 (Rf 0.93 and 0.90) and B_1 and B_2 (Rf 0.74 and 0.67).

The first diastereoseparation of this mixture in fractions A and B was carried out by means of fractionated crystallization with methanol. The A isomers showed wider solubility in methanol and other common solvents than the B isomers. Then radial tlc was performed on A and B mixtures. Finally A_1, A_2, B_1, B_2 fractions, endowed with unusually narrow melting points, were obtained (Table 2); among them the hplc on chiral phase revealed A_2 as an enantiomeric pair (***dl*$_3$**) and B_2 as a *meso* form (***meso*$_2$**) [7]. A_1 mixture was well resolved on normal-phase hplc. By repeated collection of the eluates from the two chromatographic peaks it was possible to obtain a milligram scale separation of ***dl*$_1$** and ***dl*$_2$** racemic compounds (Table 1). The complex chromatographic behavior of B_1 mixture resulted in an unsuccessful separation by normal-phase hplc of the other diastereoisomers (***dl*$_4$** and ***meso*$_2$**).

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