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Synthesis and Antiinflammatory, Analgesic Activity of 3,3′-(1,2-Ethanediyl)-bis[2-aryl-4-thiazolidinone] Chiral Compounds. Part 10†

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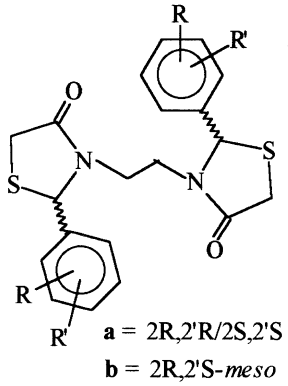
Abstract—In this note, the synthesis and structure–activity relationships of a new series of 2*R*,2′*R*/2*S*,2′*S* and 2*R*,2′S-*meso* 3,3′-(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinones] are described. Antiinflammatory activity was investigated by the carrageenin-induced paw edema test and analgesic activity by acetic acid writhing and hot plate tests in rats. All compounds displayed ulcerogenic effects and acute toxicity much lower than indomethacin and phenylbutazone. *Meso* isomers (**b**) showed better pharmacological profiles than corresponding racemates (**a**). Methoxy substitution patterns of the aryls on stereogenic carbons are generally the most favorable on the pharmacological profile.

In recent years, our research has focused on the study of 3,3′-(1,2-ethanediyl)-bis[2-substituted-4-thiazolidinones] endowed with antiinflammatory and analgesic activities and low ulcerogenic action.[1–4]

Owing to the presence of 2 and 2′ equivalent stereogenic centers, bisthiazolidinones can be obtained as *rac*. 2*R*,2′*R*/2*S*,2′*S* and 2*R*,2′*S*-*meso* isomers, which usually exhibit stereoselective pharmacological properties. Among them, [2*R*,2′*S*-*meso*] 3,3′-(1,2-ethanediyl)-bis[2-(3,4-dimethoxyphenyl)-4-thiazolidinone] **1b**, endowed with the most interesting pharmacological properties[4] ('lead compound'), was worth further investigation.

In this paper, we report the synthesis and pharmacological screening of a new series of its structural analogues. The pattern of 2 and 2′ phenyl substituents has been modified in order to go deep into the structure–activity relationships. Therefore, we explored 2,4- and 2,5-dimethoxy congeners (**6** and **7**) of the lead. Dihalogen compounds **9**, **10** and **11** have been designed taking into account that F or Cl, especially when in *meta* position, had already been found favorable for pharmacological activity.[2] To investigate what position (3-MeO or 4-MeO) was critical for activity, we prepared and evaluated also monosubstituted compounds **2** and **3** and compound **8**, the latter as a compromise because of the presence of 3-F, while methoxy group was maintained in *para*. Afterwards, we replaced 3-MeO with the electron-releasing methyl group or electron-withdrawing



	R	*R'*
1[4]	3-OCH_3	4-OCH_3
2	H	3-OCH_3
3	H	4-OCH_3
4	H	3-CH_3
5	H	3-NO_2
6	2-OCH_3	4-OCH_3
7	2-OCH_3	5-OCH_3
8	3-F	4-OCH_3
9	2-Cl	6-Cl
10	2-Cl	6-F
11[4]	3-Cl	4-Cl

Figure 1.

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Table 1. Yields, melting points,[a] IR[b] and ^{1}H NMR[c] data of bisthiazolidinones **2–10**

Compd	Yields (%)	Mp (°C)	ν C=O	CH_2CH_2[d]	2,2′-CH[e]	5,5′-CH_2[f]	Aromatic protons	Other protons
2a	58	136–139	1670	2.55, 3.99	5.92	3.68, 3.74 (J = 15.4)	6.85–7.31 (m, 4H)	3.80 (s, 3H, OCH_3)
2b	38	161–164	1672	2.85, 3.70	5.57	3.67, 3.76 (J = 15.2)	6.83–7.33 (m, 4H)	3.83 (s, 3H, OCH_3)
3a	56	110–115	1660	2.48, 3.89	5.91	3.66, 3.71 (J = 15.6)	6.84–7.29 (m, 4H)	3.79 (s, 3H, OCH_3)
3b	38	180–185	1668	2.79, 3.61	5.55	3.65, 3.74 (J = 15.5)	6.90,7.25 (m, 4H)	3.82 (s, 3H, OCH_3)
4a	59	128–133	1684	2.50, 3.93	5.99	3.68, 3.73[g]	7.13–7.25 (m, 4H)	2.34 (s, 3H, CH_3)
4b	36	216–220	1670	2.79, 3.67	5.55	3.65, 3.75 (J = 15.6)	7.19 (m, 4H)	2.36 (s, 3H, CH_3)
5a	53	203–205	1660	2.64, 3.66	6.01	3.67, 3.88 (J = 15.6)	7.64–8.22 (m, 4H)	—
5b	41	248–250	1675	2.68, 3.53	5.9	3.62, 3.87 (J = 15.7)	7.59–8.16 (m, 4H)	—
6a	45	110–115	1665	2.64, 3.91	6.13	3.61, 3.70 (J = 15.3)	6.43–7.08 (m, 3H)	3.78, 3.81 (2s, 6H, OCH_3)
6b	47	166–170	1680	2.78, 3.81	5.94	3.60, 3.70 (J = 15.4)	6.45–7.07 (m, 3H)	3.82, 3.83 (2s, 6H, OCH_3)
7a	49	177–180	1675	2.70, 4.01	6.2	3.64[g]	6.71–6.84 (m, 3H)	3.75, 3.82 (2s, 6H, OCH_3)
7b	48	178–182	1688	2.83, 3.87	5.99	3.61, 3.71 (J = 15.3)	6.70–6.85 (m, 3H)	3.77, 3.82 (2s, 6H, OCH_3)
8a	49	203–208	1658	2.50, 3.93	5.89	3.66, 3.73 (J = 15.8)	6.88–7.11 (m, 3H)	3.88 (s, 3H, OCH_3)
8b	46	175–179	1670	2.73, 3.48	5.44	3.55, 3.64 (J = 15.6)	6.79–7.01 (m, 3H)	3.81 (s, 3H, OCH_3)
9a	60	198–202	1674	2.65, 3.82	6.63	3.73, 3.78 (J = 15.3)	7.20–7.38 (m, 3H)	—
9b	33	214–217	1685	2.66, 3.82	6.63	3.75, 3.86 (J = 15.4)	7.21–7.83 (m, 3H)	—
10a	47	150–155	1673	2.73, 4.01	6.56	3.71, 3.88 (J = 15.1)	6.97–7.33 (m, 3H)	—
10b	48	195–199	1690	2.86, 3.81	6.24	3.60, 3.65 (J = 15.0)	6.97–7.33 (m, 3H)	—

Analyses were ±0.4% (CHN) compared with theoretical values.
[a]Benzene was the crystallization solvent for **2–5** compounds; EtOH for **6–10**.
[b]Expressed in cm^{-1}, Nujol mull.
[c](δ) $CDCl_3$ solution except for **5a**, **5b** (DMSO-d_6 solution); J values are expressed in hertz.
[d]The ethylene protons resonate as an AA′XX′ systems.
[e]2-H, 2′-H resonate as a singlet.
[f]5-CH_2, 5′-CH_2 resonate as an AB system.
[g]Unresolved system.

nitro group in pairs **4** and **5**, respectively (Fig. 1). Each new analogue was screened both as racemate (**a**) and *meso*form (**b**).

These bisthiazolidinones have been obtained, as previously reported, by the reaction of mercaptoacetic acid with *N*,*N*′-dibenzylidenethylendiamines.[1,4] After alkaline workup, the crude mixture of **a** and **b** isomers was separated by silica gel column chromatography (diethyl ether/light petroleum ether 40–60° in variable ratio mixtures). In general *meso* isomers eluted more slowly than corresponding racemates.[1,4]

Table 1 collects physical-chemical, analytical and spectroscopic IR and ^{1}H NMR data of **2–10** new diastereoisomeric pairs. Also in this series enantiomeric pairs **a** are obtained in higher yields than isomers **b** and display lower melting points and wider solubility in common organic solvents than the corresponding *meso* forms. HPLC on Chiralcel OD stationary phase has corroborated the assignment; moreover, the racemates display good separation factors and the *meso* forms generally have intermediate elution times.[5]

Structures assigned to synthesized compounds are in close accordance with elemental analyses and IR, ^{1}H NMR results (Table 1).

Diagnostic peculiarities in the ^{1}H NMR spectra of 3,3′-(1,2-ethanediyl)-bis[2-aryl-4-thiazolidinones] exist, namely (i) the isochronism of the corresponding protons in the heterocyclic rings, (ii) the 2,2′-CH resonance of 2*R*,2′*R*/2*S*,2′*S* isomers at higher frequencies than those of *meso* isomers ($\Delta\delta \cong 0.35$ ppm in $CDCl_3$) and (iii) the symmetrical AA′XX′ systems due to the heterotopic geminal protons of ethylenediamine central chain.[3,4,6]

To evaluate the antiinflammatory and analgesic activities, tested compounds suspended in 10% arabic gum in a volume of 0.5 mL/100 g b.w. were administered orally to rats[7] by gavage at an initial dose of 100 mg/kg, whereas those found active were also administered at 50 and 25 mg/kg b.w. Male Wistar rats (180–200 g) divided into groups of five animals each, were used. The response from the compound-treated animals was compared with that of reference drugs, indomethacin (INDO) (5 mg/kg) and phenylbutazone (PBZ) (50 mg/kg), both orally administered. Detailed procedures have been previously described[3] relative to the following animal models: carrageenin-induced paw edema test,[8] acetic acid writhing test,[9] hot plate test,[10] gastric damage evaluation[11] and acute toxicity (LD_{50}) on mice.[12] Cytotoxic activity was determined following the modified Meyer method based on immobilization of brine shrimp (*Artemia salina*) larvae.[13–15]

In line with our previous findings, these pairs of bisthiazolidinones also exhibited very low toxicity in vitro and in vivo. In fact, LC_{50} values always superior to 1000 μg/mL were found in the *A. salina* assay and LD_{50}, determined in mice, po, was usually higher than 1000 mg/kg. It is worth remembering that indomethacin and phenylbutazone, similarly tested, showed LD_{50} = 15 and 300 mg/kg, respectively.[16]

Antiinflammatory, analgesic and ulcerogenic evaluations results are collected in Tables 2 and 3, relatively to positive results only. Diastereomeric pairs **1** and **11**, previously assayed at 50 mg/kg dose,[4] have been retested at 100 and 25 mg/kg doses to study the dose-dependent effect.

From Table 2, it is evident that lead compound **1b** has the most significant dose-dependent activity over the 3 h. The *rac* **1a**, at 100 mg/kg, displays higher potency, 3 h

after the injection of the irritant. In 3-OCH_3 bisthiazolidinones, *meso*form **2b** displays a dose-dependent effect at the first hour (−65.30 at 100, −38.29% at 50 mg/kg) while *rac* **2a** displays significant activity only at 60 min and at high dose. On the contrary in compounds **3** that maintain the 4-OCH_3 substituent on aryl groups, high dose-dependent potency is displayed 120 min and 180 min after carrageenin injection. Indeed, at 100 mg/kg **3b** reaches almost complete edema inhibition. At such a dose corresponding *rac* **3a** maintains over 66% of the activity of the *meso* isomer, at the third hour. It seems that the 3- or 4-OCH_3 group is alternatively able to enhance activity either at the first hour or at the second and third hour. Such findings seemingly suggest that the 3-OCH_3 substituted compounds could interact with the first inflammatory mediators (such as histamine, prostaglandins generated by COX-1), whereas the 4-OCH_3 substituted ones could be involved in the inhibition of later mediators (such as prostaglandins produced by COX-2).

Thus, in order to verify whether influence was exerted by the nature of the substituent on benzene rings, we have explored other monosubstituted compounds (**4**, **5**). At 100 mg/kg, the presence of the electron-releasing methyl group (**4b**) provoked 55.81% inhibition only at the third hour, while *rac* **4a** was practically inactive. The presence of the nitro group on the contrary is greatly

Table 2. Carrageenin-induced paw edema test and incidence of gastrointestinal lesions in rats

Compd	Dose (mg/kg; po)	% Edema variation (X±SE)			% Animal with	
		60 min	120 min	180 min	Hyperemia	Ulcers
1a	100	−2.13±1.00	−30.13*±1.54	−76.62***±1.90	20	0
1b	100	−58.28**±1.12	−62.33***±1.71	−58.01**±0.20	20	0
	50	−53.62**±1.15	−59.49**±1.44	−44.26**±1.10	20	0
	25	−38.33**±1.20	−53.28**±1.28	−40.09**±1.35	20	0
2a	100	−34.40**±0.12	−26.3±0.09	−18.31±0.10	20	0
2b	100	−65.30***±0.08	−12.7±0.10	20.1±0.12	20	0
	50	−38.29**±1.10	−8.15±1.30	29.59±2.00	20	0
3a	100	39.52±1.15	−26.62±1.12	−64.98***±1.18	20	0
3b	100	20.23±0.13	−75.90***±1.83	−96.42***±1.00	40	0
	50	14.08±1.25	−49.00**±1.61	−66.97***±1.60	20	0
4a	100	43.41±0.18	−0.13±1.40	−23.6±1.03	20	0
4b	100	25.6±0.80	−13.05±1.27	−55.81**±1.09	80	20
5a	100	−35.52**±0.10	−0.31±0.11	23.43±0.90	40	0
5b	100	−83.30***±1.11	−67.30***±0.10	21.11±1.00	40	0
7a	100	33.55±1.83	−24.62±1.02	−70.22***±1.31	40	0
7b	100	−12.48±1.01	−13.51±1.08	−39.06**±1.10	40	0
8a	100	8.6±1.90	−23.83±1.21	−38.94**±1.15	20	0
8b	100	22.13±0.80	25.12±1.24	30.11±1.04	20	40
11a	100	14.04±1.00	−31.41*±1.27	−27.48±1.00	40	0
11b	100	−61.12***±0.90	−61.10***±1.15	−61.20***±0.09	40	0
INDO	5	−15.4±0.09	−10.85±0.18	−50.86**±0.05	100	80
PBZ	50	−20.27±0.14	−5.25±0.08	−39.02**±0.18	100	80

Only active compounds have been reported.
*$p<0.05$; **$p<0.01$; ***$p<0.001$ compared to controls. Student's t test.

Table 3. Acetic acid writhing and hot plate tests in rats (100 mg/kg; po)

Compd	No writhings (X±SE)	% Inhibition	Reaction time (s) X±SE		
			60 min	120 min	180 min
Controls	20.5±1.8	—	11.2±2.0	12.0±1.8	11.3±2.0
1a[a]	9.6±1.8	53**	30.4**±1.5	26.7**±1.8	16.8*±1.9
1b	10.0±2.0	51**	26.6**±1.1	26.8**±2.1	36.4***±2.0
2a	19.5±1.9	4	13.4±2.1	13.0±2.6	12.9±2.0
2b	8.1±1.7	60**	12.1±1.4	12.0±1.9	10.7±2.0
3a	15.8±1.7	22*	11.2±1.5	15.1±1.7	13.9±1.9
3b	11.3±1.5	44*	17.0*±1.5	15.6±1.6	15.0±2.2
4b	7.3±1.3	64**	18.4*±1.3	11.1±1.8	11.0±1.9
5b	17.8±0.9	13	17.1*±1.9	19.4*±2.3	22.0*±2.2
8a	16.4±2.0	20	13.4±2.5	16.8*±1.9	13.5±1.3
8b	10.2±1.3	50**	16.1*±1.3	12.3±1.6	12.5±1.8
11a[a]	15.2±0.8	24*	20.0*±1.2	18.4*±1.7	19.8*±1.2
11b	4.6±1.5	77**	18.7*±1.0	22.5*±2.2	22.9*±1.9
INDO[b]	4.0±0.3	80**	26.1**±1.3	27.1**±1.4	28.0**±2.2
PBZ[a]	3.6±0.5	82**	28.3**±1.8	30.0**±2.0	31.7**±2.5

Only active compounds have been reported.
*$p<0.05$; **$p<0.01$; ***$p<0.001$ compared to controls. Student's t test.
[a]50 mg/kg po.
[b]5 mg/kg po.

beneficial in that higher levels of inhibition were shown in initial stages for **5a** and even more for **5b** that reached 83.30% of inhibition at the first hour.

Among other phenyl disubstituted compounds, only 2,5-dimethoxy substituted **7a**, **7b** inhibited edema, but only 2 h after carrageenin injection. On the contrary, in 3-F,4-OCH_3 substituted bisthiazolidinones **8** activity, unexpectedly, is lost. Over the 3 h, dichloro substituted derivative **11b**[4] shows an antiinflammatory effect similar to that of lead compound, even if limited to the highest dose. Corresponding *rac* **11a** displays half the potency of the *meso*form only at the second hour after administration of the irritant. Finally compounds **6**, **9** and **10**, all bearing *ortho* substituents, whatever their electronic nature, gave very disappointing results (data not reported).

It is worth noting that all tested compounds produce gastric damage lower than the reference drugs; with the exception of **4b** and **8b**, they have no ulcerogenic effect.

Moreover, bisthiazolidinones **1**–**11** have been assayed orally at 100 mg/kg for analgesic properties but the responses from the two tests are not always homogenous. Table 3 collects the most significant results. Once again, the 3,4-dimethoxy derivatives **1a** and **1b** exhibited the highest activity in both tests, followed by 3,4-dichloro analogues **11a** and **11b**, the latter being less potent in hot plate test. *Meso* isomers bearing electron-releasing groups (**2b**, **3b**, **4b** and **8b**) were able to reduce to about 50% the number of writhings with respect to controls. Responses relative to racemates especially in the hot plate test are difficult to be related to each other.

In general, it must be taken into account that the interpretation of the pharmacological profiles of 2*R*,2′*S*-*meso* isomers is relatively straightforward, whereas that of *rac* 2*R*,2′*R*/2*S*,2′*S*, because of the different contributions from *RR* and *SS* enantiomers, is not.

Our ongoing research will be addressed to the study of the mechanism of action of the most potent compounds **1b**, **2b**, **3b** starting from the assessment of COX-1/COX-2 selectivity by in vitro assays. Hopefully, the knowledge of the structure of ovine COX-1[17] and murine COX-2[18] active sites, by means of modeling methods, will help us to elucidate the observed diastereoselectivity of action and to better explain the influence of phenyl substitution pattern on pharmacological profile. These results will be reported in the near future.

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In this assay appropriate amounts of compounds (10, 100 and 1000 μg/mL) were assayed: LC_{50} is the minimum concentration expressed in μg/mL which causes 50% of the larvae to lose mobility. Finney's statistical method of Probit analysis[15] was used to calculate the concentration of the drug that kill 50% of the brine shrimp within the 24 h exposure period, that is the LC_{50} of the drug with 95% confidence intervals.
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