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Original article

3,3'-Di(1,3-thiazolidine-4-one) system. V. Synthesis and pharmacological properties of 3,3'(1,2-ethanediyl)bis-(2-heteroaryl-1,3-thiazolidine-4-one) derivatives

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Summary — A class of anti-inflammatory, analgesic and histamine H_1- and H_2-receptor antagonists the 2,2'-diheteroarylbisthiazolidinones and their 1,1'-disulfones, obtained as [RR, SS] and [RS, SR] isomers, is described. The heteroaryl substitution at 2 and 2' carbons generally improves the pharmacological activities with respect to those of the previously studied 2,2'-diaryl analogues. In particular the 2,2'-dithienyl derivatives exhibit analgesic properties and, as [RS, SR] isomers **6b**, **11b**, **12b** H_2-histamine receptor antagonism as well. The most effective acute anti-inflammatory agents appear to be the 2,2'-di(3-pyridyl) compounds **8a**, **8b**, **14a**, **14b** which also display analgesic activity. Moreover, an H_1-histamine receptor antagonism is almost selectively exerted by the 2,2'–di(2-pyridyl) derivatives **7a**, **7b**, **13a**, **13b**. The relationships between the assessed activities and the chirality and/or the sulfur oxidation state of the molecules are discussed. The anti-cancer potential was also evaluated against P 388 murine lymphocytic leukemia; however, the results were not significant.

Résumé — Système 3,3'-di(1,3-thiazolidin-4-one). V. Synthèse et propriétés pharmacologiques de dérivés 3,3'(1,2-ethanediyl)bis(2-hétéroaryl-1,3-thiazolidin-4-one). *Une classe d'agents anti-inflammatoires, analgésiques et antagonistes des récepteurs H_1 et H_2 de l'histamine, les (1,2-éthanediyl)-3,3' bis 2-(hétéroaryl-1,3-thiazolidin-4-one), obtenues sous forme d'isomères de configuration (RR, SS) et (RS, SR) est décrite. La substitution par des groupes hétéroaromatiques dans les positions chirales 2 et 2' améliore généralement toutes les activités pharmacologiques par rapport à celles des bisthiazolidinones 2,2'-diaryl substituées précédemment étudiées. Tous les dérivés du thiophène en particulier montrent une activité analgésique et sous forme d'isomères (RS, SR) **6b**, **11b**, **12b** une activité antagoniste du récepteur H_2 de l'histamine. Parmi les composés essayés, les plus actifs comme agents anti-inflammatoires sont les dérivés 3-pyridyl substitués **8a**, **8b**, **14a**, **14b** qui manifestent aussi une activité analgésique. Les 2-pyridyl bisthiazolidinones **7a**, **7b**, **13a**, **13b** présentent en outre une activité sélective d'antagonistes du récepteur H_1 de l'histamine. Ces observations sont discutées en relation avec la chiralité et/ou l'état d'oxydation des atomes de soufre des molécules. Enfin, l'activité vis-à-vis de la leucémie lymphoïde P 388 de la souris a été recherchée; les dérivés étudiés n'ont manifesté aucun effet significatif vis-à-vis de la leucémie lymphoïde P 388 de la souris.*

3,3' (1,2-ethanediyl)bis(2-heteroaryl-1,3-thiazolidine-4-one) compounds / 1,1'-disulfones / synthesis / configuration isomers / anti-inflammatory agents / analgesics / H_1 and H_2 histaminic antagonists / anti-leukemic screening / SARs

Introduction

In accordance with our laboratories' program directed towards the synthesis of new bithiazolidinones with potential biological applications, several series of these compounds have been prepared and screened for anti-inflammatory and related activities [1, 2, 3]. The starting point of this research was the 2,2'-diaryl-bithiazolidinones obtained as RS and RR/SS isomers, only the former, however, being endowed with anti-inflammatory/analgesic/antipyretic action when 2-, 3-chloro and 2-, 3-fluorophenyl substituted (fig 1).

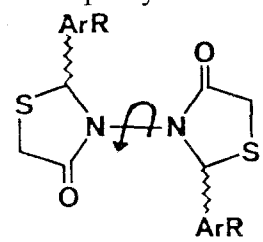

Fig 1.

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With the aim of optimizing the pharmacological properties and investigating the structure–activity relationships, an investigation was developed in a 2-fold direction: a) the replacement of 2 and 2' phenyls with different groups such as alkyl, cycloalkyl and furyl: this last derivative showed improved anti-inflammatory–analgesic profiles and an unexpected anti-histaminic activity [2]; b) the insertion of an ethylene chain between nitrogens: the 3,3'(1,2-ethanediyl) bisthiazolidinones (fig 2; X = S) displayed anti-histaminic effects which were more significant than the anti-inflammatory properties, especially when fluorophenyl-substituted [2], the antipyretic and analgesic properties also being improved.

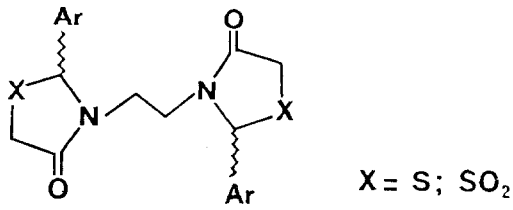


Fig 2.

Interestingly, the subsequent oxidation of sulphur atoms (fig 2, X = SO_2) again modified bioactivity towards the anti-inflammatory property [3].

The SARs outlined prompted us to continue our investigation by exploring the 3,3' (1,2-ethanediyl)bis (2-heteroaryl-1,3-thiazolidinone) derivatives and their disulfones. The heterocycles employed included 2-furyl, 2-thienyl, 2- and 3-pyridyl rings and some of their methyl substituted derivatives.

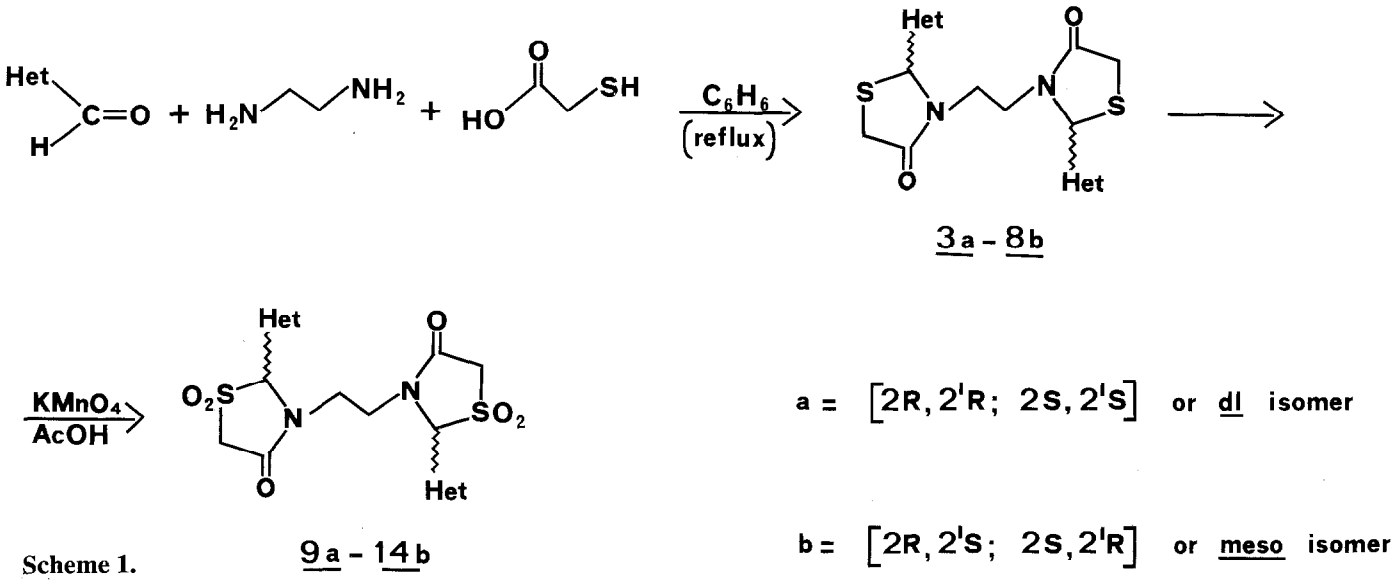
This report describes the synthesis and the preliminary screening of the above-mentioned compounds as anti-inflammatory–analgesic and anti-histaminic agents.

Furthermore, since some monocyclic 2-heteroarylsubstituted-4-thiazolidinones investigated by other authors from our department had shown significant antitumor activity [4], we also thought it interesting to submit our bicyclic analogues to the anti-leukemic screening on P 388 lymphocytic murine leukemia, in agreement with the NCI of Bethesda, MD, USA.

Chemistry

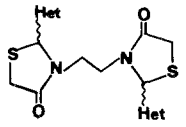
The synthesis of the 3,3' (1,2-ethanediyl)bis (2-heteroaryl-1,3-thiazolidine-4-one) compounds **3–8** has been performed by reacting ethylenediamine with appropriate heteroaromatic aldehydes and mercapto-acetic acid in refluxing dry benzene (scheme 1). The yields of such compounds (table I) are generally not satisfactory; however, no attempt was made to increase them by using the isolated intermediate bis-azomethines, since our main interest was directed towards the SAR investigation.

The subsequent oxidation to the 1,1'-disulfones **9–14** was carried out by means of $KMnO_4$ in glacial acetic acid (table II). When we are dealing with furylderivatives, the synthetic and oxidative reactions occur with remarkable decomposition (see *Experimental protocols*).



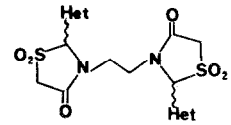
Scheme 1.

Table I. 3,3' (1,2-ethanediyl) bis [2-heteroaryl-1,3-thiazolidine-4-one] compounds. [a]Recryst solv: EtOH except for compounds **3b** and **5b** (DMSO).



Comp.	Het	2,2' carbons chirality	M.p. °C[a)]	Yield %	Formula
3 a	2-furyl	RR,SS	148-50	23	$C_{16}H_{16}N_2O_4S_2$
3 b	"	RS,SR	216-19	15	" "
4 a	5-Me-2-furyl	RR,SS	125-28	25	$C_{18}H_{20}N_2O_4S_2$
4 b	"	RS,RS	193-96	18	" "
5 a	2-thienyl	RR,SS	126-28	35	$C_{16}H_{16}N_2O_2S_4$
5 b	"	RS,SR	232-35	15	" "
6 a	5-Me-2-thienyl	RR,SS	128-30	25	$C_{18}H_{20}N_2O_2S_4$
6 b	"	RS,SR	193-95	15	" "
7 a	2-pyridyl	RR,SS	183-85	55	$C_{18}H_{20}N_4O_2S_2$
7 b	"	RS,SR	196-99	35	" "
8 a	3-pyridyl	RR,SS	142-45	20	$C_{18}H_{20}N_4O_2S_2$
8 b	"	RS,SR	203-05	30	" "

Table II. 3,3' (1,2-ethanediyl) bis [2-heteroaryl-1,3-thiazolidine-4-one 1,1-dioxide] compounds. [a]**10b** was obtained in very poor yield and in mixture with decomposition products. [b]Recryst solv: DMSO/EtOH except for compounds **9a** and **10a** (EtOH).



Comp. [a)]	Het	2,2' carbons configuration	M.p. °C[b)]	Yield %	Formula
9 a	2 furyl	RR,SS	180-83	20	$C_{16}H_{16}N_2O_8S_2$
9 b	"	RS,SR	195-98	30	" "
10 a	5-Me-2-furyl	RR,SS	183-85	15	$C_{18}H_{20}N_2O_8S_2$
11 a	2-thienyl	RR,SS	197-99	60	$C_{16}H_{16}N_2O_6S_4$
11 b	"	RS,SR	210-13	50	" "
12 a	5-Me-2-thienyl	RR,SS	189-92	50	$C_{18}H_{20}N_2O_6S_4$
12 b	"	RS,SR	198-200	60	" "
13 a	2-pyridyl	RR,SS	242-43	70	$C_{18}H_{20}N_4O_6S_2$
13 b	"	RS,SR	246-49 (dec.)	75	" "
14 a	3-pyridyl	RR,SS	242-43	75	" "
14 b	"	RS,SR	256-59	70	" "

The presence of two chiral centres in the 2 and 2' positions allows these compounds to be obtained in racemic (2R, 2'R; 2S, 2'S) and *meso* (2R, 2'S; 2S, 2'R) forms* which have different solubilities, melting points and IR and ^{1}H NMR spectroscopic features (table III).

The assigned structures of all compounds are in close accordance with analytical and spectral data.

Hereafter, for greater convenience, the (2R, 2'R; 2S, 2'S) isomers will be indicated by "a" and the (2R, 2'S; 2S, 2'R) isomers by "b".

Pharmacology

The pharmacological activities of the bisthiazolidinones under investigation were evaluated by *in vivo* testing. In particular, the carrageenin rat foot edema test was used to assess the acute anti-inflammatory activity by oral administration of compounds at 50 mg/kg in comparison with indomethacin (5 mg/kg) and phenylbutazone (5 and 50 mg/kg). The % inhibition of edema was determined 60, 120 and 180 min after the injection of the irritant (table IV) with the aim of shedding some light on the inflammatory mediators inhibited, if that was the case [7].

The analgesic properties were evaluated by the acetic acid writhing and hot plate tests performed on rats (table V); the last assay, when carried out at 50 ± 0.5°C, could be taken as indicative of the analgesic properties of NSAIDs [8].

Subacute gastric damage was evaluated by administering repeated therapeutic doses for 4 consecutive days to rats fasted for 15 h (table IV). Indomethacin and phenylbutazone, tested in comparison, were found to be extremely irritant, although administered at 5 mg/kg.

Furthermore, the simple technique of Owen and Pipkin was used to simultaneously assess *in vivo* the antagonist activity at histamine H_1- and H_2-receptors in anesthetized guinea pigs [9].

The principle of this technique is based on the presence of histamine H_1- receptors in respiratory smooth muscle and H_2- receptors in the right atrium of guinea pigs. Thus the intravenous injection of

*The 2 and 2' C atom chirality of analogous compounds reported in [2] and [3] was determined according to data provided by Farina *et al* for configurational isomers of 3,3' (1,2-ethanediyl) bis [2-phenyl-1,3-thiazolidine-4-one] [5]. However, in the course of a fuller ^{1}H NMR conformational analysis, performed by using chiral LSR and the Laocoon III computer program and variable temperature procedures, we found that the assumed chirality in those papers was contrary to what was subsequently determined in the present study. Therefore, in the present note the corrected C atom chirality is used, also confirmed by recent X-ray analysis [6].

20 μg/kg of histamine simultaneously causes bronchoconstriction and tachycardia antagonized selectively by the reference drugs, mepyramine and cimetidine orally administered 60 min after histamine treatment. The antagonist effects of the compounds are expressed both as % increase in the ventilation pressure and heart rate with respect to basal values and % inhibition with respect to histamine (table VI).

Finally, the anti-cancer screening was performed against P 388 lymphocytic leukemia in mice, under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The anti-tumor activity is expressed in terms of % T/C, where T is the median survival time of treated animals and C that of controls (table VII): none of the tested bisthiazolidinones, however, was found to be active since the % T/C was always less than ≥ 127.

Table III. IR and ^{1}H NMR data.

Compound	IR (cm^{-1})[a]		NMR δ (ppm)[b]			
	ν C = O	ν SO_2	CH_2 - CH_2[c]	5-CH_2, 5'-CH_2	2-H, 2'-H[d]	Other protons
3a	1680		2.74, 3.97	3.61, 3.79[e]	5.94	{6.40 (m,4H);7.41 (d,2H)} furyl-H
3b	1660		2.76, 3.53	3.65 (br.s)	5.87	{6.45 (m,4H);7.63 (unr.d,2H)} furyl-H
4a	1680, 1652		2.75, 3.79	3.59, 3.79[e]	5.87	2.27 (s,6H,CH_3); {5.92[f] (2H); 6.30 (d,2H)} furyl-H
4b	1661		2.96, 3.69	3.56, 3.79[e]	5.60	2.29 (s,6H,CH_3); {5.92 (br.d,2H);6.30 (d,2H)} furyl-H
5a	1680, 1650		2.67, 3.97	3.68 (br.s)	6.26	6.80 - 7.53 (m,6H,thiophene-H)
5b	1665		2.76, 3.53	3.66 (br.s)	6.06	{7.10 (m,4H); 7.57 (br.d,2H)} thiophene-H
6a	1687, 1670		2.72, 3.97	3.66 (br.s)	6.16	2.45 (s,6H,CH_3); {6.57 (d d,2H); 6.96 (d,2H)} thiophene-H
6b	1680, 1670		2.98, 3.66	3.63 (br.s)	5.77	2.45 (s,6H,CH_3); {6.55 (d d,2H); 6.88 (d,2H)} thiophene-H
7a	1685, 1673		2.76, 3.99	3.64, 3.87[e]	5.90	{7.23 (m,4H); 7.70 (m,2H); 8.55 (d,2H)} pyridine-H
7b	1660		2.90, 3.81	3.58, 3.84[e]	5.68	{7.26 (m,4H); 7.71 (m,2H); 8.53 (d,2H)} pyridine-H
8a	1675, 1668		2.49, 4.01	3.73 (br.s)	5.98	{7.26 (m,2H); 7.67 (m,2H); 8.58 (m,4H)} pyridine-H
8b	1682, 1670		2.92, 3.54	3.73 (br.s)	5.69	{7.33 (m,2H); 7.75 (m,2H); 8.56 (m,4H)} pyridine-H
9a	1710	1325, 1140, 1128	3.03, 3.76	4.31 (s)	6.20	{6.58 (m,2H); 6.82 (br.d,2H); 7.83 (unr.peak,2H)} furyl-H
9b	1680	1345, 1138	2.96, 3.90	4.32 (br.s)	6.27	{6.60 (m,2H); 6.80 (br.d,2H); 7.85 (unr.peak,2H)} furyl-H
10a	1715 - 1690	1330, 1145, 1130	3.15, 3.75	4.28 (s)	6.10	2.30 (s,6H,CH_3); {6.19[f] (d,2H); 6.68 (d,2H)} furyl-H
11a	1710, 1693	1332, 1140, 1118	2.93, 3.83	4.39 (s)	6.35	{7.22 (m,4H); 7.80 (br.d,2H)} thiophene-H
11b	1680	1338, 1135	2.98, 3.95	4.30 (br.s)	6.37	{7.20 (m,4H); 7.75 (d d,2H)} thiophene-H
12a	1700 - 1675	1330, 1145	2.96, 3.85	4.36 (s)	6.24	2.49 (s,6H,CH_3); {6.84 (d d,2H); 7.13 (d,2H)} thiophene-H
12b	1678	1338, 1140	2.98, 3.89	4.26 (br.s)	6.25	2.47 (s,6H,CH_3); {6.81 (d d,2H); 7.10 (d,2H)} thiophene-H
13a	1695	1330, 1145, 1125	3.10, 3.76	4.18 (s)	6.20	{7.30 - 8.10 (m,6H); 8.64 (d,2H)} pyridine-H
13b	1680	1340, 1135	2.88, 3.94	4.15 (s)	6.20	{7.30 - 8.10 (m,6H); 8.43 (d,2H)} pyridine-H
14a	1705	1340, 1120	2.95, 3.93	4.42 (br.s)	6.15	{7.33 - 8.00 (m,4H); 8.56 (m,4H)} pyridine-H
14b	1710 - 1698	1335, 1150	2.91, 3.98	4.28, 4.48[e]	6.14	{7.30 - 8.00 (m,4H); 8.50 (m,4H)} pyridine-H

[a]The ν CO and ν SO_2 absorptions are generally very intense with shoulders and subpeaks: the reported values refer to the main peaks. [b]The bisthiazolidinones were recorded in $CDCl_3$ except for **3b** and **5b**, which as well as all 1,1'-disulfones were registered in DMSO-d_6. [c]The ethylene protons resonate as AA'BB' systems except in unoxidized **a** compounds where approximate AA'XX' systems; these multiplets are sometimes partially masked from 5-CH_2, 5'-CH_2 or DMSO-d_6 signals. [d]Singlet or broad singlet. [e]AB system. [f]Signal partially masked from 2-H, 2'-H peak.

Table IV. Anti-inflammatory activity: carrageenin-induced paw edema and gastric damage in rats (50 mg/kg). °Administered at a 5 mg/kg dose. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to controls; Student's t-test.

Compounds	% volume edema variation			% rats with hyperaemia	% rats with ulcers
	60'	120'	180'		
Indomethacin°	- 15.14	- 10.85	- 50.86*	0/6	5/6
Phenylbutazone°	- 10.15	- 8.10	- 32.24*	5/6	4/6
Phenylbutazone	- 20.27	- 5.25	- 39.02*	6/6	5/6
3 a	18.92	6.33	15.12	5/6	0/6
3 b	- 61.06**	- 45.51**	- 37.27*	5/6	0/6
4 a	- 20.03	- 44.59*	0.03	6/6	1/6
4 b	58.65	35.45	8.35	5/6	0/6
5 a	- 25.15*	- 69.17**	- 51.20**	5/6	2/6
5 b	- 25.75*	- 5.44	2.01	2/6	0/6
6 a	- 3.52	58.99	72.02	2/6	0/6
6 b	44.53	56.72	5.02	2/6	0/6
7 a	- 7.92	- 45.00**	- 45.89**	6/6	0/6
7 b	24.24	54.53	49.19	1/6	0/6
8 a	- 5.35	- 41.26*	- 42.65*	5/6	1/6
8 b	- 38.44*	- 64.88**	- 70.46***	5/6	0/6
9 a	41.79	16.96	0.82	5/6	0/6
9 b	- 67.27**	- 55.37**	- 60.15**	4/6	0/6
11 a	- 36.49*	- 20.65	- 19.39	5/6	1/6
11 b	85.47	30.06	20.74	2/6	0/6
12 a	- 28.31*	- 23.05	- 16.69	0/6	0/6
12 b	- 68.81**	- 34.69*	- 30.43	4/6	2/6
13 a	- 1.22	- 41.86*	- 53.22**	6/6	0/6
13 b	- 48.87*	- 71.47**	- 71.49***	6/6	1/6
14 a	- 28.92*	- 56.93**	- 66.01***	4/6	0/6
14 b	- 25.76*	- 45.83**	- 51.35**	5/6	0/6

Results and Discussion

The bisthiazolidinones **3a–8b** and their 1,1' disulfones **9a–14b** were first explored as acute anti-inflammatory agents by means of the "classical" carrageenin rat foot oedema test: 60% of them displayed anti-edematous effects, all with acute approximate toxicity > 1000 mg/kg.

Encouraged by these results, we proceeded by assaying their peripheral analgesic properties and the gastric irritancy potential. Furthermore, the promising anti-histaminic properties displayed by 3,3'-di-(2-furyl-1,3-thiazolidine-4-one) previously reported [2], prompted us to submit the new heteroarylbisthiazolidinones to an evaluation of their histamine H_1- and H_2-receptor antagonist potential.

The pharmacological screening was planned so that a comparison could be made with the previously investigated 2,2'-diarylsubstituted derivatives, some pharmacological properties of which, *ie*, the acute anti-inflammatory and the anti-histaminic, have been found to be linked to the chirality and/or the oxidation state of the molecules.

Therefore in the present study the simultaneous pharmacological examination of the (RR, SS) and (RS, SR) isomers both as disulfides and disulfones, 2

Table V. Analgesic activity: acetic acid writhing test and hot plate test in rats (50 mg/kg). °Administered at a 5 mg/kg dose *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ Student's t-test.

Compound	N.of writhings (M ± SE)	% inhibition	Reaction time	
			1 h	3 h
Controls	28.6 ± 2.0		9.34 ± 0.6	9.54 ± 0.5
Indomethacin°	4.0 ± 0.3	86.01***	26.10*** ± 1.3	28.07*** ± 2.2
Phenylbutazone°	7.2 ± 0.5	74.82***	24.21*** ± 1.5	27.01*** ± 2.1
Phenylbutazone	3.6 ± 0.5	87.41***	28.3*** ± 1.8	31.7*** ± 2.5
3 a	20.6 ± 0.3	27.97*	12.46* ± 1.0	7.48 ± 2.4
3 b	2.4 ± 0.8	91.60***	13.00* ± 1.0	12.90* ± 1.3
4 a	18.4 ± 2.6	35.66*	15.22** ± 1.5	16.70** ± 1.3
4 b	24.4 ± 4.5	14.68	10.76 ± 1.1	9.22 ± 0.3
5 a	23.2 ± 2.4	18.88	10.64 ± 1.1	12.20 ± 1.8
5 b	13.4 ± 3.4	53.14*	10.28 ± 1.5	13.14** ± 0.5
6 a	7.4 ± 0.4	74.12***	17.86** ± 2.0	14.96** ± 0.8
6 b	10.2 ± 1.9	64.33**	17.28** ± 1.8	14.54** ± 0.9
7 a	32.0 ± 2.0	0	10.82 ± 2.2	8.12 ± 0.4
7 b	17.6 ± 1.2	38.46*	24.42*** ± 1.6	23.46*** ± 1.4
8 a	15.4 ± 1.8	46.15*	12.60* ± 1.1	12.68* ± 1.1
8 b	13.4 ± 2.5	53.14*	11.70* ± 0.7	12.96* ± 1.5
9 a	10.4 ± 0.9	63.63**	12.70* ± 1.2	14.12* ± 1.9
9 b	21.6 ± 2.1	24.47*	10.52 ± 0.1	18.16** ± 1.5
11 a	8.8 ± 1.3	69.23**	17.82** ± 2.2	15.32** ± 1.3
11 b	18.4 ± 2.8	35.66*	19.50** ± 2.5	16.22** ± 1.5
12 a	7.2 ± 0.5	74.82***	28.02*** ± 2.0	24.98*** ± 1.7
12 b	7.8 ± 0.9	72.72**	17.88** ± 1.3	12.94* ± 0.8
13 a	33.2 ± 1.5	0	12.32* ± 1.1	14.42** ± 1.4
13 b	24.2 ± 1.4	13.98	10.54 ± 1.6	11.22 ± 1.9
14 a	15.6 ± 2.4	45.45*	15.20** ± 1.3	13.92** ± 1.1
14 b	14.4 ± 3.3	49.65*	17.36* ± 2.5	19.07** ± 1.4

Table VI. Anti-histaminic activity: simultaneous *in vivo* assay at histamine H_1- and H_2- receptors in guinea pigs (50 mg/kg). °Administered at 20 μg/kg *iv*. ^Administered at 25 mg/kg *per os*. +With respect to basal values. *$P < 0.05$; **$P < 0.001$ with respect to basal values.

Compounds	% increase in ventilation pressure+ (M ± S.E)	% inhinbition with respect to histamine	% increase of sinusal chronotropism+ (M ± S.E)	% inhibition with respect to histamine
Histamine°	48 ± 3.21**		30 ± 2.25**	
Mepyramine^	6 ± 2.15	87.5	29 ± 3.57*	3.3
Cimetidine^	46 ± 1.51**	4.1	5 ± 1.60	83.3
3 a	30 ± 2.95*	37.5	30 ± 3.15**	0
4 a	50 ± 3.81*	0	33 ± 2.45**	0
4 b	49 ± 2.67*	0	10 ± 2.00	66.6
5 a	48 ± 2.70*	0	27 ± 3.88*	10.0
6 a	47 ± 1.20**	2.0	29 ± 2.85*	3.3
6 b	46 ± 2.22**	4.1	9 ± 1.36	70.0
7 a	26 ± 1.49*	45.8	32 ± 2.56*	0
7 b	20 ± 1.50**	58.3	28 ± 3.80*	6.6
9 a	38 ± 2.55*	20.8	30 ± 3.78*	0
9 b	44 ± 3.80*	8.3	17 ± 1.80*	43.3
11 a	48 ± 3.20*	0	28 ± 4.25*	6.6
11 b	42 ± 3.18*	12.5	14 ± 2.30	53.3
12 a	48 ± 2.78*	0	29 ± 2.90*	3.3
12 b	43 ± 2.39*	10,4	12 ± 2.50	60.0
13 a	28 ± 1.75*	41.6	30 ± 3.26*	0
13 b	24 ± 1.32*	50.0	31 ± 3.00*	0

Table VII. P 388 Lymphocytic leukemia antitumor activity in mice.

Comp.	Doses (mg/kg)	T/C %	Comp.	Doses (mg/kg)	T/C %
3 a	400	91	8 a	400	109
	200	98		200	112
	100	100		100	112
3 b	240	101	8 b	240	101
	120	102		120	105
	60	100		60	105
4 a	200	99			
	100	100	11 a	400	90
	50	104		200	93
4 b	240	97		100	94
	120	98	11 b	400	92
	60	98		200	94
5 a	400	TOX		100	97
	200	96	12 a	400	91
	100	100		200	94
5 b	240	95		100	101
	120	97	12 b	400	TOX
	60	102		200	87
6 a	200	100		100	97
	100	109	13 a	400	91
	50	101		200	98
6 b	200	94		100	90
7 a	200	97	13 b	400	89
	100	96		200	92
	50	94		100	100
7 b	400	93	14 a	400	92
	200	102		200	93
	100	97		100	97
			14 b	400	101
				200	97
				100	101

and 2' heteroarylsubstituents being equal, may allow the previously outlined SARs to be emphasized and discussed in detail.

It seems convenient, to first examine the pharmacological results presented in tables IV, V and VI within the limits of each group of heteroarylbisthiazolidinones.

The 2,2'difuryl derivatives generally exhibit significant analgesic properties in all the tests and, as (RR, SS) isomers, also have H_1- anti-histaminic action (**9a** < **3a**); the *meso* analogues, on the contrary, inhibit carrageenin paw edema from the first to the third hour (**3b** < **9b**). The 5-methyl-2-furyl substitution is beneficial only on the analgesic profile of **4a**, **4b** being inactive. The latter compound, however, shows histamine H_2-receptor antagonism, though to a lesser degree than cimetidine (66.6% *versus* 83.3%). Unfortunately, the corresponding 1,1' disulfones were obtained in such a poor yield that we could not assay them.

Isosteric replacement of furyl ring with 2-thienyl nucleus results in a powerful anti-acute inflammatory agent **5a**; much less effective is **5b**, whereas the methylsubstitution as in **6a** and **6b** produces edemigen features. Upon oxidation the anti-inflammatory properties are retained only at the first hour in **11a** and **12a**, **11b** showing on the contrary agonistic action whereas **12b** appears to improve its anti-inflammatory profile.

As regards the analgesic response, the thienyl derivatives, with the sole exception of **5a**, display significant values of protection from pain (table V), in particular the disulfones **12a** and **12b**.

Also of note is the fact that in this group can be found the most active H_2-receptor antagonists and that this activity is clearly related to asymmetric carbon chirality: 3 out of 4 (RS, SR) isomers **6b**, **11b** and **12b** significantly increase sinusal chronotropism.

Unfortunately **5a**, its sulfone **11a** and **12b** are found to induce gastric ulcers in rats, though to a lesser extent than reference drugs (table IV).

Among the 2,2'-di(2-pyridyl)bisthiazolidones, **7a** displays at the 3rd h greater antiedemigen potency than that of phenylbutazone (50 mg/kg), its (RS, SR) isomer **7b** on the contrary showing inflammatory effects. The sulphur oxidation improves the activity in *dl* form **13a** and much more in the *meso* form **13b**, which appears as more potent than reference drugs.

As regards analgesic properties (table V), it is evident that **7b** only shows significant levels of protection from pain in both assays; the sulphur oxidation being beneficial on the *dl* isomer (**13a** in hot plate test) and detrimental on the *meso* isomer **13b**.

It is interesting to note that all 2-pyridyl compounds act as histamine antagonists at their H_1-receptors, the (RS, SR) forms **7b** and **13b** displaying higher bronchospasm inhibition values than the (RR, SS) forms **7a** and **13a**. The last, however, is ulcerogenic in the subacute gastric damage assay.

The best acute anti-inflammatory activity is by far observed among the 2,2'-di(3-pyridyl)bisthiazolidones: their oedema inhibition potency increases from the first to the third hour, indicating that they probably act as inhibitors of postaglandin synthetase [7]. Phenylbutazone, under the same experimental conditions, shows inhibitory potency much lower than that of **8b**, **14a** and **14b**. The sulphur oxidation apparently

raises the *racemic* forms activity (**14a** > **8a**) while it reduces that of *meso* analogues.

In the writhing test, the 3-pyridylderivatives show significant potency, in particular as (RS, SR) isomers; in the hot plate test, the better compounds appear to be the oxidized forms (**14b** > **14a**).

Unfortunately, none of these promising derivatives was assayed as H_1- and H_2-receptor antagonists therefore the picture of their SARs will be incomplete.

Finally no active compounds have been singled out in the anticancer screening (table VII) since none reached the threshold of % T/C > 127. Nevertheless, by comparing under the same conditions the tested bisthiazolidinones, it can be seen that (RR, SS) configurational isomers generally display % T/C values higher than those of *meso* forms (**7a** > **7b**, **8a** > **8b**, **12a** > **12b**, **13a** > **13b**); the sulphur oxidation resulting in decreased ratios of % T/C as in **11a** < **5a**, **12a** < **6a** and **14a** < **8a**.

In conclusion, 3-pyridyl derivatives also in this assay appear to be very interesting molecules in which suitable structural modifications might lead to active compounds.

Conclusion

Taking these observations into account, it can be pointed out that the new 2,2'-diheteroarylbisthiazolidones under study generally possess remarkable peripheral analgesic properties associated with low acute toxicity and good gastric tolerance.

In comparison with the previously investigated 2,2'-diaryl analogues [1–3], the anti-acute inflammatory action is on the whole improved especially when 2- and 3-pyridyl substituents are present on the asymmetric C atoms.

Moreover, the observed relationships between edema inhibition potency and (RS, SR) chirality [1, 10], is again evident in sulfide and sulfone 2,2'-difuryl compounds and in oxidized 5-methyl-2-thienyl ones, as well as in 2-pyridyl (**13b** > **13a**) and in 3-pyridyl derivatives (**8b** > **8a**). On the contrary, the relationship between the sulphur oxidation and the previously observed change from anti-histaminic to anti-inflammatory activity does not emerge here. The pharmacological differences displayed by sulfide and sulfone analogues appear to be mostly determined by the nature of heteroarylsubstituent.

On the other hand, our preliminary anti-histaminic screening has emphasized the same trend. In fact, it appears that the 2-pyridyl compounds selectively interact with the H_1-receptors of guinea pig smooth muscle, whereas the 2-thienyl derivatives and the furyl isoster **9b**, stereoselectively interact with H_2-receptors of guinea pig atrium.

This finding may be rationalized by observing that the former bisthiazolidinones possess the same structural skeleton as mepyramine and other antihistamines, though lacking any cationic centre usually retained as the critical moiety of histamine H1-antagonists, and the latter ones are structurally very close to many classical H_2-antagonists such as cimetidine and its congeners [11].

Obviously these suggestions should be further investigated by more sophisticated test systems as well as by the conformational studies now in progress.

Experimental protocols

Chemical methods

Melting points were taken on a Kofler–Reichert hot-stage apparatus and are uncorrected. Infrared spectra were obtained with a Perkin–Elmer mod 257 instrument as nujol or hexachlorobutadiene mulls. ^{1}H NMR spectra were measured on a Varian T-60A spectrometer using tetramethylsilane as an internal standard. Chemical shifts are expressed in δ units. Elemental analyses were performed using a C Erba mod 1106 elemental analyzer and were in agreement with theoretical values to within ± 0.4%. The purity of compounds was verified by thin-layer chromatography (TLC) which was run on silica gel GF_{254} (Merck). Heterocyclic carboxyaldehydes were those commercially available.

*Synthesis of 3,3' (1,2-ethanediyl) bis (2-heteroaryl-1,3-thiazolidine-4-one) (**3–6**)*

To a stirred solution of the proper heteroaromatic carboxyaldehyde (0.06 mol) in dry benzene (150 ml), ethylenediamine (0.03 mol) and mercaptoacetic acid (0.09 mol) were added and then refluxed for 3 h. During this period the reaction mixtures generally separated an abundant brown residue formed by decomposition products which was removed by filtration but not further analyzed. From the cooled or progressively concentrated benzenoid solution, the **b** compounds at first, then some mixtures of **b** and **a** compounds were precipitated. By evaporating mother liquors an oily residue was obtained which repeatedly rubbed with cold EtOH, mainly yielded **a** isomers.

In the synthesis with 5-methyl-thiophenecarboxyaldehyde the initial benzenoid solution did not allow anything to precipitate. Removal of the solvent on a rotating evaporator left an oily residue from which **6b** at first, then **6a** precipitated. The crude bisthiazolidinones **3–6** thus obtained were purified by repeated crystallization with EtOH or DMSO.

Synthesis of 3,3' (1,2-ethanediyl) bis (2-(2-pyridyl)-1,3-thiazolidine-4-one) isomers 7

With 2-pyridinecarboxyaldehyde, an analog synthetic procedure was used. During the heating under reflux, however, a non miscible liquid was found to separate from the benzenoid solution; the decantation of the solvent and the subsequent dilution of this liquid with water gave solid mixtures of **7a** and **7b** isomers, successively isolated by means of numerous recrystallizations with EtOH. The spontaneous evaporation at room temperature of benzenoid mother liquors as well as the treatment of benzenoid residue afforded similar isomeric mixtures.

Synthesis of 3,3' (1,2-ethanediyl) bis (2(3-pyridyl)1,3-thiazolidine-4-one) isomers 8
With 3-pyridinecarboxyaldehyde also the reaction mixture permitted separation of a nonmiscible liquid which when diluted with water afforded a solid containing crude **8b** while the aqueous mother liquors were submitted to extraction with $CHCl_3$. This organic phase, dried with anhydrous Na_2SO_4 and evaporated *in vacuo* left an oily residue that slowly solidified. Subsequent treatment with cold EtOH furnished small quantities of crude **8a**; additional quantities were obtained from the benzenoid residue, whereas the initial benzenoid solution separated crude **8b**. Since there was an instability of **8a** with respect to warmth and to all organic solvents, the purification was accomplished by means of repeated fast washings with cold EtOH.

General synthetic procedure for 1,1' disulfones **9–14**
An aqueous solution of potassium permanganate (6 eq) was added dropwise to a well-stirred solution of the appropriate 3,3'-bisthiazolidinone in glacial acetic acid. The temperature was kept below 30°C by external cooling. After the addition was complete, a concentrated aqueous sodium bisulfite solution was added to remove the manganese dioxide. The mixture was then diluted with an equal volume of water, or neutralized with diluted NH_4OH: solid products were obtained that were filtered off and purified by crystallization.

The oxidation of furylderivatives, however, proceeds with remarkable decomposition, so the yields of disulfones are very low. A light increase is obtained by adding the permanganate and then the bisulfite solutions as quickly as possible.

Biological methods

In the screening for anti-inflammatory, analgesic, ulcerogenic and anti-histaminic activities all test compounds were administered orally by gavage suspended in 10% gum arabic in a vol of 0.5 ml/100 g of body weight in rats and guinea pigs and 0.2 ml/10 g bw in mice. Male Wistar rats (180–200 g), Swiss mice (20–25 g) and guinea pigs (≈ 500 g) of both sexes, divided into groups of 6 animals each were used.

Carrageenin-induced paw edema in rats
Edema was induced in the right hind paw of each rat by subplantar injection of 0.05 ml of a 1% carrageenin solution, according to the method of Winter *et al* [12]. The test compounds were administered orally 1 h before carrageenin injection. Paw volume was measured with a water plethysmometer (Basile, 7150) prior to irritant injection and 1 h and 3 h later. The percentage of edema inhibition in treated *versus* control animals was calculated.

Acetic acid writhing test in rats
The technique of Niemegeers *et al* [13] was used. Rats were injected *ip* with 0.5 ml of 1% aqueous solution (pH: 2.77 ± 0.11) of acetic acid (HOAc) which induced the characteristic writhing 15 min after HOAc. The rats were treated by oral gavage with the compounds to be investigated. The number of writhes occurring was counted for a 15-min period, starting 60 min after HOAc injection.

Hot plate test in rats
The experimental method of Eddy and Leimback [14] modified by Michael–Titus and Costentin [15] was used. Rats were selected for sensitivity and randomized. The reaction time between the moment when the rat reached the hot plate (50 ± 0.5°C) and that when the animal licked its hind paw was measured 60 and 180 min after drug administration.

Gastric-irritancy test in rats
Subacute gastric damage was evaluated by repeated dosing to rats (6 rats per group). The compounds were given by gavage, in the morning, to animals fasted for 15 h for 4 consecutive days. On the 5th day, the rats were killed; their stomachs were slit and examined macroscopically [16]. The number of rats showing hyperaemia and/or gastric ulcers was determined.

Acute toxicity in mice
This test was carried out on Swiss mice fasted for 18 h, with water provided *ad libitum*. The compounds were given by gavage, using 5 females and 5 males in a single dose of 1000 mg/kg. The mice were kept under observation for 7 days to monitor mortality [17].

H_1- and H_2-anti-histaminic in vivo *activity*
The technique reported by Owen and Pipkin [9] was followed. Guinea pigs, of either sex, about 500 g body weight, were anesthetized with ethyluretane, 1.25 g/kg by *ip* injection. The trachea was cannulated to permit artificial respiration. Catheters were tied into one jugular vein for the administration of histamine in saline (20 μg/kg).

Airway resistance was measured using the method of Collier *et al* [18], with some modifications. By means of a respiration pump, the lungs were inflated at a rate of 50 strokes/min (10 cc/stroke). Airway resistance in the guinea pig-pump circuit was measured by a bronchospasm transducer (V Basile); resistance was proportional to the maximum pressure required to inflate the lungs. In–flow pressure was registered on electronic recorder.

Heart rate was simultaneously measured by recording the ECG (Battaglia–Rangoni apparatus, Mod Simplex ar).

Bronchospasm and tachycardia were induced by *iv* injection of histamine·2 HCl in saline (20 μg/kg) 60 min after the oral administration of the compound to be investigated (50 mg/kg) and of the reference drugs cimetidine·HCl (25 mg/kg) and mepyramine maleate (25 mg/kg).

Anti-cancer in vivo *activity (P 388 lymphocytic screen)*
The synthesized compounds were screened by 3PS31 test system [19]. 1×10^6 leukemic cells in ascitic fluid from infected mice are implanted *ip* in CD_2F1 mice. Each compound, suspended in saline with Tween 80 was injected intraperitoneally once a day, starting one day after tumor implant and was continued daily for a total of 5 injections. The anti-tumor activity was expressed in terms of % T/C, where T was the median survival time of treated animals and C that of controls. T:C values ≥ 127% indicated activity, whereas T/C values ≤ 86% indicated toxicity. The reference compound 5-fluorouracil has a % T/C value of > 135 at a dose of 20 mg/kg when given *ip* daily for 5 days [20].

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