Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 2149–2153

BIOORGANIC & MEDICINAL CHEMISTRY

3-Arylsulphonyl-5-arylamino-1,3,4-thiadiazol-2(3*H*)ones as Anti-inflammatory and Analgesic Agents

Silvia Schenone,[a,*] Olga Bruno,[a] Angelo Ranise,[a] Francesco Bondavalli,[a] Walter Filippelli,[b] Giuseppe Falcone,[b] Lucio Giordano[b] and Maria Redenta Vitelli[b]

[a]*Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia dell'Università degli Studi di Genova, Viale Benedetto XV, 3, 16132, Genoa, Italy*

[b]*Dipartimento di Medicina Sperimentale, sezione di Farmacologia 'L. Donatelli', Facoltà di Medicina e Chirurgia, II Università degli Studi di Napoli, Via S. Andrea delle Dame 8, 80138, Naples, Italy*

Received 22 November 2000; accepted 11 April 2001

Abstract—Two series of 3-arylsulphonyl-5-arylamino-1,3,4-thiadiazol-2(3*H*)ones **2** with potential anti-inflammatory and analgesic activity were prepared and tested. Pharmacological results revealed that all the title compounds, endowed with an arylsulphonyl side chain, possess good antalgic activity and fair anti-inflammatory properties. The analgesic profile of the two series, evaluated by the acetic acid writhing test, showed that compouds **2c**, **2f** and **2h**, in particular, were the most active. Structure–activity relationships are briefly discussed.

Introduction

Many biological activities correlate with the five-membered 1,3,4-thiadiazole ring: inter alia, some years ago a number of derivatives were prepared as anti-infective[1] or antitumor[2] agents and, more recently, as anti-inflammatory[3–5] and antiplatelet[6] drugs.

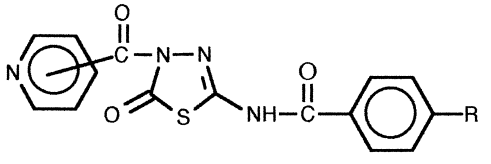
In the course of a research program aimed at designing, synthesizing, and testing new bioactive compounds acting as anti-inflammatory agents, a series of 3,5-disubstituted 1,3,4-thiadiazol-2(3*H*)ones[7] of structure **1**, a family of thiadiazole derivatives, with nicotinoyl/isonicotinoyl and benzamido substituents, have been prepared to verify whether these products could have biological properties similar to those of the classical pyrazolone-analogues (Fig. 1).

Compounds **1** exhibited fair antiphlogistic activity but lacked the well-known pyrazolone antipyretic properties. In order to improve the anti-inflammatory profile of these derivatives, we considered attaching a sulphonyl residue to a nitrogen atom of the heterocyclic ring so as to simulate the sulphonamide fragment, which is present in some successful anti-inflammatory drugs, as an interesting feature.

From this point of view, it is important to mention a clinical report[8] stating that sulphonamide-related agents "appear as a well-defined group of drugs to be considered for planning rational therapeutic strategies to control tissue injury during neutrophilic inflammation".

Keeping this in mind, we planned and synthesized two series of new derivatives **2** bearing an arylsulphonyl group on position 3 and a *p*-substitued anilino-moiety in position 5 of the thiadiazole ring (Fig. 2).

The nature of the *para*-substituent R′, present in the phenyl group of the aniline moiety, was selected among electron-donating and withdrawing groups in order to introduce a variation of the electronic properties[9] (σ_p-Hammett values ranging from -0.27 for OCH_3 to



1 : 5-Aroylamino substituted 3-nicotinoyl/isonicotinoyl-1,3,4-thiadiazol-2(3H)ones

R = C_6H_5, $C_6H_4pCH_3$, $C_6H_4pOCH_3$, C_6H_4pCl, 2-Furyl

Figure 1.

*Corresponding author. Tel.: +39-10-353-8866; fax: +39-10-353-8358; e-mail: schensil@unige.it

0968-0896/01/$ - see front matter
PII: S0968-0896(01)00121-3

+0.23 for Cl) in the title compounds to clarify any contribution to the biological activity by this structural subunit. The selection of the R substituent was made so as to verify the possible influence of increased lipophilicity from the H to CH_3 group.

Results and Discussion

Chemistry

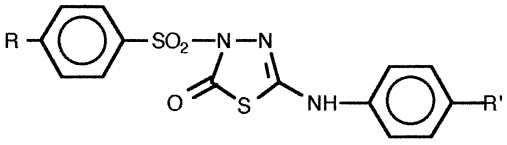
The synthetic scheme employed for the preparation of compounds **2a–l** is shown in Scheme 1.[7]

Arylsulphonyl hydrazides were treated with the selected 4-substituted arylisothiocyanates to give the corresponding arylthiosemicarbazides **3a–l** in good yields.

Intermediates **3a–l** were then cyclized by reaction with phosgene in the presence of sodium acetate, as an acid scavenger, to give the expected derivatives **2a–l** from reasonable to good yields (52–85%) (Table 3) after purification by column chromatography and crystallization.

Elemental analyses and IR and ^{1}H NMR spectral data (Table 4) confirmed the structures of the final products.

Although we had hoped for good antiphlogistic activity, better results were obtained in the analgesic profile of all the tested compounds (Table 6). In this case, antalgic activity was clearly evident in most of **2a–l**, particularly in **2c**, **2f** and **2h** (54.4, 53.8, and 51.3% of inhibition) showing an ED_{50} of 37.33 (20.47–68.08), 36.92 (18.47–73.81) and 44.95 (24.86–81.28) mg/kg, respectively. These values seem to be sufficiently safe in comparison



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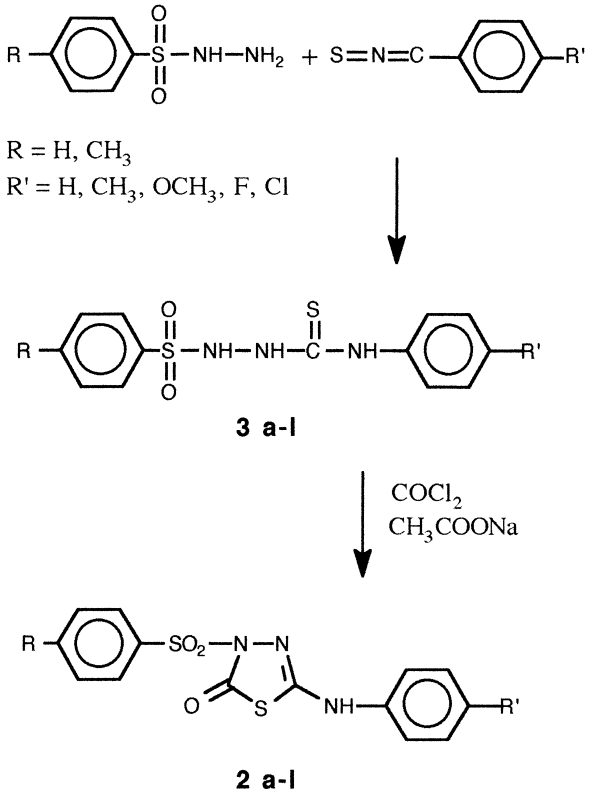
R = H, CH_3

R' = H, CH_3, OCH_3, F, Cl

Figure 2.

Table 1. Yields and melting points of arylthiosemicarbazides **3a–l**

Compound	R	R′	Formula	M_r	Yield (%)	Mp (°C)
3a	H	H	$C_{13}H_{13}N_3O_2S_2$	307.39	91	184–185
3b	H	CH_3	$C_{14}H_{15}N_3O_2S_2$	321.41	89	191–192
3c	H	OCH_3	$C_{14}H_{15}N_3O_3S_2$	337.41	95	181–182
3d	H	F	$C_{13}H_{12}N_3O_2S_2F$	325.38	85	187–188
3e	H	Cl	$C_{13}H_{12}N_3O_2S_2Cl$	341.83	90	190–191
3f	CH_3	H	$C_{14}H_{15}N_3O_2S_2$	321.41	80	191–192
3g	CH_3	CH_3	$C_{15}H_{17}N_3O_2S_2$	335.44	82	189–190
3h	CH_3	OCH_3	$C_{15}H_{17}N_3O_3S_2$	351.44	95	185–186
3i	CH_3	F	$C_{14}H_{14}N_3O_2S_2F$	339.40	92	190–191
3l	CH_3	Cl	$C_{14}H_{14}N_3O_2S_2Cl$	355.86	85	187–188



Scheme 1.

Table 2. IR and ^{1}H NMR of compounds **3a–l**

Compound	IR (cm^{-1})	^{1}H NMR δ (ppm)
3a	3330 (N–H)	6.90–8.30 (m, 10HAr), 9.70, 9.81 and 10.09 (3s, 3H, 3NH, disappear with D_2O).
3b	3340, 3130	2.29 (s, 3H, CH_3), 6.90–7.40 (m, 4HAr), 7.45–8.10 (m, 5HAr), 9.48–9.95 (m, 3H, 3NH, disappears with D_2O).
3c	3338, 3120	3.76 (s, 3H, OCH_3), 6.86 and 7.26 (2d AB, J = 8.4 Hz, 4HAr), 7.53–8.06 (m, 5HAr), 9.20–9.50 (m, 1H, NH, disappears with D_2O), 2H of 2NH not detectable.
3d	3340	6.80–8.25 (m, 9HAr), 8.68–9.85 (m, 3H, 3NH, disappears with D_2O).
3e	3350, 3290, 3140	6.90–8.20 (m, 9HAr), 9.12–9.64 (m, 2H, 2NH, disappears with D_2O), 1H of NH not detectable.
3f	3325, 3225, 3130	2.40 (s, 3H, CH_3), 6.80–8.10 (m, 9HAr), 9.40–10.12 (m, 3H, 3NH, disappears with D_2O).
3g	3340, 3145	2.30 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 6.80–7.55 (m, 6H Ar), 7.81 (d, J = 7.8 Hz, 2HAr), 9.12 (m, 2H, 2NH, disappears with D_2O), 1H of NH not detectable.
3h	3290, 3160	2.41 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.91 and 7.30 (2d, J = 9 Hz, 4HAr), 7.45 and 7.83 (2d, J = 8.4 Hz, 4H Ar), 9.00–9.50 (m, 3H, 3NH, disappears with D_2O).
3i	3235, 3315, 3350–2800 (OH)	2.41 (s, 3H, CH_3),7.07 and 7.31 (2d, J = 8.4, 4HAr), 7.46 and 7.81 (2d, J = 7.8 Hz, 4HAr), 9.20–9.80 (m, 3H, 3NH, disappears with D_2O).
3l	3285, 3140	2.41 (s, 3H, CH_3), 7.13–7.63 (m, 6HAr), 7.80 (d, J = 9, 2HAr), 9.23–9.70 (m, 2H, 2NH, disappears with D_2O), 1H of NH not detectable.

with the corresponding acute toxicity values (LD_{50}, oral administration) for **2c**, **2f**, and **2h** which were 496, 365, and 347 mg/kg, respectively.

The sulphonyl side chain could be responsible for the appearance of these general antinociceptive properties, which were not observed in the preliminary screenings for compounds **1**, and in fact resulted to be inactive.

The evaluation of the anti-inflammatory activity of compounds **2a–l** showed that no convincing improvement, in comparison to compounds **1**, was achieved by introducing a sulphonyl fragment on the thiadiazolone heterocyclic ring. In fact, antiphlogistic properties were present at a good level only in compounds **2c** and **2g** with a rapid onset of the maximum effect (1 h after treatment) followed by a slow decrease with the passing of time (Table 5). Other compounds exhibited only a moderate degree of action, slowly increasing 4 h after being administered.

It is, however, worth noting that compound **2c** disclosed the best biological profile with regard to anti-inflammatory and analgesic properties, being the most potent for both activities.

Regarding the structure–activity relationships at present, few comments on the analgesic data are possible concerning the influence of the R′-substituent of the aniline ring. It is evident that a good electron-donating group (i.e., $R' = OCH_3$) favours the antipain properties: in both series, compounds **2c** and **2h** have the best level of activity. A convincing discussion is, however, premature, since compounds **2b** and **2g**, bearing a weak electron-donating group ($R = CH_3$, $\sigma = -0.17$) both display the smallest analgesic effect on the inside of the two series. The negative value of the lipophilic parameter[9] of the OCH_3 group ($\pi = -0.02$) may play a major

Table 3. Yields and melting points of 3-arylsulphonyl-5-arylamino-1,3,4-thiadiazolo-2(3H)ones **2a–l**

Compound	R	R′	Formula	M_r	Yield%	Mp (°C)
2a	H	H	$C_{14}H_{11}N_3O_3S_2$	333.38	85	173–174
2b	H	CH_3	$C_{15}H_{13}N_3O_3S_2$	347.41	68	174–175
2c	H	OCH_3	$C_{15}H_{13}N_3O_4S_2$	363.41	52	152–153
2d	H	F	$C_{14}H_{10}N_3O_3S_2F$	351.37	55	118–119
2e	H	Cl	$C_{14}H_{10}N_3O_3S_2Cl$	367.82	60	197–199
2f	CH_3	H	$C_{15}H_{13}N_3O_3S_2$	347.41	72	141–142
2g	CH_3	CH_3	$C_{16}H_{15}N_3O_3S_2$	361.43	65	145–146
2h	CH_3	OCH_3	$C_{16}H_{15}N_3O_4S_2$	377.43	70	156–157
2i	CH_3	F	$C_{15}H_{12}N_3O_3S_2F.H_2O$	383.41	70	128–129
2l	CH_3	Cl	$C_{15}H_{12}N_3O_3S_2Cl$	381.85	73	184–185

Table 5. Carrageenan rat paw edema test: anti-inflammatory activity

Compound	Dose (mg/kg po)	Edema inhibition (%) relative to control at:	
		1st hour	4th hour[a]
Indomethacin	2.5	41	59
	5	44	68
	10	52	83
2a	50	21	24
2b	50	13	12
2c	50	50	41
2d	50	29	32
2e	50	17	34
2f	50	33	37
2g	50	46	39
2h	50	17	34
2i	50	25	29
2l	50	29	46

[a]ED_{50} of indomethacin 1.385 (0.984–1.949) mg/kg at the fourth hour.

Table 6. Acid acetic writhing test: analgesic activity

Compound	Dose (mg/kg po)	Mean no. of writhes in 25 min after treatment ± SE	Inhibition % relative to controls
Controls		44.8 ± 5.9	
Indomethacin	5	22.4 ± 3.5	50.0
2a	50	25.3 ± 5.3	43.5
2b	50	26.7 ± 6.1	40.4
2c	12.5	27.3 ± 3.4	39.0
	25	24.7 ± 1.8	44.8
	50	20.4 ± 2.9	54.4
2d	50	24.7 ± 4.3	44.8
2e	50	25.8 ± 5.8	42.4
2f	12.5	26.9 ± 5.1	40.0
	25	23.8 ± 3.2	46.8
	50	20.7 ± 4.7	53.8
2g	50	28.6 ± 3.8	36.2
2h	12.5	28.7 ± 4.1	36.0
	25	25.0 ± 3.8	44.2
	50	21.8 ± 2.6	51.3
2i	50	26.1 ± 6.3	41.7
2l	50	22.9 ± 3.3	48.8

Table 4. IR and ^1H NMR of compounds **2a–l**

Compound	IR (cm^{-1})	^1H NMR δ (ppm)
2a	3315 (NH), 1680 (C=O)	6.90–8.41 (m, 10HAr), 9.80–10.70 (s, 1H, NH, disappears with D_2O).
2b	3340, 1705	2.30 (s, 3 H, CH_3), 7.00–7.58 (m, 4 H Ar), 7.60–8.30 (m, 5 H Ar), 9.62–10.38 (br s, 1 H, NH, disappears with D_2O).
2c	3350, 1690	3.85 (s, 3 H, OCH_3), 6.70–7.45 (m, 4H Ar), 7.58–8.30 (m, 5HAr), NH not detectable.
2d	3360, 1720	6.65–8.32 (m, 9HAr), NH not detectable.
2e	3380, 1710	7.10–8.30 (m, 9HAr), NH not detectable.
2f	3380, 1715	2.46 (s, 3H, CH_3), 7.10–7.55 (m, 7HAr), 8.03 (q, $J = 7.8$ Hz, 2HAr), NH not detectable.
2g	3380, 1715	2.31 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.98–7.27 (m, 4H Ar), 7.40 and 8.03 (2dAB, $J = 8.4$ Hz, 4HAr), NH not detectable.
2h	3315, 1710	2.43 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 6.98 and 7.39 (2dAB, $J = 8.4$ Hz, 4HAr), 7.55 and 7.97 (2d, $J = 9$ Hz, 4HAr), 9.95 (s, 1H, NH, disappears with D_2O).
2i	3450–3100 (NH + OH), 1715	2.47 (s, 3H, CH_3), 6.80–7.60 (m, 6HAr), 8.01 (d, J, = 8.4 Hz, 2HAr), NH not detectable.
2l	3335, 1720	2.45 (s, 3H, CH_3), 7.25–7.75 (m, 6HAr), 8.00 (d, $J = 8.4$ Hz, 2HAr) NH not detectable.

role in determining the antalgic activity. This fact could also account for the prevailing antiphlogistic feature of **2c** in comparison with that of its homologue **2h** bearing a more lipophilic CH_3 group ($\pi = +0.56$) on the arylsulphonyl substituent.

In conclusion, these thiadiazolone derivatives, with a sulphonyl side chain, seem to be promising and susceptible to development as peripheral antinociceptive agents. Moreover, a comparison of our results to those of some close related substituted thiazolidine-2,4-diones,[10] points out better antiphlogistic and antipain properties for the present thiadiazolo-2(3*H*)one derivatives.

Experimental

Chemistry

Starting materials were purchased from Aldrich-Italia (Milan). Melting points were determined with a Büchi 530 apparatus and are uncorrected. IR spectra were measured in KBr with a Perkin-Elmer 398 spectrophotometer. ^{1}H NMR spectra were recorded in $(CD_3)_2SO$ solution on a Varian Gemini 200 (200 MHz) instrument, chemical shifts are reported as δ (ppm) relative to TMS as internal standard; *J* in Hz. ^{1}H patterns are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

All compounds were tested for purity by TLC (Merck, Silica gel 60 F_{254}, $CHCl_3$ as eluant).

Analyses for C, H, N were within ± 0.3% of the theoretical value.

General procedure for thiosemicarbazides (3a–l). A solution of the suitable isothiocyanate (21 mmol) in anhydrous tetrahydrofuran (THF) (10 mL) was added dropwise to a suspension of benzenesulphonyl or p-toluenesulphonyl hydrazide (20 mmol) in anhydrous THF (40 mL). The mixture was continuously stirred for over three days and kept at room temperature to complete the reaction (TLC monitored), the solvent was evaporated under reduced pressure and the residue treated with water (50 mL). The white solid which was obtained was filtered and crystallized from dry ethanol (Tables 1 and 2).

General procedure for 1,3,4-thiadiazol-2(3H)-ones (2a–l). A phosgene solution (20% in toluene, 12 mL, ~24 mmol) was added dropwise by cooling to a suspension of each thiosemicarbazide **3a–l** (20 mmol) and anhydrous sodium acetate (4.1 g, 50 mmol) in anhydrous THF (50 mL). The reaction mixture was stirred overnight at room temperature, the solvent was evaporated under reduced pressure and the residue treated with water (50 mL). The resulting suspension was extracted with $CHCl_3$ (3×40 mL) and dried ($MgSO_4$). After removal of the solvent, the residue was purified by column chromatography (Florisil® 60–100 mesh, $CHCl_3$ as eluant) to give a solid residue which was crystallized from Et_2O petroleum ether (1:1) (Tables 3 and 4).

Pharmacology

The tested compounds were administered orally by gavage in 1% methylcellulose suspension, using a dose of 50 mg/kg (~140 μmol/kg). Indomethacin was included and used as reference drug in all the tests for comparison purposes at the dose of 5 mg/kg (14 μmol/kg). The estimation of ED_{50} and LD_{50} values was afforded using the Litchfield and Wilcoxon I formula, by means of the computer program PHARM-PCS [7].[11]

The following experimental procedures were employed.

Anti-inflammatory activity

The paw edema inhibition test was used on rats.[12] Groups of five rats of both sexes (body weight 220–280 g), pregnant females excluded, were given a dose of the test compound. Thirty minutes later, 0.2 mL of 1% carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw. The paw volume was measured by a water plethysmometer (Socrel) and then measured again 1, 2, 3, and 4 h later. The mean variation of the paw volume at each time interval was compared to that of the control group at the same time intervals and percentage inhibition values were calculated. The experimental results at the first and fourth hour are listed in Table 5.

Analgesic activity

The acetic acid writhing test was used on mice.[13] Groups of 10 mice (weight 20–25 g) of both sexes, pregnant females excluded, were given a dose of the test compound. Thirty minutes later the animals were injected intraperitoneally with 0.25 mL/mouse of 0.5% acetic acid solution and writhes were counted during the following 25 min. The mean number of writhes for each experimental group and percentage inhibition compared to the control group were calculated. The experimental results are listed in Table 6.

Microanalyses of compounds **2a–l**

Compound	% Calculated/found		
	C	H	N
2a	50.44	3.33	12.60
	50.16	3.35	12.63
2b	51.86	3.77	12.13
	51.72	3.78	12.15
2c	49.58	3.61	11.56
	49.51	3.66	11.46
2d	47.86	2.87	11.96
	48.17	2.96	11.80
2e	45.72	2.74	11.42
	45.71	2.66	11.28
2f	51.86	3.77	12.10
	52.09	3.74	12.24
2g	53.17	4.18	11.63
	52.93	4.23	11.65

2h	50.92	4.01	11.13
	50.76	3.91	10.99
2i.H_2O	46.99	3.68	10.96
	46.93	3.62	11.02
2l	47.18	3.17	11.00
	47.19	2.97	11.02

Microanalyses of compounds **3a–l**

Compound	% Calculated/found		
	C	H	N
3a	50.79	4.26	13.67
	50.80	4.23	13.86
3b	52.32	4.70	13.07
	52.61	4.77	13.22
3c	49.84	4.48	12.45
	50.10	4.70	12.51
3d	47.99	3.72	12.91
	47.83	3.68	12.86
3e	45.68	3.54	12.29
	46.00	3.59	12.44
3f	52.32	4.70	13.07
	52.60	4.75	13.10
3g	53.71	5.11	12.53
	53.96	5.21	12.65
3h	51.27	4.88	11.96
	51.30	4.75	12.19
3i	49.54	4.16	12.38
	49.79	4.13	12.50
3l	47.25	3.97	11.81
	47.41	3.92	11.79

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