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New 1,3,4-thiadiazole derivatives endowed with analgesic and anti-inflammatory activities

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Abstract—Two series of *N*-[5-oxo-4-(arylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-amides were synthesized and tested in vivo for their analgesic and anti-inflammatory activities. All the new compounds possess good antalgic action in the acetic acid writhing test and some terms of the series showed also fair anti-inflammatory activity in the carrageenan rat paw edema test. Ulcerogenic and irritative action on the gastrointestinal mucose, in comparison with indomethacin is low.

1. Introduction

The identification of compounds able to treat both acute and chronic pain is challenging in pharmaceutical research,[1] pain is in fact a very important problem present in 90% of diseases, from the simple back pain to pain associated with different forms of cancer. The classical therapies for pain treatment are mainly the non-steroidal-anti-inflammatory drugs (NSAIDs) and opiates, whose lead compounds, acetylsalicylic acid and morphine, respectively, were isolated in 19th century.[2]

NSAIDs show side effects such as gastrointestinal irritation and lesions, renal toxicity and inhibition of platelet aggregation, while the use of opioids is limited to severe pain because of adverse secondary reactions as respiratory depression, dependence, sedation, and costipation.[3,4] Molecular biology techniques and development of selective ligands for the different receptors classes involved in pain led a further insight in the research of the nociceptive transmission. At the moment it is known that 10–15 neurotransmitters or neuromodulators are involved in the pain processing pathway;[1] so it is potentially possible to develop novel analgesic classes of compounds apart from NSAIDs and opioids, preferably devoid of severe side effects.

During recent years there has been a large investigation on different classes of thiadiazole compounds, many of which were found to possess an extensive spectrum of pharmacological activities. In particular, derivatives of differently substituted 1,3,4-thiadiazole are known to exhibit antimicrobial[5] and antitubercolar[6–8] activities, other compounds acting on SNC as anticonvulsant[9–11] or as antidepressant and anxiolitic[12] agents; recently a family of 1,3,4-thiadiazoles phosphodiesterase 7 selective inhibitors was also reported.[13]

Moreover, many reports indicate that acylthiosemicarbazides and their corresponding cyclized 1,3,4-thiadiazole derivatives possess anti-inflammatory[14–17] and analgesic[18] activities.

Being involved in a research program on non-steroidal anti-inflammatory and anti-pains agents, we focused our attention on the 1,3,4-thiadiazole ring, in particular we reported the synthesis and biological activity of two series of 2,4-disubstituted derivatives **1**[19] and **2**[20] (see Fig. 1), not previously studied for their potential pharmaceutical applications, which showed good anti-inflammatory activity in the carrageenan rat paw edema

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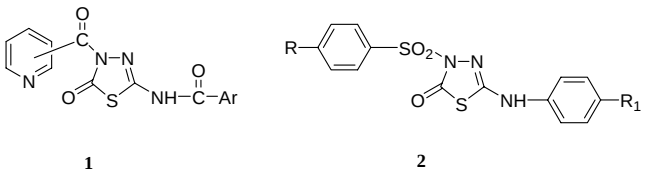


1 2

Figure 1.

test. Appreciable antalgic activity was also present in compounds **2**, which possess the sulfonamide fragment.

To ameliorate the pharmacological profile of these compounds and to find selectivity between the two activities, anti-inflammatory and analgesic, we thought worthwhile to synthesize some new derivatives **3**, bearing an arylsulfonyl group in position 4 as in **2** and a substituted aroyl or a furoyl moiety in position 2 of the thiadiazole ring already present in compounds **1**.

The newly synthesized compounds **3** were subjected to screens for their anti-inflammatory and analgesic activities. Three of the most active compounds **3e**, **3i**, and **3s** were also tested for the irritative and ulcerogenic action on gastric mucosa.

Moreover, in order to explore a possible mechanism of action, the affinity of the most active compound **3e** for different receptors involved in pain transmission was also investigated.

2. Chemistry

The desired *N*-[5-oxo-4-(arylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-amides **3a**–**t** were synthesized as shown in Scheme 1.

Benzenesulfonyl hydrazide or 4-methylbenzensulfonyl hydrazide were treated with different acylisothiocyanates in anhydrous THF to afford the corresponding acylthiosemicarbazides **4a**–**t** in good yields.

Compounds **4a**–**t** were cyclized with phosgene in anhydrous THF, in the presence of dry sodium acetate, necessary to eliminate hydrochloric acid evolving during the reaction.

The expected 1,3,4-thiadiazole derivatives **3a**–**t** were obtained as white solids in good to excellent yields.

The structures of these new compounds were confirmed by elemental and spectral analysis.

3. Pharmacology

The analgesic activity of compounds **3a**–**t**, orally administered, was evaluated through the acetic acid writhing test in mice.

All the compounds were initially screened at a dose of 50 mg/kg and showed a fair antinociceptive activity with inhibition values >40%, as reported in Table 1. The more active terms **3e**, **3i**, **3j**, **3k**, **3n**, **3p**, **3q**, **3r**, **3s**, and **3t** were further tested at different doses (25 and 12.5 mg/kg) to study their analgesic profile, and their ED_{50} values were calculated. Indomethacin was used as the reference compound at a dose of 5 mg/kg.

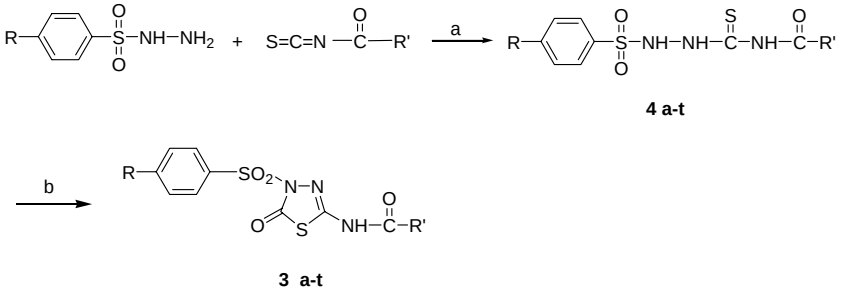
Compounds **3e**, **3i**, **3j**, **3k**, **3n**, **3p**, **3q**, **3r**, **3s**, and **3t** showed a dose dependent inhibition at the different doses tested, the best compound being **3s**, with an inhibition value of 48.8% at a dose of 12.5 mg/kg.

At this dose, also the other active compounds **3e**, **3i**, **3j**, **3k**, **3n**, **3p**, **3q**, and **3t** maintained inhibition values higher than 40%.

The synthesized compounds **3** were also tested for their anti-inflammatory activity in the carrageenan rat paw edema test, at a dose of 50 mg/kg po, using indomethacin as reference compound (5 mg/kg); the edema inhibition % relative to control was measured after 1 and 4 h.

Compounds **3c**, **3e**, **3i**, and **3j** showed good anti-inflammatory activity (Table 2).

Three of the most active compounds in both analgesic and anti-inflammatory assays, namely **3e**, **3i**, and **3s**, were also tested for their ulcerogenic activity on the gastric mucosa; the technique involves exposing rats to brief periods of cold stress, which is not itself sufficient to cause mucose damage, but specifically sensitizes the stomach to irritant or ulcerogenic actions of NSAIDs. The assessment of gastric ulcerogenicity of some well-known anti-inflammatory/analgesic drugs using this assay showed good agreement with clinical reports of the occurrence of gastric ulceration and haemorrhage.[21]



4 a-t

3 a-t

Scheme 1. Reagents and conditions: (a) THF anhyd, rt, 3 days; (b) CH_3COONa anhyd, phosgene, THF anhyd, 12 h, rt.

Table 1. Acid acetic writhing test: analgesic activity of compounds **3a–t**

Compound	R	R′	Dose (mg/kg po)	Mean No. of writhes in 25 min after treatment ± SE	Inhibition % relative to controls	ED_{50} mg/kg (fiducial limits)
Control			Acetic acid 0.5%	46.1 ± 5.7		
Indomethacin			5	21.6 ± 3.9	53.0	
3a	H	Phenyl	50	24.1 ± 4.3	47.7	
3b	H	4-Methylphenyl	50	23.9 ± 5.1	48.1	
3c	H	4-Methoxyphenyl	50	26.5 ± 3.1	42.5	
3d	H	4-Chlorophenyl	50	27.6 ± 2.9	40.1	
3e	H	4-Fluorophenyl	12.5	26.0 ± 3.1	43.6	38.010
			25	24.3 ± 2.8	47.2	(16.767–86.164)
			50	22.4 ± 4.1	51.4	
3f	H	2-Fluorophenyl	50	26.3 ± 5.2	42.9	
3g	H	3-Fluorophenyl	50	25.6 ± 3.8	44.5	
3h	H	2,4-Difluorophenyl	50	24.7 ± 4.8	46.4	
3i	H	4-Trifluoromethylphenyl	12.5	25.8 ± 5.4	44.0	43.464
			25	23.9 ± 3.7	48.1	(17.804–106.105)
			50	22.8 ± 3.8	50.5	
3j	H	2-Furoyl	12.5	25.6 ± 4.3	44.4	47.860
			25	24.8 ± 5.4	46.2	(19.539–117.231)
			50	22.8 ± 4.9	50.5	
3k	CH_3	Phenyl	12.5	26.1 ± 4.5	43.3	26.908
			25	22.8 ± 3.3	50.5	(11.870–60.998)
			50	21.3 ± 2.8	53.8	
3l	CH_3	4-Methylphenyl	50	24.6 ± 3.1	46.6	
3m	CH_3	4-Methoxyphenyl	50	25.2 ± 4.2	45.3	
3n		4-Clorophenyl	12.5	26.6 ± 2.9	42.3	44.130
			25	25.0 ± 3.3	45.7	(16.180–20.357)
			50	23.0 ± 3.8	50.1	
3o	CH_3	4-Fuorophenyl	50	24.1 ± 4.7	47.7	
3p	CH_3	2-Fuorophenyl	12.5	27.6 ± 2.3	40.1	50.245
			25	25.1 ± 4.1	45.5	(20.478–23.278)
			50	22.9 ± 5.2	50.3	
3q	CH_3	3-Fluorophenyl	12.5	26.1 ± 2.5	43.4	30.638
			25	23.8 ± 5.1	48.3	(13.458–69.748)
			50	21.0 ± 3.6	54.4	
3r	CH_3	2,4-Difluorophenyl	12.5	28.3 ± 5.3	38.6	42.615
			25	24.8 ± 6.1	46.2	(21.453–84.650)
			50	22.5 ± 4.7	51.2	
3s	CH_3	4-Trifluoromethylphenyl	12.5	23.6 ± 2.7	48.8	23.409
			25	21.9 ± 4.2	52.5	(8.601–63.709)
			50	20.8 ± 3.3	54.8	
3t	CH_3	2-Furoyl	12.5	26.1 ± 3.9	43.3	57.182
			25	24.9 ± 5.1	45.9	(21.143–154.652)
			50	23.1 ± 3.7	49.8	

The ulcerogenic and irritative action on the gastrointestinal mucose of the tested 1,3,4-thiadiazoles **3**, in comparison with indomethacin is low; in fact at a dose 100 mg/kg **3e**, **3i**, and **3s** caused ulcer in 20–30% of animals and hyperemia in 40–50%, while indomethacin at a dose of 5 mg/kg caused ulcer and hyperemia in 60% and 80% of animals, respectively (Table 2).

The pharmacological studies on animals were carried out in agreement with the legal regulation of DL 116/92 (Italy).

To investigate the possible mechanism of action of this interesting family of 1,3,4-thiadiazoles, compound **3e**, that showed either antinociceptive or anti-inflammatory activity, was screened, according to standard protocols, for inhibition of cyclooxygenase-1 (COX-1) and -2 (COX-2) and inducible nitric oxide synthetase (iNOS), target enzymes involved in inflammation and pain.

Compound **3e** did not inhibit the activity of these enzymes, showing at a 10^{-5} M concentration an inhibition of control value of −2, 3, 5 for COX-1, COX-2, and i-NOS, respectively.

4. Results and conclusions

From these data a preliminary SAR can be drawn for compounds **3**.

The first observation arising is that these new 1,3,4-thiadiazoles are more active under the two tested actions, either than compounds **1**, devoid of the analgesic activity, or than compounds **2**, where the anti-inflammatory activity was present but weak.

The presence of the tolyl substituent on the sulfonamide moiety in 4 is evidently more suitable for increasing the

Table 2. Carrageenan rat paw edema test: anti-inflammatory activity of compounds **3a**–**t**; ulcerogenic activity of compounds **3e**, **i**, and **s**

Compound	Anti-inflammatory activity			Ulcerogenic activity		
	Dose (mg/kg po)	Edema inhibition (%) relative to control at:		Dose (mg/kg po)	% of animal with ulcer	% of animals with hyperemia
		1st h	4th h			
Indomethacin	5	45	68	5	60	80
3a	50	52	42			
3b	50	55	48			
3c	50	58	50			
3d	50	19	24			
3e	50	77	61	100	20	40
3f	50	26	20			
3g	50	31	29			
3h	50	36	34			
3i	50	58	63	100	30	40
3j	50	59	53			
3k	50	26	42			
3l	50	32	48			
3m	50	26	42			
3n	50	32	35			
3o	50	26	29			
3p	50	31	38			
3q	50	36	43			
3r	50	26	36			
3s	50	45	24	100	30	50
3t	50	54	48			

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

analgesic activity, being **3s**, bearing the 4-trifluoromethyl-phenyl substituent on the amide chain, the most active compound, with an inhibition of 54.8% at a dose of 50 mg/kg and of 48.8% at a dose of 12.5 and showing an ED_{50} of 23.409. Very interesting were also found compounds **3n**, **3p**, **3q**, and **3r** possessing halogenated substituents on the amide part, showing an inhibition of 50.1, 50.3, 54.4, and 51.2, respectively, at a dose of 50 mg/kg. Compounds **3k** and **3t** bearing an unsubstituted phenyl ring and a 2-furoyl group gave an inhibition of 53.8 and 49.8, respectively.

Compound **3e** with a *p*-fluoro phenyl substituent showed to be the most active compound (51.4 of inhibition at 50 mg/kg) among the benzoyl sulfonamido derivatives. It is interesting to notice that also in this series the *p*-trifluoromethyl-phenyl and the 2-furoyl substitutions present in **3i** and **3j** confirmed its importance, showing inhibition values of 50.5%.

Considering the anti-inflammatory activity, it is prevalent in the benzoyl-sulfonamido series, being **3i** and **3e** the most active compounds; **3i** showed a better value of edema inhibition after 4 h (63%) than after 1 h (58%), possessing a longer standing of action as compared to **3e** (77% after 1 h and 61% after 4 h). Also compounds **3c** and **3j** showed interesting inhibition values of 58% and 59%, respectively, after 1 h and slightly lower after 4 h; it is interesting to say that compounds **3e**, **3i**, and **3j** have been found active also in the analgesic test.

In conclusion, compounds **3** here reported have generally shown very interesting analgesic activity, best shown when the 1,3,4-thiadiazole scaffold is substituted by the benzoyl and *p*-tolyl sulfonamido group on N-4 together with halogenated substituents on the para position on the aromatic ring of the amide moiety; this activity is separate in some cases from the anti-inflammatory one, that is present in different extent in some of the compounds **3**, but prevalent and good in **3i**, **3e**, **3c**, and **3j**.

The mechanism of action of these molecules is still unknown, as shown by the negative results of tests on COX-1, COX-2, and i-NOS, but should be considered that the mechanism of pain transmission is very complex and many other different neuromodulators and receptors could be involved; work to understand the enzymatic pathway on which these agents act is still in progress.

Nevertheless we fill, on the ground of the results on the in vivo tests reported, that compounds **3** can be considered good starting point for future work in developing analgesic agents with low gastric ulcerogenicity, devoid of anti-inflammatory activity.

5. Experimental

5.1. Chemistry

Starting materials were purchased from Aldrich-Italia (Milan).

Melting points were determined with a Büchi 540 apparatus and are uncorrected. IR spectra were measured in KBr with a Perkin-Elmer 398 spectrophotometer. ^1H 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 (Merk, Silica gel 60 F_{254}, $CHCl_3$ as eluant).

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

5.1.1. Method A. Preparation of compounds 4a–t. To a suspension of benzenesulfonyl hydrazide or 4-methylbenzensulfonyl hydrazide (20 mmol) in anhydrous tetrahydrofuran (THF) (40 mL) a solution of the suitable acylisothiocyanate (21 mmol) in anhydrous THF (10 mL) was added dropwise, cooling with an ice-water bath. The mixture was stirred for over three days 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 precipitated was filtered and crystallized from absolute ethanol.

5.1.1.1. *N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}benzamide (4a). Yield 76%; mp 157–158 °C. ^{1}H NMR: δ 7.09–8.20 (m, 10H Ar), 13.66 (m, 2H, 2NH, disappear with D_2O), 15.18 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3360, 3330, 3200 (3NH), 1670 (CO). Anal. ($C_{14}H_{13}N_3O_3S_2$) C, H, N, S.

5.1.1.2. 4-Methyl-*N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}benzamide (4b). Yield 92%; mp 139–140 °C. ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 7.17–8.57 (m, 9H Ar), 9.56, 12.56, and 13.80 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3500, 3330, 3220 (3NH), 1680 (CO). Anal. ($C_{15}H_{15}N_3O_3S_2$) C, H, N, S.

5.1.1.3. 4-Methoxy-*N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}benzamide (4c). Yield 82%; mp 133–134 °C. ^{1}H NMR: δ 3.88 (s, 3H, OCH_3), 6.83–8.10 (m, 9H Ar), 8.45, 9.00, and 12.54 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3365, 3125 (2NH), 1660 (CO). Anal. ($C_{15}H_{15}N_3O_4S_2$) C, H, N, S.

5.1.1.4. 4-Chloro-*N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}benzamide (4d). Yield 83%; mp 155–156 °C. ^{1}H NMR: δ 7.30–8.18 (m, 9H Ar), 9.06, 12.99, and 14.42 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3320, 3200 (2NH), 1680 (CO). Anal. ($C_{14}H_{12}N_3O_3S_2Cl$) C, H, N, S.

5.1.1.5. 4-Fluoro-*N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}benzamide (4e). Yield 73%; mp 161–162 °C. ^{1}H NMR: δ 7.13–8.03 (m, 9H Ar), 8.34, 8.87, and 12.24 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3269, 3190 (2NH), 1683 (CO) Anal. ($C_{14}H_{12}N_3O_3S_2F$) C, H, N, S.

5.1.1.6. 2-Fluoro-*N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}benzamide (4f). Yield 60%; mp 128–129 °C. ^{1}H NMR: δ 7.13–8.19 (m, 9H Ar), 8.40, 9.45, and 12.24 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3368, 3187 (2NH), 1680 (CO). Anal. ($C_{14}H_{12}N_3O_3S_2F$) C, H, N, S.

5.1.1.7. 3-Fluoro-*N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}benzamide (4g). Yield 73%; mp 158–159 °C. ^{1}H NMR: δ 7.26–8.04 (m, 9H Ar), 8.35, 8.91, and 12.20 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3331, 3176 (2NH), 1675 (CO). Anal. ($C_{14}H_{12}N_3O_3S_2F$) C, H, N, S.

5.1.1.8. 2,4-Difluoro-*N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}benzamide (4h). Yield 65%; mp 169–170 °C. ^{1}H NMR: δ 6.90–8.27 (m, 8H Ar), 9.33–9.37 (m, 2H, 2NH, disappears with D_2O), 12.20 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3419, 3199 (2NH), 1680 (CO). Anal. ($C_{14}H_{11}N_3O_3S_2F_2$) C, H, N, S.

5.1.1.9. *N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}-4-(trifluoromethyl)benzamide (4i). Yield 60%; mp 159–161 °C. ^{1}H NMR: δ 7.00–7.92 (m, 9H Ar), 8.43, 9.40, and 12.01 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3420, 3360, 3210 (3NH), 1670 (CO). Anal. ($C_{15}H_{12}N_3O_3S_2F_3$) C, H, N, S.

5.1.1.10. *N*-{[2-(phenylsulfonyl)hydrazino]carbonothioyl}-2-furamide (4j). Yield 53%; mp 155–156 °C. ^{1}H NMR: δ 6.64–6.66 (m, 1H fur), 7.41–8.00 (m, 7H, 5HAr + 2H fur), 8.32, 9.00, and 12.04 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3283, 3209, 3170 (3NH), 1684 (CO). Anal. ($C_{12}H_{11}N_3O_4S_2$) C, H, N, S.

5.1.1.11. *N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}-carbonothioyl)benzamide (4k). Yield 53%; mp 130–131 °C. ^{1}H NMR: δ 2.40 (s, 3H, CH_3), 7.10–8.00 (m, 9H Ar), 8.48, 9.12, and 12.68 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3540, 3420, 3180 (3NH), 1680 (CO). Anal. ($C_{15}H_{15}N_3O_3S_2$) C, H, N, S.

5.1.1.12. 4-Methyl-*N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)benzamide (4l). Yield 88%; mp 170–172 °C. ^{1}H NMR: δ 2.38 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.19–7.99 (m, 8H Ar), 8.36, 8.98, and 12.56 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3545, 3220 (2NH), 1670 (CO). Anal. ($C_{16}H_{17}N_3O_3S_2$) C, H, N, S.

5.1.1.13. 4-Methoxy-*N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)benzamide (4m). Yield 90%; mp 155–156 °C. ^{1}H NMR: δ 2.38 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 6.90–8.18 (m, 8H Ar), 13.77 (m, 2H, 2NH, disappears with D_2O). IR: cm^{-1} 3350, 3215 (2NH), 1710 (CO). Anal. ($C_{16}H_{17}N_3O_4S_2$) C, H, N, S.

5.1.1.14. 4-Chloro-*N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)benzamide (4n). Yield 98%; mp 185–186 °C. ^{1}H NMR: δ 2.37 (s, 3H, CH_3), 7.30–8.13 (m, 8H Ar), 13.76 (m, 3H, 3NH, disappears with D_2O). IR: cm^{-1} 3340, 3230, 3060 (3NH), 1662 (CO). Anal. ($C_{15}H_{14}N_3O_3S_2Cl$) C, H, N, S.

5.1.1.15. 4-Fluoro-*N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)benzamide (4o). Yield 73%; mp 185–186 °C. ^{1}H NMR: δ 2.42 (s, 3H, CH_3), 7.10–7.40

(m, 4H Ar), 7.70–7.93 (m, 4H Ar), 8.34, 8.90, and 12.34 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3352, 3246 (2NH), 1663 (CO). Anal. ($C_{15}H_{14}N_3O_3S_2F$) C, H, N, S.

5.1.1.16. 2-Fluoro-*N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)benzamide (4p). Yield 47%; mp 150–151 °C. ^1H NMR: δ 2.45 (s, 3H, CH_3), 7.12-8.20 (m, 8H Ar), 8.38, 9.45, and 12.22 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3430, 3410, 3215 (3NH), 1683 (CO). Anal. ($C_{15}H_{14}N_3O_3S_2F$) C, H, N, S.

5.1.1.17. 3-Fluoro-*N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)benzamide (4q). Yield 61%; mp 127–128 °C. ^1H NMR: δ 2.43 (s, 3H, CH_3), 7.17–7.90 (m, 8H Ar), 8.35, 9.05, and 12.20 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3440, 3300, 3218 (3NH), 1684 (CO). Anal. ($C_{15}H_{14}N_3O_3S_2F$) C, H, N, S.

5.1.1.18. 2,4-Difluoro-*N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)benzamide (4r). Yield 39%; mp 195–196 °C. ^1H NMR: δ 2.45 (s, 3H, CH_3), 6.90–8.25 (m, 7H Ar), 9.20 and 9.40 (2br s, 2H, 2NH, disappear with D_2O). IR: cm^{-1} 3426, 3216 (2NH), 1677 (CO). Anal. ($C_{15}H_{13}N_3O_3S_2F_2$) C, H, N, S.

5.1.1.19. *N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)-4-(trifluoromethyl)benzamide (4s). Yield 70%; mp 186–187 °C. ^1H NMR: δ 2.43 (s, 3H, CH_3), 7.20–8.20 (m, 8H Ar), 8.35, 9.00, and 12.16 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3247, 3133 (2NH), 1660 (CO). Anal. ($C_{16}H_{14}N_3O_3S_2F_3$) C, H, N, S.

5.1.1.20. *N*-({2-[(4-methylphenyl)sulfonyl]hydrazino}carbonothioyl)-2-furamide (4t). Yield 60%; mp 156–157 °C. ^1H NMR: δ 2.42 (s, 3H, CH_3), 6.60–6.70 (m, 1H fur), 7.20–7.86 (m, 6H, 2H fur + 4H Ar), 8.31, 8.98, and 12.00 (3br s, 3H, 3NH, disappear with D_2O). IR: cm^{-1} 3500, 3255, 3126 (3NH), 1670 (CO). Anal. ($C_{13}H_{13}N_3O_4S_2$) C, H, N, S.

5.1.2. Method B. Preparation of compounds 3a–t. To a suspension of each acylthiosemicarbazide **4a–t** (20 mmol) and anhydrous sodium acetate (4.1 g, 50 mmol) in anhydrous THF (50 mL) was added a phosgene solution (20% in toluene, 12 mL, ~24 mmol). 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 solid thus obtained was recrystallized from absolute ethanol.

5.1.2.1. *N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]benzamide (3a). Yield 89%; mp 209–210 °C. ^1H NMR: δ 7.40–8.30 (m, 10H Ar), 9.02 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3285 (NH), 1709, 1665 (2CO). Anal. ($C_{15}H_{11}N_3O_4S_2$) C, H, N, S.

5.1.2.2. 4-Methyl-*N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]benzamide (3b). Yield 91%; mp 203–204 °C. ^1H NMR: δ 2.42 (s, 3H, CH_3), 7.20–8.26 (m, 9H Ar), 8.53 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3510 (NH), 1723, 1705 (2CO). Anal. ($C_{16}H_{13}N_3O_4S_2$) C, H, N, S.

5.1.2.3. 4-Methoxy-*N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]benzamide (3c). Yield 81%; mp 224–225 °C. ^1H NMR: δ 3.84 (s, 3H, CH_3), 7.02–8.09 (m, 9H Ar), 12.73 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3333 (NH), 1696, 1667 (2CO). Anal. ($C_{16}H_{13}N_3O_5S_2$) C, H, N, S.

5.1.2.4. 4-Chloro-*N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]benzamide (3d). Yield 81%; mp 220–221 °C. ^1H NMR: δ 7.10–8.07 (m, 9H Ar), 9.63 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3340 (NH), 1750, 1648 (2CO). Anal. ($C_{15}H_{10}N_3O_4S_2Cl$) C, H, N, S.

5.1.2.5. 4-Fluoro-*N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]benzamide (3e). Yield 71%; mp 232–233 °C. ^1H NMR: δ 7.16–8.14 (m, 9H Ar), 9.04 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3546 (NH), 1718, 1680 (2CO). Anal. ($C_{15}H_{10}N_3O_4S_2F$) C, H, N, S.

5.1.2.6. 2-Fluoro-*N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]benzamide (3f). Yield 90%; mp 219–220 °C. ^1H NMR: δ 7.20–8.20 (m, 9H Ar), 9.43 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3403 (NH), 1721, 1672 (2CO). Anal. ($C_{15}H_{10}N_3O_4S_2F$) C, H, N, S.

5.1.2.7. 3-Fluoro-*N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]benzamide (3g). Yield 68%; mp 226–227 °C. ^1H NMR: δ 7.24–8.16 (m, 9H Ar), 9.50 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3235 (NH), 1685, 1666 (2CO). Anal. ($C_{15}H_{10}N_3O_4S_2F$) C, H, N, S.

5.1.2.8. 2,4-Difluoro-*N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]benzamide (3h). Yield 59%; mp 221–222 °C. ^1H NMR: δ 6.95–8.23 (m, 8H Ar), 9.32 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3397 (NH), 1715, 1676 (2CO). Anal. ($C_{15}H_9N_3O_4S_2F_2$) C, H, N, S.

5.1.2.9. *N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-4-(trifluoromethyl) benzamide (3i). Yield 71%; mp 230–231 °C. ^1H NMR: δ 7.24–8.18 (m, 9H Ar), 9.33 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3244 (NH), 1743, 1667 (2CO). Anal. ($C_{16}H_{10}N_3O_4S_2F_3$) C, H, N, S.

5.1.2.10. *N*-[5-oxo-4-(phenylsulfonyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-2-furamide (3j). Yield 80%; mp 155–156 °C. ^1H NMR: δ 6.67–6.80 (m, 1H fur), 7.63–8.06 (m, 7H, 2H fur + 5H Ar), 12.90 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3291 (NH), 1718, 1682 (2CO). Anal. ($C_{13}H_9N_3O_5S_2$) C, H, N, S.

5.1.2.11. *N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (3k). Yield 60%; mp 205–206 °C. ^1H NMR: δ 2.47 (s, 3H, CH_3), 7.03–8.25 (m, 9H Ar), 12.42 (br s, 1H, NH, disappears

with D_2O). IR: cm^{-1} 3300 (NH), 1700, 1675 (2CO). Anal. ($C_{16}H_{13}N_3O_4S_2$) C, H, N, S.

5.1.2.12. 4-Methyl-*N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (3l). Yield 55%; mp: 218–220 °C. ^1H NMR: δ 2.40 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 7.05–8.01 (m, 8H Ar), 9.05 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3350 (NH), 1715, 1660 (2CO). Anal. ($C_{17}H_{15}N_3O_4S_2$) C, H, N, S.

5.1.2.13. 4-Methoxy-*N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (3m). Yield 90%; mp: 200–201 °C. ^1H NMR: δ 2.43 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 7.00–8.09 (m, 8H Ar), 12.71 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3502 (NH), 1721, 1665 (2CO) Anal. ($C_{17}H_{15}N_3O_5S_2$) C, H, N, S.

5.1.2.14. 4-Chloro-*N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (3n). Yield 81%; mp 244–245 °C. ^1H NMR: δ 2.43 (s, 3H, CH_3), 7.10–8.58 (m, 8H Ar), 9.40 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3282 (NH), 1675, 1664 (2CO). Anal. ($C_{16}H_{12}N_3O_4S_2Cl$) C, H, N, S.

5.1.2.15. 4-Fluoro-*N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (3o). Yield 73%; mp 222–224 °C. ^1H NMR: δ 2.43 (s, 3H, CH_3), 7.15–8.01 (m, 8H Ar), 9.04 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3284 (NH), 1680, 1665 (2CO). Anal. ($C_{16}H_{12}N_3O_4S_2F$) C, H, N, S.

5.1.2.16. 2-Fluoro-*N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (3p). Yield 70%; mp 192–193 °C. ^1H NMR: δ 2.48 (s, 3H, CH_3), 7.17–8.16 (m, 8H Ar), 9.40 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3407 (NH), 1724, 1675 (2CO). Anal. ($C_{16}H_{12}N_3O_4S_2F$) C, H, N, S.

5.1.2.17. 3-Fluoro-*N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (3q). Yield 60%; mp 192–193 °C. ^1H NMR: δ 2.47 (s, 3H, CH_3), 7.26–8.00 (m, 8H Ar), 9.34 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3234 (NH), 1680, 1664 (2CO). Anal. ($C_{16}H_{12}N_3O_4S_2F$) CH, N, S.

5.1.2.18. 2,4-Difluoro-*N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}benzamide (3r). Yield 75%; mp 224–225 °C. ^1H NMR: δ 2.43 (s, 3H, CH_3), 7.16–7.94 (m, 7H Ar), 13.03 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3423 (NH), 1723, 1675 (2CO). Anal. ($C_{16}H_{11}N_3O_4S_2F_2$) C, H, N, S.

5.1.2.19. *N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}-4-(trifluoromethyl)benzamide (3s). Yield 75%; mp 237–238 °C. ^1H NMR: δ 2.47 (s, 3H, CH_3), 7.20–8.08 (m, 8H Ar). IR: cm^{-1} 3251 (NH), 1720, 1668 (2CO). Anal. ($C_{17}H_{12}N_3O_4S_2F_3$) C, H, N, S.

5.1.2.20. *N*-{4-[(4-methylphenyl)sulfonyl]-5-oxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}-2-furamide (3t). Yield 58%; mp 116–117 °C. ^1H NMR: δ 2.47 (s, 3H, CH_3), 6.60–6.70 (m, 1H fur), 7.25–8.03 (m, 6H, 4H Ar + 2H fur), 9.29 (br s, 1H, NH, disappears with D_2O). IR: cm^{-1} 3545 (NH), 1721, 1675 (2CO). Anal. ($C_{14}H_{11}N_3O_5S_2$) C, H, N, S.

5.2. 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} values was afforded using the Litchfield and Wilcoxon I formula, by means of the computer program PHARM-PCS [7].[22]

5.2.1. Analgesic activity: acetic acid writhing test. The acetic acid writhing test was used on mice.[23] Groups of 10 mice (*Mus musculus*, 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. Experimental results are listed in Table 1.

5.2.2. Anti-inflammatory activity: rat paw edema test. The paw edema inhibition test was used on rats.[24] Groups of 10 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 0.95% NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw. The paw volume was measured by a water plethysmometer (Basile) 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 (five rats treated with carrageenan, but not treated with test compound) at the same time intervals and percent inhibition values were calculated. Experimental results at the first and fourth hours are listed in Table 2.

5.2.3. Ulcerogenic action. Groups of 10 rats (body weight 220–280 g) of both sexes, pregnant females excluded, were exposed to brief periods of cold stress and then were treated with an oral dose (100 mg/kg) of a test compound, except the control group. The animal were sacrificed 6 h after the dosing and their stomachs and small intestines were macroscopically examined to asses the incidence of hyperemia and ulcers. Experimental results are listed in Table 2.

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