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A facile regioselective synthesis of novel *spiro*-thioxanthene and *spiro*-xanthene-9′,2-[1,3,4]thiadiazole derivatives as potential analgesic and anti-inflammatory agents

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ABSTRACT

The 1,3-dipolar cycloaddition of nitrile imines to 9*H*-thioxanthone-9-thione and 9*H*-xanthone-9-thione afforded novel *spiro*-thioxanthene-9′,2-[1,3,4]thiadiazoles **6a-g** and *spiro*-xanthene-9′,2-[1,3,4]thiadiazoles **7a-g** in good yields. Some of the newly synthesized compounds were tested for anti-inflammatory and analgesic activities comparable to ibuprofen. Compounds **6a,d,e** and **7a,d,e** showed significant activity compared to standard drug. The toxicity studies revealed that neither death nor other behavioral or toxicological changes were observed on rats up to a dose as high as 200 mg/kg.

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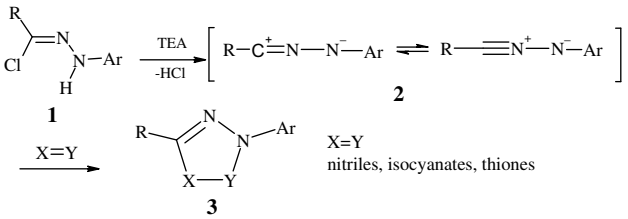
Recently, we described the synthesis of some novel five-membered heterocycles via 1,3-dipolar cycloaddition using nitrile imines as 1,3-dipole. The heterocumulenic cation **2** was found to undergo cycloaddition to multiple bonds of isocyanates, nitriles and thiones to furnish pyrazoles **3** and thiadiazoles (X=S) (Scheme 1).[1–5] We wondered whether these cycloadditions could be applied to syntheses of *spiro*-thioxanthene-9′,2-[1,3,4]thiadiazole and xanthene derivatives.

1,3-Dipolar cycloaddition is a versatile synthetic strategy for the constructions of five-membered ring heterocycles.[6,7] Also, some of xanthone-9-thiones and thioxanthone-9-thiones exhibit antiviral,[8] local anaesthetic,[9] bronchodilator[10] and anticonvulsant.[11] Moreover, xanthene derivatives are useful pharmaceuticals such as muscarinic receptor antagonist,[12] cancer chemotherapy,[13] trypanothione reductase inhibitor,[14] chemosensitizers against chloroquine-resistant Plasmodium falciparum,[15] nonpeptidic inhibitors,[16] mG1uR1 enhancer[17,18] and CCR1 antagonist.[19,20] Moreover, the 1,3,4-thiadiazole ring system exhibit biologically activity, for example leishmanicidal,[21] anticonvulsant.[22,23] Thus, syntheses of xanthene derivatives are of immense interest. According to the literature replacement, of oxygen by sulfur in the xanthone core also changed the pharmacological profiles of patented compounds.[24] Here we report the syntheses of various *spiro*-thioxanthene-9′,2-[1,3,4]thiadiazole derivatives via the cycloaddition of numerous nitrile imines **5a–g** to 9*H*-thioxanthene-9-thione and 9*H*-xanthene-9-thione **4a,b**, the structures of which are confirmed by X-ray single crystal structural analysis. Besides, the evaluation of anti-inflammatory and analgesic activities of some products has been studied.

The 1,3-dipolar cycloadditions of the nitrile imines generated in situ from hydrazonoyl chlorides **5a–g** and triethylamine in benzene to thioxanthene-9-thione **4a** afforded novel 3,5-disubstituted *spiro*-thioxanthene-9′,2-[1,3,4]thiadiazoles **6a–g** in good yields (76–88%) under dry conditions (Scheme 2). The mixture of an equimolar of **4a**, *C*-phenyl-*N*-phenyl hydrazonoyl chloride **5a** in dry benzene in presence of triethylamine was refluxed for 3 h led to the compound **6a**. This cycloaddition is chemoselective as it occurs on only C=S of **4a** furnishing exclusively the *spiro*-thioxanthene-9′,2-[1,3,4]thiadiazole **6a**. The mechanism of the reaction may proceed via two pathways: **A**: regioselectively, as the electron rich



Scheme 1.

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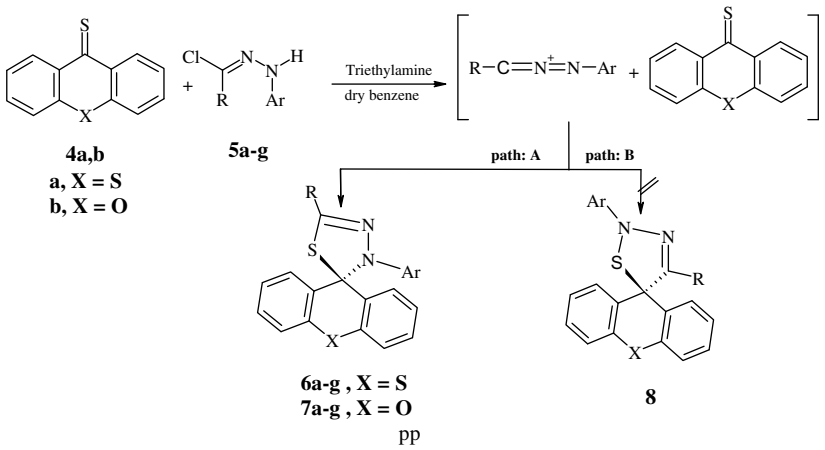


6,7a; R = Ar = C_6H_5

6,7b; R = $COCH_3$, Ar = C_6H_5

6,7c; R = $COCH_3$, Ar = p-C_6H_4-Br

6,7d; R = $COCH_3$, Ar = p-C_6H_4-Cl

6,7e; R = $COCH_3$, Ar = p-C_6H_4-NO_2

6,7f; R = $COOC_2H_5$, Ar = p-C_6H_5

6,7g; R = $COOC_2H_5$, Ar = p-C_6H_4-CH_3

Scheme 2.

nitrogen of the dipole adds to the carbon of thione **4a** and only one regioisomer is obtained exclusively in high yields; **B**: the electron rich carbon of the dipole adds to the carbon of thione **4a** and furnish the other regioisomer **8** (Scheme 2). The spectroscopic analyses (IR, NMR, MS), beside the X-ray crystallographic study (Fig. 1) conformed the structure **6a** and not structure **8**.

The crystallographic data and details of structure determination of thiadiazole derivative **6a** are shown in Table 1. Some selected bond lengths, bond angles, and torsion angles are given in Table 2.

Also, the cycloaddition of nitrile imine generated in situ from *C*-acetyl-*N*-aryl (phenyl, 4-bromophenyl, 4-chlorophenyl and 4-nitrophenyl) **5b–e** and triethylamine to **4a** afforded 3-aryl-5-acetyl-*spiro*-thioxanthene-9′,2-[1,3,4]thiadiazole (**6b–e**) as only

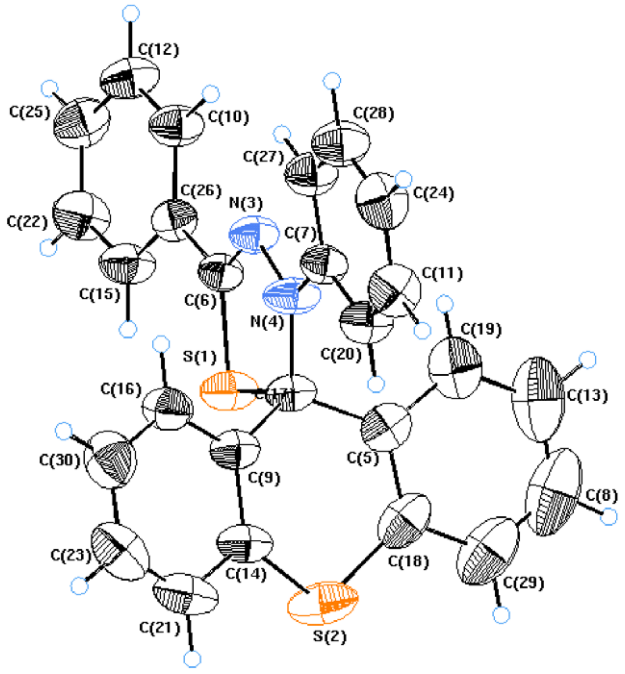


Figure 1. Single crystal X-ray diffraction of **6a**; the crystallographic numbering does not reflect the systematic numbering.

Table 1
Crystal data and details of structure determination of **6a**

Crystal data	Compound **6a**
Empirical formula	$C_{26}H_{18}N_2S_2$
Formula weight	422.572
Shape/color	Cube/yellow
Crystal system	Triclinic
Space group	$P\bar{1}$
Temperature (K)	298
Wavelength (Å)	0.71073
Unit cell determinations a (Å)	8.1982 (4)
b (Å)	11.1808 (5)
c (Å)	12.1107 (9)
α (°)	104.565 (2)
β (°)	93.377 (2)
γ (°)	106.703 (5)
Volume (Å^3)	1018.73 (10)
Z	2
Density (calculated) (mg m^{-3})	1.378
θ range for data collection (°)	2.910–21.967
Index ranges	
h	$0 \rightarrow 8$
k	$-11 \rightarrow 11$
l	$-12 \rightarrow 12$
Reflections, measured	2931
Independent	2443
Observed	1725
Data/restraints/parameters	1725/0/271
Goodness-of-fit	1.036
R indices (all data), R	0.062
wR	0.090
R indeces ($I \geqslant 3\sigma(I)$)	0.049
Max./min. electron density (eÅ^{-3})	0.26/−0.33

one pure product in high yields (Scheme 2). The structures of **6b–e** were elucidated using H NMR and MS. The ^{1}H NMR of compound **6c** as an example, showed $COCH_3$ at δ 2.57 in addition to the aromatic protons (12H) around δ *6.86–7.57*, also the mass spectrum of **6c** showed the prominent ion peak at m/z 467 (M^+, 75).

On the other hand, the thiooxo derivative **4a** reacted with nitrile imine generated in situ from *C*-carboethoxy-*N*-aryl (phenyl and 4-tolyl) **5f,g** as described above to afford the only one product **6f,g**, respectively (Scheme 2). The structure of the products **6f,g** were established by elemental analysis and spectral data (IR, NMR and MS). The ^{1}H NMR spectrum of **6f** as an example, showed

Table 2
Selected bond lengths (Å), bond angles (°), and torsion angles (°) for **6a**

S1–C6	1.748 (3)	S2–C14–C9	123.3 (3)
S1–C17	1.867 (3)	S2–C14–C21	117.3 (2)
S2–C14	1.741 (3)	S1–C17–N4	102.3 (2)
S2–C18	1.757 (4)	S1–C17–C5	107.4 (2)
N3–N4	1.369 (3)	S1–C17–C9	107.2 (2)
N3–C6	1.293 (4)	N4–C17–C5	111.3 (2)
N4–C6	2.215 (4)	N4–C17–C9	111.8 (2)
N4–C7	1.417 (3)	C5–C17–C9	115.8 (2)
N4–C17	1.477 (3)	S2–C18–C5	122.5 (3)
C5–C17	1.510 (4)	S2–C18–C29	117.3 (3)
C9–C14	1.387 (4)	C6–C26–C10	121.5 (3)
C9–C17	1.523 (4)	C6–C26–C15	120.5 (3)
C6–S1–C17	90.35 (13)	C17–S1–C6–N3	−2.1 (2)
C14–S2–C18	103.0 (2)	C6–S1–C17–N4	2.2 (2)
N4–N3–C6	112.6 (2)	C6–S1–C17–C5	−115.1 (2)
N3–N4–C7	118.5 (2)	C6–S1–C17–C9	119.9 (2)
N3–N4–C17	118.1 (2)	C17–S1–C6–C26	−179.0 (2)
C6–N4–C7	150.8 (2)	C14–S2–C18–C5	−20.2 (3)
C6–N4–C17	85.6 (2)	C18–S2–C14–C9	23.0 (3)
C7–N4–C17	123.2 (2)	C18–S2–C14–C21	−161.0 (3)
C17–C5–C18	120.9 (3)	C14–S2–C18–C29	162.4 (4)
S1–C6–N3	116.5 (2)	N4–N3–C6–S1	1.1 (2)
S1–C6–C26	121.3 (2)	C6–N3–N4–C7	−174.6 (4)
N3–C6–C26	122.1 (3)	C6–N3–N4–C17	0.9 (2)
N4–C7–C20	121.2 (3)	N4–N3–C6–C26	178.0 (4)
N4–C7–C27	119.7 (3)	C7–N4–N3–C6	−174.6 (4)

CH_3 at δ 1.25 as triplet, J = 7.0 Hz, CH_2 at δ 4.25 as quartet, J = 7.0 Hz, in addition to the aromatic protons (13H) at δ 6.89–7.63.

The scope of the reaction was extended further by replacing the sulfur atom at position 10 in thioxanthene-9-thione **4a** with oxygen and the study of the reactivity of the xanthene-9-thione **4b** towards the same nitrile imines generated from hydrazonoyl chlorides **5a–g** under the same reaction conditions. The latter derivative **4b** gave the corresponding cycloaddition products **7a–g**. In the case of *C*-phenyl-*N*-phenyl hydrazonoyl chloride **5a** was reacted with **4b** under the same reaction condition to afford 3,5-diphenyl-*spiro*-xanthene-9′,2-[1,3,4]thiadiazole **7a** as only one product.

Also, the cycloaddition of initrile imine generated in situ from *C*-acetyl-*N*-aryl **5b–e** and triethylamine to **4b** afforded 3-aryl-5-acetyl-*spiro*-xanthene-9′,2-[1,3,4]thiadia-zole (**7d–e**) in high yields (Scheme 2). The structures of the products of this cycloaddition are in accordance with their NMR spectroscopic data and elemental analyses. The ^{1}H NMR of compound **7e** as an example, showed $COCH_3$ at δ 2.63, two doublet for 4-nitrophenyl at 7.15 and 8.07 with coupling constant J = 8.40 Hz in addition to the remaining aromatic protons (8H) around δ *7.33–7.66* as multiplet band, also the mass spectrum of **7e** showed the prominent ion peak at *m*/*z* 417 (M^+, 36), *m*/*z* 316 (M^+–C_3H_3NOS, 42), *m*/*z* 269 (M^+–$C_6H_2N_3O_2$, 20) and *m*/*z* 211 (M^+–$C_9H_8N_3O_3$, 100).

On the other hand, the thiooxo derivative **4b** reacted with nitrile imine generated in situ from *C*-carboethoxy-*N*-aryl (phenyl and 4-tolyl) **5f,g** as described above to afford the only one product 7f,**g**, respectively (Scheme 2). The structure of the products **6f,g** were established by elemental analysis and spectral data (IR, NMR and MS). The ^{1}H NMR spectrum of **7g** as an example, showed CH_3 at δ 1.25 as triplet, J = 7.0 Hz, CH_3 as singlet at δ 2.23, CH_2 at δ 4.26 as quartet, J = 7.0 Hz, and the aromatic protons (12H) at δ *6.95–7.85*. Finally, the *spiro* structure of **6** and **7** is clearly evident from the lack of a ^{13}C signal for the thiooxo group and the presence of a signal at 81.86 ppm due to a quaternary carbon of both compounds as expected for the spiro-thiadiazolo derivatives. Its ^{13}C NMR spectrum of **7g** showed the spiro-carbon at 81.86, C=N at 148.03 and C=O at 159.78 ppm.

The anti-inflammatory activity of the synthesized compounds was evaluated by the carrageenan induced paw edema method. The compounds were tested at an oral dose of 70 mg/kg body weight and were compared with the standard drug ibuprofen at the same oral dose. The tested compounds showed anti-inflammatory activity ranging from 50% to 86% (Table 4), and the standard drug ibuprofen showed 92% inhibition after 4 h. The *spiro*-xanthene-9′,2-[1,3,4]thiadizole and its thioxanthene **6a, 7a** having a 4-nitrophenyl group at position 3 and acetyl group at position 5 showed the maximum activity (84–86%). Also, the *spiro* compounds which having two phenyl groups at position 3 and 5, showed high activities (85% and 82%), respectively. Whereas when the 4-nitro group was replaced by the hydrogen, chlorine and bromine showed good activity around (76–71%), respectively.

The compounds that showed anti-inflammatory activity higher than 80% were tested for analgesic activity (Table 3). Compounds **6a,d,e** and **7a,d,e** showed analgesic activity ranging from 57% to 73%, whereas the standard drug ibuprofen showed 84% at a 70 mg/kg oral dose. Among all the tested compounds, the *spiro*-thiadiazole derivative having the 4-nitrophenyl group **6e** showed maximum activity (73%). When this group was replaced by the 4-bromophenyl (**6d**) and phenyl (**6a**), there was a significant decrease in the activity. The rats were observed for possible incidence of death or other behavioral changes. The toxicity studies revealed that neither death nor other behavioral or toxicological changes were observed on rats up to a dose as high as 200 mg/kg.

All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed by Vario, Elementar apparatus (Shimadzu). The IR spectra (KBr, cm^{-1}) were recorded on a Perkin-Elmer 1650 spectrometer (USA). ^{1}H NMR spectra were recorded in DMSO-d_6 with tetramethylsilane as an internal standard on a JEOL EX-270 or on JEOL ECA-500. Mass spectra were recorded on 70 ev EI Ms-QP 1000 EX (Shimadzu, Japan). The starting materials hydrazonoyl chloride (**5a–g**) were prepared as the same in the literature.[25,26] Reactions were monitored by TLC on silica gel 60 GF254 (0.25 mm).

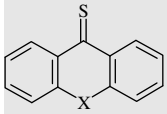
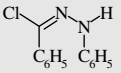
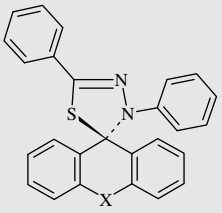
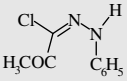
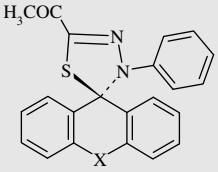
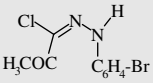
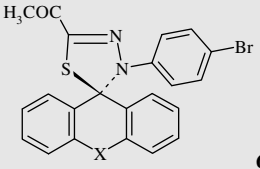
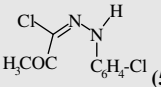
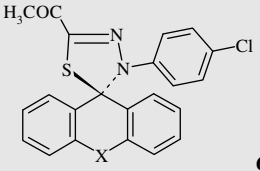
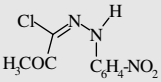
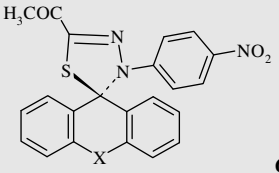
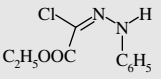
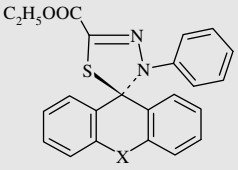
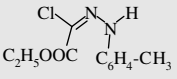
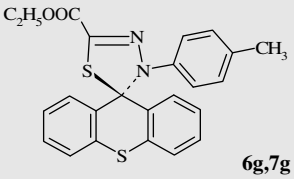
Spiro-thioxanthene- or spiro-xanthene-9′,2-[1,3,4]thiadiazole derivatives (**6a–g, 7a–g**)**:** *general procedure.* A mixture of compound **4a,b** (0.01 mol) and the appropriate hydrazonoyl chlorides **5a–g** (0.01 mol) in dry benzene (30 mL) and 4 drops of triethylamine, was stirred under reflux for 3–5 h (TLC control). The solvent was evaporated under reduced pressure. The solid produced was washed three times with 30 mL methanol and crystallized from an appropriate solvent to produce **6a–g** and **7a–g**, respectively, in high yields.

3,5-Diphenyl-spiro-thioxanthene-9′,2-[1,3,4]thiadiazole (**6a**). The compound was obtained from **4b** and *N*-phenylbenzenecarbohydrazonoyl chloride **5a**, as yellow needles (from dioxane), mp 215–217 °C; IR: 3038 (CH aryl), 1594 (C=N); ^{1}H NMR (δ, ppm): 6.79 −7.63 (m, 18H, Ar–H); MS (*m*/*z*), 422 (M^+, 100); Analysis: $C_{26}H_{18}N_2S_2$ (422.56); Requires: C, 73.90; H, 4.30; N, 6.63; S, 15.17. Found: C, 73.87; H, 4.27; N, 6.59; S, 15.14.

5-Acetyl-3-phenyl-spiro-thioxanthene-9′,2-[1,3,4]thiadiazole (**6b**). The compound was obtained from **4b** and 2-oxo-*N*-phenylpropane hydrazonoyl chloride **5b**, as yellow needles (from ethanol/dioxane) (1:1), mp 186–188 °C; IR: 1668 (CO), 1592 (C=N). ^{1}H NMR (δ, ppm); 2.60 (s, 3H, CH_3), 6.91–7.61 (m, 13H, Ar–H); Analysis: $C_{22}H_{16}N_2OS_2$ (388.50); Requires: C, 68.01; H, 4.15; N, 7.21; S, 16.50. Found: C, 67.97; H, 4.12; N, 7.19; S, 16.48.

5-Acetyl-3- (4-bromophenyl)-spiro-thioxanthene-9′,2-[1,3,4]thiadiazole (**6c**). The compound was obtained from **4b** and 2-oxo-*N*-(4-bromophenyl)propane hydrazonoyl chloride **5c**, as yellow crystals (from dioxane), mp 211–213 °C; IR: 1668 (CO), 1594 (C=N). ^{1}H NMR (δ, ppm); 2.57 (s, 3H, CH_3), 6.86–7.57 (m, 12H, Ar–H); MS (*m*/*z*), 469 (M^++2, 36), 467 (M^+, 75); Analysis: $C_{22}H_{15}BrN_2OS_2$ (467.39); Requires: C, 56.53; H, 3.23; N, 5.99; S, 13.72. Found: C, 56.50; H, 3.19; N, 5.95; S, 13.70.

Table 3
C–S, C–N bond formation at the tertiary carbon of 9*H*-xanthen-9-thione with nitrile imines

Substrate	Hydrazonoyl chloride **5a–g**	Product[a] (**6**, **7a–g**)	No: Time[b] (**h**)	Yield[c] (%)
 **4a**,**b**	 **(5a)**	 **(6a)[d],7a**	**3a**; 3 **4a**; 3	88 77
	 **(5b)**	 **6b,7b**	**3b**; 3 **4b**; 4	86 75
	 **(5c)**	 **6c,7c**	**3c**; 4 **4c**; 3	79 80
	 **(5d)**	 **6d.7d**	**3d**; 4 **4d**; 4	76 81
	 **(5e)**	 **6e,7e**	**3e**; 5 **4e**; 3	78 72
	 **(5f)**	 **6f,7f**	**3f**; 3 **4f**; 2	83 75
	 **(5g)**	 **6g,7g**	**3g**; 3 **4g**; 2	89 80

[a] Products were characterized by IR, ^{1}H, ^{13}C NMR, MS.
[b] Reactions were monitored by TLC.
[c] Yields of the isolated products.
[d] The structure was confirmed by X-ray.

5-Carboxyethyl-3-phenyl-spiro-thioxanthene-9′,2-[1,3,4]thiadiazole (**6f**). The compound was obtained from **4b** and chloro(phenylhydrazono)ethylacetate **5f**, as yellow crystals (from ethanol), mp 190–193 °C; IR: 1698 (CO), 1592 (C=N). ^{1}H NMR (δ, ppm); 1.25 (t, 3H, J = 7.0 Hz, CH_3CH_2), 4.25 (q, 2H, J = 7.0 Hz, CH_3CH_2), 6.89–7.63 (m, 13H, Ar–H); Analysis: $C_{23}H_{18}N_2O_2S_2$ (418.52); Requires: C, 66.00; H, 4.33; N, 6.69: S, 15.32. Found: C, 66.01; H, 4.29; N, 6.65; S, 15.29.

Table 4
Pharmacological activities anti-inflammatory and analgesic activities (Writhing test)

Compound	Dose (mg/kg, 1% CMC)	Anti-inflammatory activity (mean inhibition ± SEM %)	Dose (mg/kg, 1% CMC)	Analgesic activity (mean inhibition ± SEM, %)
Control		—		—
6a	70	85 ± 1.9[b]	70	59 ± 0.5[a]
6b	70	75 ± 1.7[a]	—	—
6d	70	82 ± 1.7[b]	70	57 ± 0.9[a]
6e	70	86 ± 1.7[b]	70	73 ± 0.9[a]
6g	70	71 ± 2.6[a]	—	—
7a	70	82 ± 0.9[a]	70	71 ± 1.2[a]
7b	70	79 ± 0.8[a]	—	—
7c	70	76 ± 2.1[a]	—	—
7d	70	82 ± 2.0[c]	70	72 ± 1.1[a]
7e	70	84 ± 2.0[c]	70	56.6 ± 1.1[a]
Ibuprofen	70	92 ± 1.0	70	83.5 ± 0.7[a]

Anti-inflammatory and analgesic activities of the test compounds were measured with respect to the control and compared with respect to the standard drug.
[a] $p < 0.0001$.
[b] $p < 0.001$.
[c] $p < 0.05$.

5-Carboxyethyl-3-(4-tollyl)-spiro-thioxanthene-9′,2-[1,3,4]thiadiazole (**6g**). The compound was obtained from **4b** and chloro(4-tollylhydrazono)ethylacetate **5g**, as yellow crystals (from dioxane), mp 255–267 °C; IR: 1696 (CO), 1590 (C=N). ^{1}H NMR (δ, ppm); 1.27 (t, 3H, J = 7.0 Hz, CH_3CH_2), 2.20 (s, 3H, CH_3), 4.25 (q, 2H, J = 7.0 Hz, CH_3CH_2), 6.80–7.59 (m, 12H, Ar–H); MS (m/z), 432 (M^+, 100); Analysis: $C_{24}H_{20}N_2O_2S_2$ (432.55); Requires: C, 66.63; H, 4.66; N, 6.47: S, 14.82. Found: C, 66.59; H, 4.63; N, 6.45; S, 14.79.

3,5-Diphenyl-spiro-xanthene-9′,2-[1,3,4]thiadiazole (**7a**). The compound was obtained from **4a** and *N*-phenylbenzene-carbohydrazonoyl chloride **5a**, as yellow needles (from ethanol), mp 195–197 °C; IR: 3031 (CH aryl), 1590 (C=N). ^{1}H NMR (δ, ppm); 6.72–7.68 (m, 18H, Ar–H); MS: m/z (%), 406 (M^+, 85), 297 (12), 211 (27), 193 (84), 90 (100); Analysis: $C_{26}H_{18}N_2OS$ (406.50); Requires: C, 76.82; H, 4.46; N, 6.89; S, 7.88. Found: C, 76.79; H, 4.39; N, 6.86; S, 7.85.

5-Acetyl3-phenyl-spiro-xanthene-9′,2-[1,3,4]thiadiazole (**7b**). The compound was obtained from **4a** and 2-oxo-*N*-phenylpropane hydrazonoyl chloride **5b**, as yellow needles (from ethanol), mp 190–192 °C; IR: 1663 (CO), 1595 (C=N). ^{1}H NMR (δ, ppm); 2.61 (s, 3H, CH_3), 6.92–7.65 (m, 13H, Ar–H); MS: m/z (%), 372 (M^+, 84), 271 (84), 270 (72), 269 (11), 212 (100); Analysis: $C_{22}H_{16}N_2O_2S$ (372.44); Requires: C, 70.94; H, 4.33; N, 7.52; S, 8.61. Found: C, 70.91; H, 4.29; N, 7.40; S, 8.57.

Pharmacology Animals both sex of Swiss mice weighing 25–30 g were used in analgesic activity and adult male of Sprague–Dawley rats weighing between 150 and 180 g were used in anti-inflammatory activity, taking into account international principle and local regulations concerning the care and use of laboratory animals.[27] The animals were housed in groups of six and acclimatized to room conditions for at least 2 days before the experiments. Food and water were freely available up to the time of experiments. The food was withdrawn on the day before the experiment, but free access to water was allowed. All the compounds (70 mg/kg body mass) and the reference NSAID ibuprofen (70 mg/kg body mass) were suspended in 1% carboxymethyl cellulose (CMC) and administered orally using an animal feeding needle. The control groups received appropriate volumes of vehicle (1% CMC, oral) only.

Anti-inflammatory assay. This activity was performed by the following procedure of Winter et al.[28] on groups of six animals each. A freshly prepared suspension of carrageenin (1.0% m/V, 0.1 mL) was injected in the plantar region of the right hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with the test compounds (70 mg/kg body mass) suspended in 1.0% CMC given orally 1 h before the carrageenin treatment. The volume was measured before and after 4 h of carrageenin treatment using a pleythysmometer.

Analgesic assay (Writhing test). Mice were kept individually in the test cage before acetic acid injection and habituated for 30 min. Screening of analgesic activity was performed after po administration of test compounds at a dose of 70 mg/kg body mass. The compounds, which exhibited good anti-inflammatory activity comparable to that of ibuprofen, were screened for analgesic activity. All compounds were dissolved in 1.0% CMC solution. One group was kept as control and received po 1% CMC. After 1 h of drug administration, 0.10 mL of 1% acetic acid solution was given to mice intraperitoneally. The acetic acid induced writhing test[29] showed stretching movements involving arching of the back, elongation of the body, and extension of hind limbs which were counted for 5–15 min of acetic acid injection.

Acute toxicity determination (LD50). Male Sprague–Dawley rats (200–250 g) were housed individually in stainless steel cages. Rats were divided into four groups under 12 h light–dark periods. Different doses were used for each group separately (25, 50, 75, 100 mg/kg BW) as oral gavages. The rats were observed for possible incidence of death or other behavioral changes.

Statistical analysis. All statistical analyses were done by spss version 10 by one-way ANOVA using Dunnett's test.

CCDC-685500 (**6a**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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