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Preparation of 5-aryl-3-alkylthio-l,2,4-triazoles and corresponding sulfones with antiinflammatory–analgesic activity

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Abstract—In this study, a series of 5-aryl-3-alkylthio-1,2,4-triazoles and corresponding sulfones were prepared with the objective of developing better analgesic–antiinflammatory compounds with minimum ulcerogenic risk. The structures of the compounds were elucidated by spectral and elemental analysis. The compounds were assayed per os in mice for their antiinflammatory and analgesic activity as well as the ulcerogenic risk and acute toxicity. Several of these compounds showed significant activity. Alkylsulfone derivatives were found to be much more potent analgesic–antiinflammatory agents than the corresponding alkylthio analogs. Compounds **9** and **11** were the most active of the series in both analgesic and antiinflammatory activity tests. In contrast to reference compound acetyl salicylic acid, these compounds did not induce gastric lesions in the stomach of experimental animals at the doses that exhibited analgesic/antiinflammatory activity.

1. Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation in arthritis for decades. NSAIDs reduce the pain and swelling associated with arthritis by blocking the metabolism of arachidonic acid by cyclooxygenase enzyme (COX) thereby the production of prostaglandin.[1] Since prostaglandins are cytoprotective, administration of NSAIDs in long term may lead to development of threatening GI ulcers, bleeding, and renal disorders.[2–4] Recently discovery of two closely related cyclooxygenase (COX) isoforms, COX-1 and COX-2, which differ in their distribution and physiological roles, made it possible to separate the pharmacological effects from the general side effects of traditional NSAIDs. Therefore, fully selective and reversible COX-2 inhibitors with better safety profile have been marketed as a new generation of NSAIDs.[5,6] Recent reports evidence that coxibs can lead to adverse cardiovascular effects.[7] Therefore, although there are a number of antiinflammatory–analgesic drugs available in the market, development of novel compounds having antiinflammatory–analgesic activity with improved profile is still a necessity.

Over 10 years our interest has focused on the synthesis of novel heterocyclic systems which have analgesic/anti-inflammatory activity. We have synthesized various substituted 1,2,4-triazole-5-thiones and their some condensed derivatives and examined for their analgesic–antiinflammatory activity. A considerable number of the prepared compounds have anti-inflammatory activity comparable to or higher than the reference compounds besides lower ulcerogenic risks in the stomach.[8–12]

In the literature, differently substituted 3-alkylthio-1,2,4-triazole derivatives and corresponding sulfones have emerged as novel antiinflammatory compounds.[13–17] Moreover, cyclooxygenase-2 (COX-2) enzyme inhibitors which are a wide variety of heterocyclic compounds possess either an aminosulfonyl or methylsulfonyl group as common structural feature.[6] Recently, compound **I**, 5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-ethylthio-thiadiazole, has been attracting attention as a novel class COX-2 inhibitor (Fig. 1).[18]

Keywords: 5-Aryl-3-alkylthio-1,2,4-triazoles; 3-(Methyl/ethylsulfonyl)-5-aryl-1,2,4-triazoles; $KMnO_4$ catalyzed oxidation; Analgesic–antiinflammatory activity.
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Thus, in continuation to our lasting interest toward chemistry and pharmacological properties of 1,2,4-triazoles, in present study, we have designed and synthesized a series of 1,2,4-triazole derivatives having methyl/ethylsulfonyl functionality and determined their analgesic–antiinflammatory activities in an inflammatory pain model in mice.

2. Results and discussion

The general synthetic strategy employed to prepare the sulfone derivatives was based on $KMnO_4$-catalyzed oxidation reaction. As shown in Scheme 1, the starting compounds, 5-aryl-1,2,4-triazole-3-thiones (**1**–**5**), were obtained by the reaction of 4-acylthiosemicarbazide with KOH 10% under reflux, followed by the acidification with concentrated hydrochloric acid.[8] Alkylation of starting compounds with iodomethane/or ethane provided the methyl/ethyl thioether intermediates (**1a**–**5b**).[19] Treatment of 3-alkylthio compounds with $KMnO_4$ in acetic acid at 20 °C temperature resulted in the oxidation of sulfur (**6**–**15**) in 22–94% yields.[13]

According to applied condition on oxidation of alkylthio derivatives with $KMnO_4$, both sulphoxide and sulfone derivatives can be obtained. However, when 3-alkylthio compounds were treated with $KMnO_4$ in acetic acid, only one compound, which was determined to be a sufone on the basis of its spectral and elemental analysis, was obtained.

IR spectrum of the compounds exhibited NH stretching at 3226–3274 cm^{-1} and two series of SO_2 stretching bands, one between 1311 and 1332 cm^{-1} and the other one between 1123 and 1146 cm^{-1}. In the ^1H NMR, appearance of a downfield shift at SCH_3 (SCH_2–) and NH proton signals for sulfone compounds when compared with the alkylether derivatives indicated that oxidation reaction occurred. But it was hard to say that isolated product has either one or two oxygens in its molecule. Additional evidence was provided by mass spectra of the compounds. In the EI-MS spectra of the compounds, molecular ion peaks were observed for all compounds at different intensity verifying the molecular weight of a sulfone compound. The fragment peaks corresponding to loss of RSO_2^+ and $RSO_2CN_2^+$ from the molecular ion were consistent with the postulated structure. Furthermore, elemental analysis also confirmed all the structures proposed.

In the pharmacological study, we have investigated antiinflammatory and analgesic activity as well as the ulcerogenic risk and acute toxicity. Both thioether intermediates (**1a**–**5b**) and corresponding sulfones (**6**–**15**) were evaluated. In order to screen the antiinflammatory profile of the synthesized compounds, carrageenan-induced hind paw edema model in mice was used.[20] The analgesic activity of the compounds was studied by using the PBQ-induced writhing test in mice.[21] The animals were first administered at 100 mg/kg (body weight) dose of the test drugs in both screening tests. Test compounds which possessed more than 25% effect, even some of them are not significant statistically, were considered for further evaluation and the experiments were repeated for the selected compounds at three different dose levels (25, 50, and 200 mg/kg) (Tables 2 and 3). Ulcerogenic effect on acute administration was studied

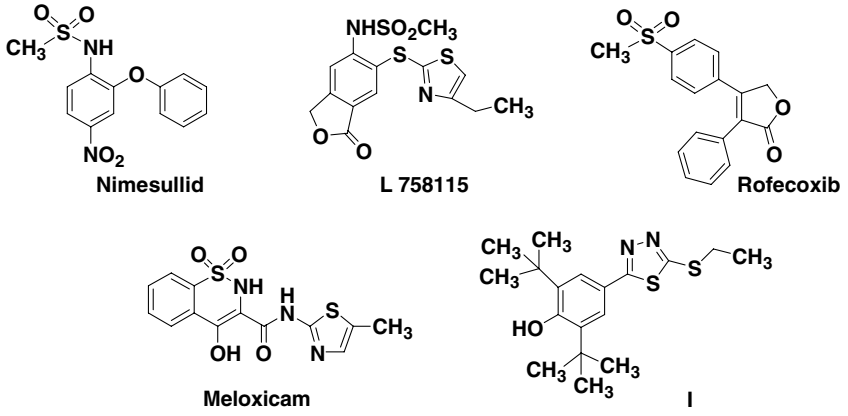
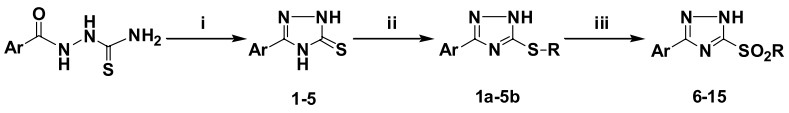


Figure 1. Structurally different COX-2 inhibitors.



Scheme 1. Synthetic pathway of compounds. Reagents and conditions: (i) 1—KOH 10%; 2—HCl; (ii) CH_3-/C_2H_5I in 1 M NaOH; 64–93%; (iii) $KMnO_4$ in AcOH, 20 °C; 22–94%. Ar = **1**: C_6H_5–; **2**: 2-ClC_6H_4–; **3**: 4-ClC_6H_4–; **4**: 3-BrC_6H_4–; **5**: 4-BrC_6H_4–; R = **a**: CH_3–, **b**: C_2H_5–; **6**: Ar = C_6H_5–, R = $–CH_3$; **7**: Ar = C_6H_5–, R = $–C_2H_5$; **8**: Ar = 2-ClC_6H_4–, R = $–CH_3$; **9**: Ar = 2-ClC_6H_4–, R = $–C_2H_5$; **10**: Ar = 4-ClC_6H_4–, R = $–CH_3$; **11**: Ar = 4-ClC_6H_4–, R = $–C_2H_5$; **12**: Ar = 3-BrC_6H_4–, R = $–CH_3$; **13**: Ar = 3-BrC_6H_4–, R = $–C_2H_5$; **14**: Ar = 4-BrC_6H_4–, R = $–CH_3$; **15**: Ar = 4-BrC_6H_4–, R = $–C_2H_5$.

to assess the safety of compounds. For the purpose of comparison, a nonselective COX inhibitor, acetylsalicylic acid, was used as positive control which caused severe bleeding at 100 mg/kg dose. According to obtained pharmacological results some preliminary conclusions can be drawn as follows:

As shown in Table 1, compounds **2b**, **3a**, **3b**, **7**–**11**, and **15** possess antiinflammatory activity at 100 mg/kg dose, po comparable with that of indomethacin or higher. The compounds had a good antiinflammatory profile coupled with notable analgesic properties (Table 2).

However, selected compounds when administered at a half dose (50 mg/kg, po) exhibited almost similar activity profile to 100 mg/kg dose level. But the compounds when administered at either increasing dose in a twofold (200 mg/kg) or decreasing dose to half (25 mg/kg), the activity was found to be decreased dramatically (Tables 2 and 3).

In spite of the high gastric ulcer incidence in reference compound, acetylsalicylic acid, the compounds were generally found safe from the viewpoint of ulcer induction at 50 mg/kg dose level. Only compounds **3b** and **8** caused some gastric lesions at 100 mg/kg dose on microscopic examination.

In order to probe structural requirements for optimal analgesic–antiinflammatory activity in this series, the substituents on the phenyl ring, length of alkyl group attached to sulfur and oxidation of sulfur to sulfone have been examined. In regard to structure the most important variable affecting the activity was the oxidation of sulfur to sulfone. Analgesic and anti-inflammatory activity of the compounds having an alkylsulfone were found to be greater than those of alkylthio (compare **1b/7**; **2a/8**; **2b/9**; **3b/11**; etc.). When compared the effect of substituent on the phenyl ring, a chlorine substituent always resulted in good activity irrespective of the position of chlorine. Whereas, replacing the chlorine by bromine produced inactive compounds with side effects except compound **15**. It showed both analgesic and anti-inflammatory activity at 50 mg/kg dose despite it was highly less when compared with 4-Cl analog (**11**). Compounds **1a**–**5a**, **6**, **8**, **10**, **12**, **14** and **1b**–**5b**, **7**, **9**, **11**, **13**, **15** carrying methyl and ethyl substituents, respectively, were synthesized to investigate whether lipophilicity of derivatives influences the activity profile of the compounds or not. As shown in Tables 1–3, although lipophilicity did not produce notable superiority in activity of alkylthio intermediates, an increase in both analgesic and antiinflammatory activities was observed with elongation of alkyl chain in sulfone derivatives (compare **6/7**; **8/9**; **10/11**; etc.). Among the compounds with ethyl, compounds **9** and **11** having 2-chlorophenyl and 4-chlorophenyl exhibited the highest analgesic and antiinflammatory activity, with percentage inhibition values 54.9%, 59.5% and 37.9%, 40.2%, respectively, at 50 mg/kg dose level.

The acute toxicity assay showed that none of the evaluated compounds produced lethal effects and did not induce any appreciable behavioral change at the administered doses during observation period.

Table 1. Preliminary antiinflammatory effects of the compounds against carrageenan-induced paw edema in mice at 100 mg/kg dose ($n = 6$)

Test samples	Swelling thickness ($\times 10^{-2}$mm) ± SEM (inhibition %)			
	90 min	180 min	270 min	360 min
Control	44.8 ± 5.8	51.5 ± 6.3	58.8 ± 7.5	68.8 ± 6.6
1a	38.1 ± 3.5 (14.9)	44.5 ± 3.4 (13.6)	47.6 ± 2.4 (19.0)	54.4 ± 2.3 (20.9)
1b	46.0 ± 6.8	48.3 ± 6.8	51.2 ± 6.9	54.7 ± 6.7
2a	42.7 ± 5.7 (4.7)	48.8 ± 5.5 (5.2)	52.8 ± 5.4 (10.2)	57.0 ± 5.7 (17.2)
2b	33.2 ± 5.7 (25.9)	37.8 ± 5.6 (26.6)	42.2 ± 5.5 (28.2)	47.5 ± 5.7 (30.9)
3a	34.0 ± 5.4 (24.1)	38.3 ± 5.2 (25.6)	43.0 ± 4.7 (26.9)	48.0 ± 4.8 (**30.2**)*
3b	34.3 ± 6.1 (23.4)	38.2 ± 6.2 (25.8)	40.7 ± 6.9 (30.8)	44.2 ± 6.9 (35.8)
4a	47.5 ± 3.8	57.3 ± 3.7	67.7 ± 4.2	79.5 ± 3.7
4b	48.5 ± 6.6	52.3 ± 6.7	55.3 ± 6.5	58.3 ± 6.5
5a	46.9 ± 5.9	54.1 ± 5.8	63.3 ± 6.1	70.9 ± 6.9
5b	59.6 ± 2.5	69.2 ± 2.6	78.2 ± 3.1	88.3 ± 3.3
6	44.2 ± 4.7 (1.3)	45.2 ± 5.0 (12.2)	47.5 ± 56.7 (19.2)	56.7 ± 3.7
7	33.2 ± 3.6 (25.9)	35.2 ± 4.3 (**31.7**)*	39.2 ± 3.8 (**33.3**)**	43.5 ± 4.1 (**36.8**)**
8	35.0 ± 3.4 (21.9)	39.0 ± 3.1 (24.3)	41.3 ± 3.6 (**29.8**)*	44.5 ± 3.7 (**35.3**)**
9	29.8 ± 3.6 (**33.4**)*	33.8 ± 3.6 (**34.4**)**	35.0 ± 3.9 (**40.5**)**	38.5 ± 3.8 (**44.0**)***
10	37.5 ± 4.7 (16.3)	42.8 ± 4.3 (16.9)	46.2 ± 4.9 (21.4)	51.2 ± 6.3 (25.6)
11	27.2 ± 3.8 (**39.3**)**	31.0 ± 3.6 (**39.8**)**	34.5 ± 3.9 (**41.3**)***	35.3 ± 4.5 (**48.7**)***
12	48.2 ± 3.4	55.9 ± 3.4	65.8 ± 4.1	80.5 ± 3.6
13	68.8 ± 4.9	74.9 ± 4.9	85.6 ± 5.5	91.7 ± 4.2
14	51.2 ± 3.8	55.2 ± 3.9	59.5 ± 3.2	64.0 ± 3.5
15	32.1 ± 2.7 (28.3)	38.3 ± 2.6 (25.6)	45.7 ± 2.9 (22.3)	49.9 ± 2.1 (27.5)
Indomethacin	31.9 ± 3.5 (28.8)	34.8 ± 3.1 (**32.4**)*	36.5 ± 3.3 (**37.9**)**	39.7 ± 2.2 (**42.3**)***

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ significant from control.

Table 2. Dose-dependent analgesic effects against PBQ-induced writhings and ulcer score of synthesized compounds in mice ($n = 6$)

Compound	Dose, per os mg/kg							
	25		50		100		200	
	Number of writhings ± SEM (% inhibition)	Ratio of ulceration	Number of writhings ± SEM (% inhibition)	Ratio of ulceration	Number of writhings ± SEM (% inhibition)	Ratio of ulceration	Number of writhings ± SEM (% inhibition)	Ratio of ulceration
Control	51.4 ± 5.1	0/6	51.4 ± 5.1	0/6	47.3 ± 4.9	0/6	51.4 ± 5.1	0/6
ASA	—	—	—	—	21.2 ± 2.0 (**55.2**)***	5/6	23.8 ± 2.2 (**53.6**)***	5/6
1a	—	—	—	—	40.2 ± 3.7 (15.0)	0/6	—	—
1b	—	—	—	—	46.1 ± 5.2 (2.5)	0/6	—	—
2a	—	—	—	—	41.8 ± 4.2 (11.6)	0/6	—	—
2b	48.5 ± 4.2 (5.6)	0/6	37.8 ± 3.2 (26.4)	0/6	35.2 ± 3.4 (25.6)	0/6	41.3 ± 3.9 (19.6)	2/6
3a	44.7 ± 3.3 (13.0)	0/6	34.1 ± 2.8 (**33.7**)*	0/6	34.1 ± 2.5 (**27.9**)*	0/6	43.3 ± 2.1 (15.8)	0/6
3b	45.1 ± 2.8 (12.3)	0/6	34.2 ± 3.4 (**33.5**)**	0/6	33.8 ± 3.0 (**28.5**)*	2/6	44.8 ± 3.7 (12.8)	5/6
4a	—	—	—	—	46.8 ± 5.1 (1.1)	0/6	—	—
4b	—	—	—	—	51.5 ± 2.7	0/6	—	—
5a	—	—	—	—	58.9 ± 3.1	2/6	—	—
5b	—	—	—	—	49.2 ± 3.7	1/6	—	—
6	—	—	—	—	37.4 ± 3.2 (20.9)	0/6	—	—
7	38.2 ± 3.4 (25.7)	0/6	26.7 ± 1.8 (**48.1**)***	0/6	27.5 ± 2.4 (**41.9**)***	0/6	48.5 ± 3.2 (5.6)	3/6
8	40.1 ± 3.9 (21.9)	0/6	30.4 ± 3.0 (**40.9**)***	0/6	32.2 ± 2.1 (**31.9**)**	1/6	45.3 ± 2.7 (11.9)	3/6
9	38.2 ± 3.0 (25.7)	0/6	23.4 ± 2.4 (**54.5**)***	0/6	23.4 ± 2.9 (**50.5**)***	0/6	45.9 ± 4.2 (10.7)	1/6
10	42.8 ± 3.9 (16.7)	0/6	29.4 ± 2.7 (**42.8**)***	0/6	31.0 ± 2.3 (**34.5**)**	0/6	46.8 ± 3.3 (8.9)	2/6
11	37.8 ± 2.9 (**26.5**)*	0/6	20.8 ± 2.1 (**59.5**)***	0/6	20.4 ± 2.1 (**56.9**)***	0/6	44.1 ± 3.8 (18.5)	1/6
12	—	—	—	—	48.2 ± 2.5	0/6	—	—
13	—	—	—	—	45.2 ± 3.1 (4.4)	2/6	—	—
14	—	—	—	—	49.4 ± 3.7	1/6	—	—
15	40.9 ± 3.7 (20.4)	0/6	35.9 ± 3.9 (**30.2**)*	0/6	35.2 ± 4.9 (25.6)	0/6	50.6 ± 3.7 (1.6)	3/6

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ significant from control.

Table 3. Dose-dependent antiinflammatory effects of selected compounds against carrageenan-induced paw edema at mice in different doses ($n = 6$)

Test samples	Dose (mg/kg)	Swelling thickness ($\times 10^{-2}$mm) ± SEM (inhibition %)			
		90 min	180 min	270 min	360 min
Control		53.2 ± 4.7	59.4 ± 4.1	64.8 ± 5.2	72.0 ± 5.1
2b	25	49.3 ± 2.6 (7.3)	54.8 ± 2.9 (7.7)	60.5 ± 3.8 (6.6)	68.9 ± 3.5 (4.3)
	50	45.2 ± 3.2 (15.0)	42.8 ± 3.4 (27.9)	46.8 ± 4.1 (27.8)	50.7 ± 3.2 (**29.6**)*
	200	55.8 ± 2.4	61.2 ± 3.1	67.9 ± 4.0	75.9 ± 3.5
3a	25	43.5 ± 3.0 (18.2)	48.9 ± 3.7 (17.7)	51.5 ± 3.2 (20.5)	58.9 ± 3.3 (18.2)
	50	37.4 ± 3.2 (29.7)	41.2 ± 3.5 (**30.6**)*	42.2 ± 3.2 (**34.9**)**	47.1 ± 3.8 (**34.6**)**
	200	54.7 ± 4.2	61.2 ± 3.9	65.9 ± 4.0	76.8 ± 3.4
3b	25	45.9 ± 3.8 (13.7)	49.9 ± 3.1 (15.9)	53.8 ± 3.6 (16.9)	59.7 ± 3.1 (17.1)
	50	39.1 ± 4.5 (26.5)	43.2 ± 4.9 (27.3)	45.8 ± 4.1 (**29.3**)*	43.1 ± 3.0 (**40.1**)**
	200	51.8 ± 2.1 (2.6)	59.9 ± 2.6	63.5 ± 3.0 (2.0)	74.2 ± 2.4 (3.4)
7	25	48.6 ± 3.6 (8.6)	54.9 ± 2.7 (7.7)	58.1 ± 3.0 (10.3)	62.4 ± 2.9 (13.3)
	50	39.2 ± 3.1 (26.3)	41.3 ± 3.3 (**30.5**)*	44.6 ± 3.0 (**31.2**)**	44.2 ± 3.2 (**38.6**)**
	200	47.9 ± 5.4 (9.9)	52.3 ± 5.3 (11.9)	57.6 ± 5.9 (11.1)	65.2 ± 5.2 (9.4)
8	25	49.1 ± 4.2 (7.7)	56.9 ± 3.8 (4.2)	60.2 ± 3.9 (7.1)	67.1 ± 3.4 (6.8)
	50	39.1 ± 2.7 (26.5)	41.2 ± 3.4 (**30.6**)*	43.1 ± 3.1 (**33.5**)**	45.5 ± 3.9 (**36.8**)**
	200	49.8 ± 2.1 (6.4)	54.6 ± 2.7 (8.1)	59.8 ± 2.4 (7.7)	65.1 ± 2.2 (9.6)
9	25	47.2 ± 3.7 (11.3)	54.4 ± 3.9 (8.4)	59.1 ± 4.2 (8.8)	61.5 ±4.0 (14.6)
	50	36.2 ± 2.3 (**31.9**)**	35.8 ± 2.8 (**39.7**)**	41.4 ± 3.0 (**36.1**)**	40.5 ± 2.9 (**43.8**)***
	200	43.1 ± 4.2 (18.9)	45.8 ± 3.9 (22.9)	51.9 ± 4.2 (19.9)	56.5 ± 4.6 (21.5)
10	25	55.2 ± 4.3	62.8 ± 4.8	69.5 ± 4.5	75.4 ± 5.4
	50	43.6 ± 3.4 (18.0)	46.2 ± 3.8 (22.2)	47.9 ± 4.2 (26.1)	50.2 ± 4.6 (**30.3**)*
	200	59.4 ± 3.7	65.8 ± 4.4	69.4 ± 4.2	76.1 ± 5.7
11	25	45.8 ± 3.0 (13.9)	49.6 ± 3.4 (16.5)	57.9 ± 3.1 (10.6)	61.2 ± 3.7 (15.0)
	50	35.4 ± 3.0 (**33.5**)*	36.1 ± 3.2 (**39.2**)**	37.2 ± 3.1 (**42.6**)***	39.4 ± 3.5 (**45.3**)***
	200	45.5 ± 5.7 (14.5)	48.9 ± 5.2 (17.7)	55.4 ± 5.4 (14.5)	58.4 ± 4.8 (18.9)
15	25	54.6 ± 3.7	62.8 ± 4.0	67.8 ± 4.6	75.1 ± 5.2
	50	40.4 ± 2.9 (24.1)	43.5 ± 2.1 (26.8)	49.1 ± 2.5 (24.2)	50.4 ± 2.7 (**30.0**)*
	200	57.4 ± 5.2	61.5 ± 4.9	68.5 ± 5.7	76.2 ± 5.5
Indomethacin	10	34.5 ± 3.0 (**35.2**)**	39.1 ± 3.1 (**34.2**)**	37.5 ± 2.9 (**42.1**)***	40.2 ± 2.4 (**44.2**)***

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ significant from control.

3. Conclusion

We have described the preparation of 5-aryl-3-alkylthio-1,2,4-triazoles and their corresponding sulfones. Several compounds have been evaluated as potential analgesic–antiinflammatory agents devoid of ulcerogenic potential. In conclusion, this preliminary investigation showed that analgesic–antiinflammatory activity of 5-aryl-3-alkylthio-1,2,4-triazoles can be significantly modified by substitution on the phenyl ring and sulfur or oxidation of sulfur to sulfone. Among the synthesized compounds, **9** and **11** possessed the most prominent and consistent activity with no ulcerogenic effect. Therefore, such compounds would represent a fruitful matrix for the development of a new class of analgesic–antiinflammatory agents and would deserve further investigation and derivatization as a promising scaffold.

The possibility that these compounds would be selective COX-2 inhibitors will be investigated in our future studies.

4. Materials and methods

4.1. Chemistry

Melting points were detected with a Thomas Hoover capillary melting point apparatus (Philadelphia, PA;USA) and are uncorrected. IR spectra (KBr) were recorded on Perkin-Elmer 1720X FT-IR spectrometer (Beaconsfield, UK). ^{1}H NMR spectra were taken on Bruker 400 MHz FT-NMR instrument (Karlsruhe, Germany) in DMSO-d_6 using TMS as internal standard. All chemical shift values were recorded as δ (ppm). Mass spectra were measured on a Agilent 1100 HPLC with MSD detector. The purity of the compounds was checked on silicagel-coated aluminium sheets (Merck, 1.005554, silicagel $HF_{254-361}$, Type 60, 0.25 mm, Darmstadt, Germany) by thin-layer chromatography. The elementary analysis of the resulting compounds was performed with Leco CHNS 932 analyzer (Philadelphia, USA) at the Scientific and Technical Research Council of Turkey, Instrumental Analysis Laboratory in Ankara. All chemicals were from Aldrich Chemical Co. (Steinheim, Germany).

4.2. Synthesis of 5-aryl-3-mercapto-1,2,4-triazoles (1–5)

Compounds **1**–**5** were prepared as per the method reported earlier.[8]

4.3. Synthesis of 3-(alkylthio)-5-aryl-1,2,4-triazoles (1a–5b)

For the synthesis of compounds, synthetic route previously published by John M. Kane et al.[19] was followed with a slight modification. The corresponding 5-aryl-3-mercapto-1,2,4-triazole (5.2 mmol) was dissolved in 1 M aqueous NaOH (12 ml). Then a solution of CH_3I/C_2H_5I (8.5 mmol) in EtOH (2.5 ml) was added. After being stirred for 3 h at room temperature, the reaction mixture was neutralized with 10% HCl. The obtained precipitate was collected. Several of methyl/ethyl thioether intermediates (except for **4a**, **4b**, **5a**, and **5b**) have already been reported previously.[22–27]

4.3.1. 3-(Methylthio)-5-(3-bromophenyl)-1,2,4-triazole (4a). Yield 64%; mp 139–140 °C. ^{1}H NMR δ (ppm): **2.65** (s, 3H, CH_3), 7.25 (d, J = 7.8 Hz, 1H, arom.H), 7.45 (d, J = 8.0 Hz, 1H, arom.H), 7.89 (d, J = 7.8 Hz, 1H, arom.H), 8.13 (s, 1H, arom.H).

4.3.2. 3-(Ethylthio)-5-(3-bromophenyl)-1,2,4-triazole (4b). Yield 67%; mp 84–85 °C. ^{1}H NMR δ (ppm): 1.45 (t, 3H, CH_3), **3.24** (q, 2H, CH_2), 7.28 (t, 1H, arom.H), 7.52 (d, J = 8.0 Hz, 1H, arom.H), 7.94 (d, J = 7.8 Hz, 1H, arom.H), 8.20 (t, 1H, arom.H), 10.30 (br, 1H, NH).

4.3.3. 3-(Methylthio)-5-(4-bromophenyl)-1,2,4-triazole (5a).[28] Yield 93%; mp 149–150 °C. ^{1}H NMR δ (ppm): **2.58** (s, 3H, CH_3), 7.45 (d, J = 8.5 Hz, 2H, arom.H), 7.85 (d, J = 8.4 Hz, 2H, arom.H), 13.50 (br, 1H, NH).

4.3.4. 3-(Ethylthio)-5-(4-bromophenyl)-1,2,4-triazole (5b). Yield 79%; mp 139–140 °C. ^{1}H NMR δ (ppm): 1.43 (t, 3H, CH_3), **3.24** (q, 2H, CH_2), 7.57 (d, J = 8.6 Hz, 2H, arom.H), 7.90 (d, J = 8.4 Hz, 2H, arom.H), 9.70 (br, 1H, NH).

4.4. Synthesis of 3-(methyl/ethylsulfonyl)-5-aryl-1,2,4-triazoles (6–15)

Powdered potassium permanganate (0.885 g; 5.6 mmol) was added portionwise to a stirred solution of 3-alkylthio compound (1.89 mmol) in acetic acid 66% (10 ml) at 20 °C over a period of 1 h. After the mixture had been stirred at room temperature for additional 2 h, it was decolorized with 12 ml of $NaHSO_3$ solution (20%). The resulting precipitate was filtered and crystallized to afford the desired compound.

Yields, melting points, and spectral and analytical data of synthesized compounds are given below.

4.4.1. 3-(Methylsulfonyl)-5-phenyl-1,2,4-triazole (6). Yield 67%; mp 151–152 °C (xylene), lit.[26] 142–144 °C (benzene–EtOH); IR (KBr): 3243 (N–H), 1334, 1320, 1133 (SO_2) cm^{-1}. ^{1}H NMR δ (ppm): **3.24** (s, 3H, CH_3), 7.35–7.40 (m, 3H, arom.H), 7.95–8.00 (m, 2H, arom.H), 14.50 (br, 1H, NH); MS (70 eV, EI): *m*/*z* (%): 225 ($M+2^{+\cdot}$, 3%), 223 ($M^{+\cdot}$, 100%), 144 ($M^{+\cdot}-SO_2CH_3$, 4%), 104 ($M^{+\cdot}-CH_3SO_2CN_2^{\cdot}$, 32%); Anal. Calcd for $C_9H_9N_3O_2S$: C, 48.42; H, 4.06; N, 18.82; S, 14.36. Found: C, 48.91; H, 4.20; N, 18.57; S, 14.14.

4.4.2. 3-(Ethylsulfonyl)-5-phenyl-1,2,4-triazole (7). Yield 47%; mp 140–141 °C (water); IR (KBr): 3247 (N–H), 1325, 1123 (SO_2) cm^{-1}. ^{1}H NMR δ (ppm): 1.29 (t, J = 7.4 Hz, 3H, CH_3), **3.32** (q, J = 7.4 Hz, 2H, CH_2), 7.36–7.42 (m, 3H, arom.H), 7.94–8.00 (m, 2H, arom.H), 12.80 (br, 1H, NH); MS (70 eV, EI): *m*/*z* (%): 239 ($M+2^{+\cdot}$, 2%), 237 ($M^{+\cdot}$, 58%), 145 ($M^{+\cdot}-SO_2C_2H_4$, 38%), 104 ($M^{+\cdot}-C_2H_5SO_2CN_2^{\cdot}$, 62%), 103 ($M^{+\cdot}-C_2H_5SO_2CN_2H^{\cdot}$, 100%); Anal. Calcd for $C_{10}H_{11}N_3O_2S$: C, 50.62; H, 4.67; N, 17.71; S, 13.51. Found: C, 50.35; H, 4.53; N, 17.31; S, 13.32.

4.4.3. 3-(Methylsulfonyl)-5-(2-chlorophenyl)-1,2,4-triazole (8). Yield 79%; mp 198–199 °C (water); IR (KBr): 3251 (N–H), 1323, 1160 (SO_2) cm^{-1}. ^1H NMR δ (ppm): **3.24** (s, 3H, CH_3), 7.20–7.35 (m, 4H, arom.H), 11.90 (br, 1H, NH); MS (70 eV, EI): *m/z* (%): 259 ($M+2^{+\cdot}$, 23%), 257 ($M^{+\cdot}$, 100%), 178 ($M^{+\cdot}-SO_2CH_3$, 3%), 138 ($M^{+\cdot}-CH_3SO_2CN_2^{\cdot}$, 86%), 137 ($M^{+\cdot}-CH_3SO_2CN_2H^{\cdot}$, 65%); Anal. Calcd for $C_9H_8ClN_3O_2S$: C, 41.95; H, 3.13; N, 16.31; S, 12.44. Found: C, 42.24; H, 2.76; N, 16.10; S, 12.19.

4.4.4. 3-(Ethylsulfonyl)-5-(2-chlorophenyl)-1,2,4-triazole (9). Yield 45%; mp 127–128 °C (water); IR (KBr): 3241 (N–H), 1320, 1129 (SO_2) cm^{-1}. ^1H NMR δ (ppm): 1.30 (t, $J = 7.4$ Hz, 3H, CH_3), **3.34** (q, $J = 7.4$ Hz, 2H, CH_2), 7.30–7.50 (m, 3H, arom.H), 7.70–7.80 (m, 1H, arom.H), 12.20 (br, 1H, NH); MS (70 eV, EI): *m/z* (%): 273 ($M+2^{+\cdot}$, 7%), 271 ($M^{+\cdot}$, 17%), 178 ($M^{+\cdot}-SO_2C_2H_4$, 57%), 138 ($M^{+\cdot}-C_2H_5SO_2CN_2^{\cdot}$, 100%), 137 ($M^{+\cdot}-C_2H_5SO_2CN_2H^{\cdot}$, 99%); Anal. Calcd for $C_{10}H_{10}ClN_3O_2S$: C, 44.20; H, 3.71; N, 15.46; S, 11.80. Found: C, 44.63; H, 3.13; N, 15.33; S, 11.58.

4.4.5. 3-(Methylsulfonyl)-5-(4-chlorophenyl)-1,2,4-triazole (10).[28] Yield 94%; mp 199–200 °C (water); IR (KBr): 3269 (N–H), 1313, 1157 (SO_2) cm^{-1}. ^1H NMR δ (ppm): **3.25** (s, 3H, CH_3), 7.39 (d, $J = 8.6$ Hz, 2H, arom.H), 7.98 (d, $J = 8.6$ Hz, 2H, arom.H), 14.80 (br, 1H, NH); MS (70 eV, EI): *m/z* (%): 259 ($M+2^{+\cdot}$, 37%), 257 ($M^{+\cdot}$, 100%), 178 ($M^{+\cdot}-SO_2CH_3$, 6%), 138 ($M^{+\cdot}-CH_3SO_2CN_2^{\cdot}$, 33%), 137 ($M^{+\cdot}-CH_3SO_2CN_2H^{\cdot}$, 45%); Anal. Calcd for $C_9H_8ClN_3O_2S$: C, 41.95; H, 3.13; N, 16.31; S, 12.44. Found: C, 42.47; H, 3.01; N, 16.05; S, 12.21.

4.4.6. 3-(Ethylsulfonyl)-5-(4-chlorophenyl)-1,2,4-triazole (11). Yield 76%; mp 185–186 °C (water); IR (KBr): 3240 (N–H), 1329, 1137 (SO_2) cm^{-1}. ^1H NMR δ (ppm): 1.45 (t, $J = 7.4$ Hz, 3H, CH_3), **3.35** (q, $J = 7.4$ Hz, 2H, CH_2), 7.42 (d, $J = 8.3$ Hz, 2H, arom.H), 7.93 (d, $J = 8.3$ Hz, 2H, arom.H), 11.45 (br, 1H, NH); MS (70 eV, EI): *m/z* (%): 273 ($M+2^{+\cdot}$, 28%), 271 ($M^{+\cdot}$, 81%), 179 ($M^{+\cdot}-SO_2C_2H_4$, 36%), 138 ($M^{+\cdot}-C_2H_5SO_2CN_2^{\cdot}$, 35%), 137 ($M^{+\cdot}-C_2H_5SO_2CN_2H^{\cdot}$, 100%); Anal. Calcd for $C_{10}H_{10}ClN_3O_2S$: C, 44.20; H, 3.71; N, 15.46; S, 11.80. Found: C, 44.05; H, 3.64; N, 15.08; S, 11.34.

4.4.7. 3-(Methylsulfonyl)-5-(3-bromophenyl)-1,2,4-triazole (12). Yield 67%; mp 152–153 °C (water); IR (KBr): 3226 (N–H), 1311, 1122 (SO_2) cm^{-1}. ^1H NMR δ (ppm): **3.35** (s, 3H, CH_3), 7.40 (t, $J = 8.1$ Hz, 1H, arom.H), 7.65 (d, $J = 7.8$ Hz, 1H, arom.H), 7.92 (d, $J = 7.8$ Hz, 1H, arom.H), 8.19 (t, $J = 8.1$ Hz, 1H, arom.H); MS (70 eV, EI): *m/z* (%): 305 ($M+4^{+\cdot}$, 8%), 304 ($M+3^{+\cdot}$, 12%), 303 ($M+2^{+\cdot}$, 99%), 301 ($M^{+\cdot}$, 100%), 222 ($M^{+\cdot}-SO_2CH_3$, 3%), 183 ($M^{+\cdot}-CH_3SO_2CN_2^{\cdot}$, 41%); Anal. Calcd for $C_9H_8BrN_3O_2S$: C, 35.78; H, 2.67; N, 13.91; S, 10.61. Found; C, 36.14; H, 2.26; N, 13.61; S, 10.63.

4.4.8. 3-(Ethylsulfonyl)-5-(3-bromophenyl)-1,2,4-triazole (13). Yield 22%; mp 142–143 °C (water); IR (KBr): 3220 (N–H), 1332, 1131 (SO_2) cm^{-1}. ^1H NMR δ (ppm): 1.35 (t, $J = 7.4$ Hz, 3H, CH_3), **3.35** (q, $J = 7.4$ Hz, 2H, CH_2), 7.30 (t, $J = 7.9$ Hz, 1H, arom.H), 7.55 (d, $J = 7.9$ Hz, 1H, arom.H), 7.93 (d, $J = 7.4$ Hz, 1H, arom.H), 8.15 (s, 1H, arom.H); MS (70 eV, EI): *m/z* (%): 319 ($M+4^{+\cdot}$, 42%), 317 ($M+2^{+\cdot}$, 61%), 315 ($M^{+\cdot}$, 53%), 223 ($M^{+\cdot}-SO_2C_2H_4$, 35%), 181 ($M^{+\cdot}-C_2H_5SO_2CN_2^{\cdot}$, 63%); Anal. Calcd for $C_{10}H_{10}BrN_3O_2S$: C, 37.99; H, 3.19; N, 13.29; S, 10.14. Found: C, 38.22; H, 2.95; N, 12.99; S, 9.95.

4.4.9. 3-(Methylsulfonyl)-5-(4-bromophenyl)-1,2,4-triazole (14).[28] Yield 68%; mp 193–194 °C (water); IR (KBr): 3274 (N–H), 1312, 1127 (SO_2) cm^{-1}. ^1H NMR δ (ppm): **3.25** (s, 3H, CH_3), 7.55 (d, $J = 8.5$ Hz, 2H, arom.H), 7.91 (d, $J = 8.5$ Hz, 2H, arom.H), 14.95 (br, 1H, NH); MS (70 eV, EI): *m/z* (%): 305 ($M+4^{+\cdot}$, 4%), 304 ($M+3^{+\cdot}$, 7%), 303 ($M+2^{+\cdot}$, 66%), 301 ($M^{+\cdot}$, 100%), 222 ($M^{+\cdot}-SO_2CH_3$, 3%), 183 ($M^{+\cdot}-CH_3SO_2CN_2^{\cdot}$, 32%); Anal. Calcd for $C_9H_8BrN_3O_2S$: C, 35.78; H, 2.67; N, 13.91; S, 10.61. Found: C, 36.26; H, 2.33; N, 13.62; S, 10.49.

4.4.10. 3-(Ethylsulfonyl)-5-(4-bromophenyl)-1,2,4-triazole (15). Yield 33%; mp 197–198 °C (water); IR (KBr): 3245 (N–H), 1329, 1138 (SO_2) cm^{-1}. ^1H NMR δ (ppm): 1.40 (t, $J = 7.5$ Hz, 3H, CH_3), **3.35** (q, $J = 7.5$ Hz, 2H, CH_2), 7.58 (d, $J = 8.2$ Hz, 2H, arom.H), 7.85 (d, $J = 8.3$ Hz, 2H, arom.H), 13.40 (br, 1H, NH); MS (70 eV, EI): *m/z* (%): 317 ($M+2^{+\cdot}$, 36%), 315 ($M^{+\cdot}$, 36%), 223 ($M^{+\cdot}-SO_2C_2H_4$, 24%), 181 ($M^{+\cdot}-C_2H_5SO_2CN_2^{\cdot}$, 76%); Anal. Calcd for $C_{10}H_{10}BrN_3O_2S$: C, 37.99; H, 3.19; N, 13.29; S, 10.14. Found: C, 38.12; H, 2.99; N, 12.95; S, 10.07.

4.5. Pharmacological procedures

4.5.1. Animals. Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Hıfzısıhha Institute (Ankara, Turkey). The animals were left for two days for acclimatization to animal room conditions and were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but allowed free access to water. A minimum of six animals was used in each group. Mice used in the present study were cared in accordance with the directory of Refiksaydam Hıfzıssıhha Institute's Animal Care Unit, which applies the guidelines of National Institutes of Health on laboratory animal welfare.

4.5.2. Preparation of test samples for bioassay. Test samples were given orally to test animals after suspending in 0.5% sodium carboxymethyl cellulose (CMC) and distilled water. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle.

4.5.3. Anti inflammatory activity

4.5.3.1. Carrageenan-induced edema test in mice.[20] For the determination of the effects on carrageenan-induced paw edema, the modified method of Kasahara et al. was employed. Sixty minutes after the oral admin-

istration of either test sample or dosing vehicle, each mouse was injected with freshly prepared (0.5 mg/25 μl) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiological saline (154 mM NaCl) into subplantar tissue of the right hind paw. As the control, 25 μl saline solution was injected into that of the left hind paw. Paw edema was measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) in 0.5% CMC was used as reference drug.

4.5.4. Antinociceptive activity

4.5.4.1. *p*-Benzoquinone-induced abdominal constriction test in mice.[21] Sixty minutes after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml/10 g body weight of 2.5% (v/v) *p*-benzoquinone (PBQ; Merck) solution in distilled water. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th minute after the PBQ injection. The data represent average of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls. Acetyl salicylic acid (ASA) (100 mg/kg) in 0.5% CMC was used as reference drug.

4.5.5. Gastric-ulcerogenic effect

4.5.5.1. Ulcerogenic effect on acute administration. Three hours after the analgesic activity experiment, mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through great curvature and examined under dissecting microscope for lesions or bleedings.

4.5.6. Acute toxicity. Single oral doses of test compounds, suspended in 0.5% carboxymethylcellulose, were administered to groups, at increasing doses, that is, 25, 50, 100, and 200 mg/kg, after a 24 h fast. Control group animals received only 0.5% carboxy methylcellulose. One hour later of dosing, food and water were allowed ad libitum. The animals were observed at 1 h after drug administration and then once daily for 72 h for signs of toxicity and mortality.

4.5.7. Statistical analysis of data. Data obtained from animal experiments were expressed as mean± standard error (±SEM). Statistical differences between the treatments and the control were tested by one-way analysis of variance (ANOVA) and Student–Newman–Keuls post hoctest. A value of $p < 0.05$ was considered to be significant.

References and notes

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28. Compounds **5a** (309266-60-2), **10** (312537-88-5), and **14** (309266-91-9) are seemed as commercial products in 'Science Finder.' Since there is no information in the literature for the preparation and spectral characteristics, these three compounds have been included in our research program and characterized by spectral data.