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Original article

Synthesis of 3,6-disubstituted 7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines as novel analgesic/anti-inflammatory compounds

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ABSTRACT

In this study, a new class of 4-amino-3-substituted-1,2,4-triazole-5-thiones (**1**–**4**) and their corresponding condensed derivatives 3,6-disubstituted 7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**1a**–**4c**) were synthesized and evaluated for their analgesic/anti-inflammatory activities. All synthesized compounds were also tested for their gastric toxicity and antioxidant activity on acute administration. Most of the compounds showed significant activity in both carrageenan-induced oedema and acetic acid-induced writhing tests besides negligible gastrointestinal toxicity. The compounds showing less ulcerogenic effect also showed less lipid peroxidation (LPO) level. Most promising results were obtained with the compounds that placed a fluoro or a chloride on the phenyl ring at the sixth position of the fused ring.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most useful clinical therapies for the treatment of inflammation, pain, and inflammation-related disorders. Unfortunately, their therapeutic and side effects are closely related to their biochemical action. The anti-inflammatory mechanism of NSAIDs is due to a reduction of prostaglandin synthesis by inhibiting cyclo-oxygenase enzyme (COX) in arachidonic acid metabolism [1]. The disruption of cytoprotective prostaglandins by all currently used NSAIDs results in unwanted adverse effects, including gastrointestinal PUB (perforation, ulceration, and bleeding), and renal toxicity. These results thus limit their therapeutic usefulness when long-term treatment is necessary [2–5]. There is still a need to synthesize novel, potent analgesic/anti-inflammatory compounds that have reduced side effects when compared with the drugs currently available in the market.

In recent decades, the literature has been enriched with progressive findings about the synthesis and pharmacological activities of 4-amino-1,2,4-triazole-5-thione ring, which is a core structure in various synthetic pharmaceuticals displaying a wide variety of biological activities [6]. A survey of literature revealed that some fused heterocyclic compounds prepared from aminotriazolethiones (e.g., triazolothiadiazoles (**I**), triazolothiadiazines (**II**)) have received much attention because they are widely used as anti-inflammatory and analgesic agents [7–11]. Recently 5-amino-6-(2,3,5-trichlorophenyl)-1,2,4-triazine (**III**), which is an isostere of 1,3,4-thiadiazine ring, has been reported as novel analgesic compound [12] (Scheme 1). Moreover, our former studies [13–19] have shown that certain compounds bearing a 1,2,4-triazole nucleus possess significant analgesic/anti-inflammatory activity with reduced gastrointestinal toxicity. We therefore considered it worthwhile to design and synthesize a novel series of 4-amino-3-substituted-1,2,4-triazole-5-thiones (**1**–**4**) and their corresponding condensed 3,6-disubstituted 7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives (**1a**–**4c**), which were expected to show anti-inflammatory/analgesic properties. This paper discusses the most common and useful procedure for synthesizing 4-amino-3-mercapto-1,2,4-triazoles, the utility of triazoles in the synthesis of triazolotriazines, and the evaluation of anti-inflammatory/analgesic activities of both derivatives.

2. Chemistry

The synthesis of 4-amino-3-substituted-1,2,4-triazole-5-thiones can be achieved by various methods starting either from carboxylic acid or its derivative esters and hydrazides. There are

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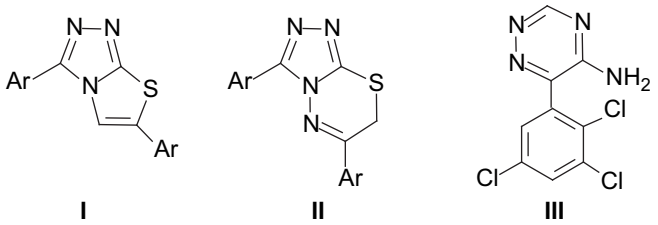


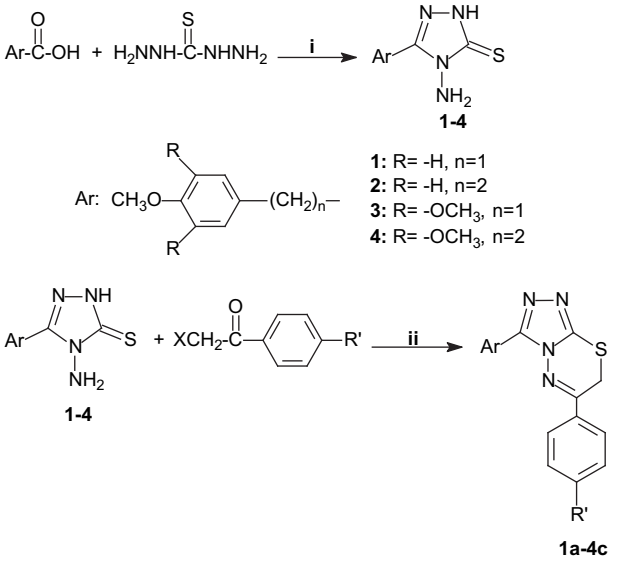
Scheme 1. Some compounds have been reported as anti-inflammatory/analgesic agents.

several reports on the synthesis of aminotriazolethiones using different multi-step synthetic routes [20,21]. It is worth noting that 4-amino-3-substituted-1,2,4-triazole-5-thiones were also obtained by a one-step synthesis. This simple method was first applied in the preparation of 3-alkyl-4-amino-5-mercapto-1,2,4-triazoles, starting from thiocarbohydrazide and ethyl esters of ortho-acids [22]. Beyer and Kroger [23] later advanced this method by refluxing thiocarbohydrazide with aliphatic monocarboxylic acids. In our study, we employed this one-step method to obtain starting compounds by combining carboxylic acids with thiocarbohydrazide at its melting temperature (160–170 °C) [9,24].

The 4-amino-3-substituted-1,2,4-triazole-5-thiones thus obtained were treated with phenacyl bromides (or chlorides) in anhydrous ethanol under reflux to obtain the title compounds 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives (**1a**–**4c**) (Scheme 2) [8–10]. All compounds were characterized by their melting points, elementary analysis, IR and ^{1}H NMR and mass spectra. The spectral data agree with the proposed structures.

While 4-amino-3-mercapto-1,2,4-triazoles may exist in *thione–thiol* tautomeric forms, in our study, the *thione* structure dominated in the solid state. Usual spectroscopic methods IR and NMR distinguished between these constitutional isomers. In the IR spectra, no absorption band was detected at about 2600–2550 cm^{-1}, attributable to the S–H group and the presence of a band at about 1188–1176 cm^{-1} indicative of C=S group was the evidence of thione form. The ^{1}H NMR spectrum of 4-amino-1,2,4-triazole-5-thiones (DMSO-d_6) exhibited NH_2 protons as a singlet at δ 5.52–5.55, integrating two protons and a NH signal as a singlet at δ 13.41–13.52 (these protons were more shielded in the spectrum of compound **3** taken in $CDCl_3$). Other peaks were observed at appropriate chemical shifts and integral values. The peak resonated at δ 166 in the ^{13}C NMR spectrum of compound **1** assigned for C=S, confirming the thione form of the triazole ring [25–28]. Compound **1** was reported in 1984 by Sattur et al. and the compound obtained was assigned as 4-amino-3-(4-methoxyphenylmethyl)-5-mercapto-1,2,4-triazole by IR spectral data [29]. However, our compounds have different m.p. Since this difference may be related with thione–thiol tautomers, an X-ray analysis of compound **1** was carried out and the structure was identified as 4-amino-3-(4-methoxyphenylmethyl)-1,2,4-triazole-5-thione. Relevant crystal data and details of the structure determinations are given in Table 1, and selected geometric parameters are given in Table 2. ORTEP-3 drawings of structure with atomic numbering are shown in Fig. 1 [30].

In compound **1**, all of the bond lengths and angles in the phenyl rings are in the normal range. It is observed that the compound exists in the thione form (Fig. 1). The C=S bond length is 1.679(3) Å, which is slightly longer than in 4-phenyl-3-[(1*H*-1,2,4-triazol-1-yl)methyl]-1*H*-1,2,4-triazole-5-thione [1.669(2) Å] [31]. In the triazole ring, the C=N bond length is in the same range, but the N–N bond is longer than those found in similar structures [C=N 1.295(2) Å and N–N 1.376(2) Å] and the bond lengths of C–N correspond with those found in the above-cited structures [C–N 1.335(3)–1.364(4) Å] [31]. As expected, all atoms of the triazole and the phenyl rings are planar. The crystal packing is governed by N–H···S and N–H···N hydrogen bonds (Fig. 2). The N–H···S intermolecular hydrogen bonds form a dimer, whereby both molecules are related by the center of inversion (Table 3, symmetry operator *i*). In addition to these centrosymmetric



1a: R= -H, n=1, R'= -H; **1b:** R= -H, n=1, R'= 4-Cl; **1c:** R= -H, n=1, R'= 4-F
2a: R= -H, n=2, R'= -H; **2b:** R= -H, n=2, R'= 4-Cl; **2c:** R= -H, n= 2, R'= 4-F
3a: R= -OCH_3, n=1, R'= -H; **3b:** R= -OCH_3, n=1, R'= 4-Cl; **3c:** R= -OCH_3, n= 1, R'= 4-F
4a: R= -OCH_3, n=2, R'= -H; **4b:** R= -OCH_3, n=2, R'= 4-Cl; **4c:** R= -OCH_3, n= 2, R'= 4-F

Scheme 2. Synthetic pathway of compounds. Reagents and conditions: (i) heat; (ii) absolute ethanol, reflux.

Table 1
Total data and details of the structure of the compounds **1** and **3a**

Formula	$C_{10}H_{12}N_4O_1S_1$	$C_{20}H_{20}N_4O_3S_1$
Formula weight	236.3	396.46
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
Cell constants		
a, [Å]	7.2658(12)	11.7489(13)
b, [Å]	6.1596(10)	8.0413(13)
c, [Å]	25.289(6)	20.228(3)
β, [°]	91.614(17)	104.430(10)
Z; D_{calc} [g cm^{-3}]	4; 1.387	4; 1.423
μ (MoK_α) [mm^{-1}]	0.271	0.205
Crystal size [mm]	0.27 × 0.30 × 0.48	0.30 × 0.48 × 0.78
Radiation	MoK_α (λ = 0.71073 Å)	MoK_α (λ = 0.71073 Å)
Temp [K]	293(2)	293(2)
θ limits [°]	2.8 - 26.29	2.8 - 26.29
Reflections collected	2340	3840
Reflections observed	1510 [$I > 2\sigma(I)$]	2490 [$I > 2\sigma(I)$]
Reflns. used in refinement	2284	3724
No. of refined parameters	149	253
R_{int}	0.0436	0.034
R / R_w values	0.0563 / 0.1986	0.0591 / 0.2178
GOF	0.849	0.769
Final shift	0.000	0.000
$(\Delta\rho)_{min}$, $(\Delta\rho)_{max}$ (e Å^{-3})	-0.549, 0.405	-0.428, 0.532

Table 2
Selected bond lengths and angles (Å, °) for compounds **1** and **3a.**

							Compound **1**	Compound **3a**
N1	–	N2					1.381 (4)	1.410 (4)
C3	–	N2					1.292 (4)	1.296 (4)
C3	–	N4					1.364 (4)	1.367 (4)
N4	–	N5					1.394(4)	1.385 (3)
C5	–	N4					1.368 (4)	1.356 (4)
C5	–	N1					1.335 (5)	1.292 (4)
C5	–	S8					1.679 (3)	1.739 (3)
C7	–	S8					–	1.796 (3)
C6	–	C7					–	1.511 (4)
C6	–	N5					–	1.280 (3)
N1	–	C5	–	S8			130.4 (2)	128.4 (2)
N2	–	N1	–	C5			113.1 (3)	106.6 (2)
N1	–	N2	–	C3			104.3 (3)	107.2 (2)
N2	–	C3	–	N4			110.7 (3)	110.2 (2)
C3	–	N4	–	N5			125.1 (3)	124.0 (2)
N2	–	C3	–	C14			125.4 (3)	125.7 (2)
C3	–	C14	–	C15			115.0 (3)	116.1 (2)
C14	–	C15	–	C16			121.2 (3)	119.3 (2)
C7	–	C6	–	C8			–	118.4 (2)
N4	–	N5	–	C6	–	C7	–	−3.3 (4)
N5	–	C6	–	C7	–	S8	–	33.6 (4)
C7	–	S8	–	C5	–	N4	–	26.5 (3)
C5	–	S8	–	C7	–	C6	–	−39.2 (3)

dimers there are also short S···S contacts (3.38 Å). Together with the N–H···S hydrogen bonds, these form a zig–zag paralel to the *b*-axis. These S···S contacts are probably another indication for the presence of the thione form. The N–H···N hydrogen bonds are formed between molecules related by translation along the *b*-axis, involving the NH_2 amino-group and the C═N double bonded ring nitrogen. In addition there is a short π···π interaction between the parallel oriented triazol rings (the centroid–centroid distance is 3.44 Å). It can be said that O–CH_3···π interactions play only a minor role. The polarisation of the methyl hydrogen will be rather small.

Formation of the triazolothiadiazines was also confirmed on the basis of IR, ^{1}H NMR, and mass spectroscopic techniques, in addition to elementary analysis. In the ^{1}H NMR spectrum of triazolothiadiazines, disappearance of the characteristic peaks belonging to primary amine and N^1–H and also the presence of SCH_2 protons, resonated at δ 3.90–4.39 ppm as a singlet integrating two protons, clearly indicated that ring closure reaction occurred. All other protons were seen at the expected chemical shifts and integral values [32,33]. The mass spectroscopic fragmentation of the compounds was studied under electron ionization. Molecular ion peaks confirmed the molecular weights of examined compounds. Either molecular ion peaks (compounds **1a–c** and **3a–c)** or tropylium peaks, formed from the cleavage of β-bond of benzene ring (compounds **2a–c** and **4a–c**), were the base peaks. In spectra, the peaks possibly attributed to the elimination of a methyl, methoxy, aryl, arylnitrile, sulfur and the peak belonging the arylalkylnitrile ion were seen at different intensities. Further spectroscopic details of these compounds are presented in the experimental part. In addition, the X-ray structure of compound **3a** was determined to confirm the assigned structure and to establish conformations of the molecule (Fig. 3, Table 1). In the structure, the six-membered thiadiazine ring, S8/C5/N4/N5/C6/C7, is distorted from planarity. Atoms C17 and N5 deviate −0.434(3) and 0.161(2) Å, respectively. Two phenyl rings are located nearly perpendicular to each other and to the dihedral angle between C8–C13 and C15–C20 ring is 84.07(9)° (Table 2). In **3a**, molecules are linked by a combination of weak C–H···O and C–H···N hydrogen bonds (Table 3, Fig. 4).

3. Pharmacology

In the pharmacological study, we investigated anti-inflammatory and analgesic activities as well as the ulcerogenic risk and antioxidant activity on acute administration. Both starting compounds **1–4** and their corresponding condensed derivatives (**1a–4c**) were evaluated. In order to screen the anti-inflammatory profile of the synthesized compounds, the carrageenan-induced hind paw edema model in mice was used [34]. The analgesic activity of the compounds was studied by using the acetic acid-induced writhing test in mice [35]. In both screening tests, 100 mg/kg (body weight) doses of the test drugs were orally administered to the animals as a first step. The compounds having more than 20% effectiveness in relieving symptoms, even some of them are not be significant statistically, were considered for further evaluation, and the experiments were repeated in two different dose levels (50 and 200 mg/kg) (Tables 4 and 5). The analgesic activity was expressed as "mean increase in latency after drug administration ± SEM" relative to controls, whereas the anti-inflammatory activity was expressed as "mean increase in paw volume ± SEM," in terms of mm and percentage inhibition in paw volume by different doses of the compounds. Ulcerogenic effect on acute administration was conducted to assess the safety of compounds at high dose levels (200 mg/kg). For the purpose of comparison, a nonselective COX inhibitor, acetylsalicylic acid, was used as a positive control. It caused severe bleeding at a dose level of 200 mg/kg. All compounds screened for ulcerogenic risk were also analyzed for lipid peroxidation.

4. Results and discussion

4.1. Anti-inflammatory activity

It is deduced from Table 4 that all compounds except compounds **3** and **1a** showed reduction in oedema volume when compared with the control group at 100 mg/kg dose, p.o. in any of the measurement intervals. Among the tested compounds, the compounds **4**, **1c**, **2b** and **4c** showed the most activity, while others were less active at 100 mg/kg/p.o dose level. Although generally all of the compounds showed either increased (compounds **1c**, **2a**, **2c**,

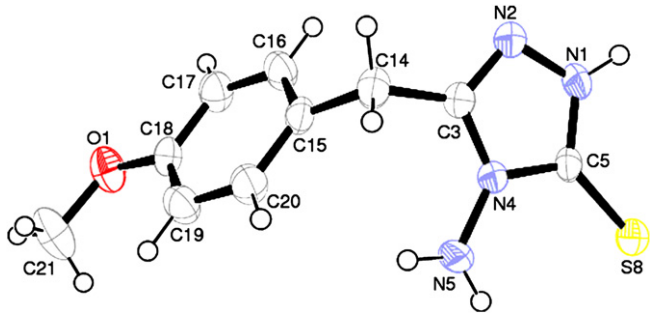


Fig. 1. ORTEP-3 view of the compound **1**, showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level.

3a–**c**, **4a**–**c**) or similar anti-inflammatory activity (compounds **1**, **2**, **4**, **1b**, **2b)** to that of 100 mg/kg dose at 200 mg/kg dose level, few (compounds **3** and **4c**) exhibited activity at a dose level of 50 mg/kg. In general, in cases of anti-inflammatory activity, compounds carrying an ethylene bridge ($n = 2$) at the 3rd position of the fused ring had higher activity than their analogs with methyl (compare compounds **4** > **3**; **2a** > **1a**; **2b** > **1b**; **4b** > **3b**; **4c** > **3c**). Among the condensed derivatives, compounds **2b** and **4c** having 4-methoxyphenylethyl and 3,4,5-trimethoxyphenylethyl at the 3rd position and 4-chlorophenyl and 4-fluorophenyl at the 6th position of the fused ring, respectively, possessed the most prominent and consistent activity.

4.2. Analgesic activity

As seen in Table 5, the compounds **2**, **3**, **4**, **2b**, **3a** and **4b** had either higher or similar analgesic activity to that of aspirin at the 100 mg/kg dose level, whereas others showed less activity when compared for percent inhibition values. When the experiments

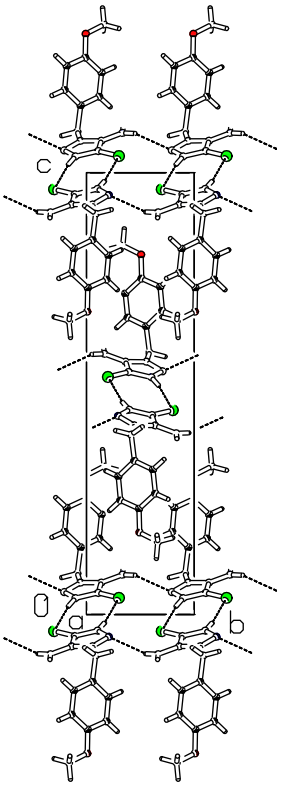


Fig. 2. Crystal packing of compound **1** showing the intermolecular interactions.

Table 3
Hydrogen bonding geometry (Å, °) of the compounds **1** and **3a**.

	D–H···A	D–H	H···A	D···A	D–H···A
Compound **1**	N1–H1···S8^{i}	0.87(4)	2.49(4)	3.348 (3)	171(3)
	N5–H52···N2^{ii}	0.86	2.33	3.056 (4)	142
	C17–H17···Cg(2)^{iii}	0.93	2.91	3.712 (4)	146
	C21–H21B···Cg(2)^{iv}	0.96	2.82	3.552 (5)	133
Compound **3a**	C10–H10···O2^{i}	0.93	2.59	3.499 (4)	166
	C23–H23B···N1^{ii}	0.96	2.57	3.522 (4)	174
	C7–H7A···Cg(3)^{iii}	0.97	2.94	3.777 (3)	144
	C12–H12···Cg(1)^{iii}	0.93	2.93	3.302 (3)	105
	C13–H13···Cg(4)^{iii}	0.93	2.79	3.639 (3)	153
	C23–H23···Cg(4)^{iv}	0.96	2.96	3.628 (4)	127

Symmetry code: for compound **1**: (i) $3-x, -y, -z$, (ii) $x, 1+y, z$, (iii) $2-x, -1/2+y, 1/2-z$, (iv) $1-x, 1/2+y, 1/2-z$; Cg(2): C15–C20 ring; for compound **3a**: (i) $x, -1+y, z$, (ii) $1-x, 1/2+y, 1/2-z$, (iii)$1-x, -y, -z$, (iv) $2-x, 1/2+y, 1/2-z$; Cg(1): N1–N2–C3–N4–C5, Cg(3): C8–C13, Cg(4): C15–C20 rings.

were repeated in 200 mg/kg/p.o. dose, the compounds **2**, **4**, **3a** and **4b** exhibited similar activity profile to that of a 100 mg/kg dose level, whereas the compounds **3** and **2b** showed less percent inhibition. As a general consideration, the compounds, except compound **4a,** when administered decreasing dose (50 mg/kg), the activity was found to be decreased. The compounds **3** and **2b** displayed a higher percent inhibition than aspirin in both 100 and 200 mg/kg/p.o. doses among the set of compounds.

4.3. Acute ulcerogenesis

The compounds, which were screened for analgesic activity, were further screened for their acute ulcerogenic risk. As a general consideration, all compounds except **2a** and **3b** were found to be safer as gastric lesion risks at high dose (200 mg/kg, p.o.) when compared to aspirin. Among the compounds, compounds **1**, **1b, 2**, and **3a** induced no ulcerogenic effect at high dose while aspirin caused severe bleeding lesions with 4/5 score at the same dose. It could be hypothesized that the compounds causing fewer gastric lesions at 200 mg/kg dose when compared to aspirin, may display diminished ulcerogenic risk at lower doses.

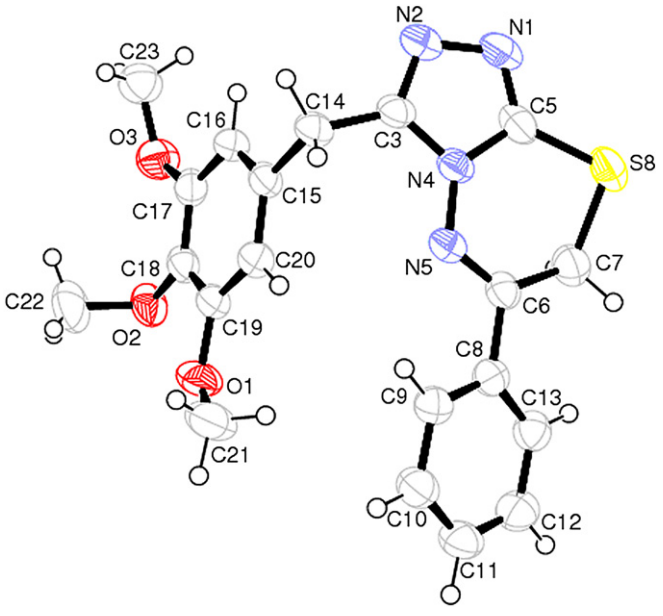


Fig. 3. ORTEP-3 view of the compound **3a**, showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level.

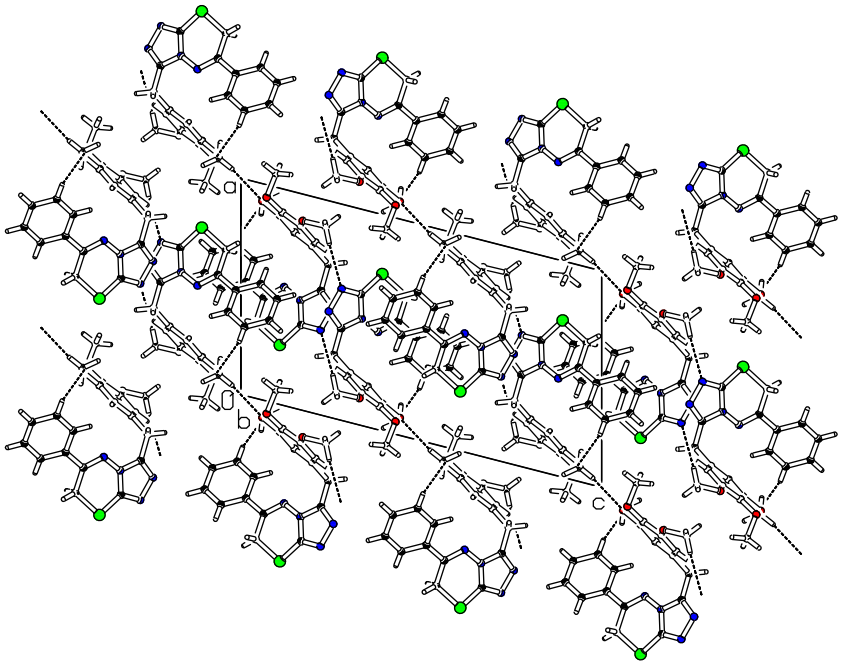


Fig. 4. Crystal packing of compound **3a** showing the intermolecular interactions.

4.4. Antioxidant activity

It has been reported in the literature that lower ulcerogenic activity of compounds is combined with a reduced TBARS content in the affected area of the gastrointestinal tract, byproducts of lipid peroxidation [36,37]. Therefore, an attempt was made to correlate the decrease in ulcerogenic effect of the compounds with that of lipid peroxidation. The lipid peroxidation is measured as nmol of TBARS/g wet weight of tissue. The aspirin showed the highest TBARS level (118.2 ± 2.1, $p < 0.001$), whereas the control group showed 78.5 ± 5.0. It was found that all derivatives showing less ulcerogenic risk also showed less lipid peroxidation production (Table 5). Thus, these studies showed that synthesized compounds have inhibited the induction of gastric mucosal lesions. The results further suggested that their diminished harmful effects on the stomach might be related to their antioxidant properties.

4.5. Acute toxicity

Test compounds used in the pharmacologic study did not show any acute toxicity. Common side effects such as mild diarrhea and depression were not recorded.

5. Conclusion

Various 4-amino-3-substituted-1,2,4-triazole-5-thiones (**1**–**4**) and their corresponding condensed derivatives 3,6-disubstituted 7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**1a**–**4c**) were synthesized with the objective of developing better analgesic/anti-inflammatory molecules with minimum ulcerogenic risk. Several derivatives have been evaluated for potential analgesic/anti-inflammatory activity devoid of ulcerogenic risk. The results are conclusive in showing that there is no noticeable increase in analgesic and anti-inflammatory activity when 3-substituted 4-amino-5-mercapto-1,2,4-triazoles are condensed with the 1,3,4-thiadizine ring. Among the condensed derivatives, the compounds **2b**, **4b** and **4c**, carrying either a 4-chloride or 4-fluoride substituent on the phenyl ring at the 6th position of the heterocyclic compound, showed better analgesic and anti-inflammatory activities, and less ulcerogenic risk, along with minimum lipid peroxidation. These findings parallel to those of Prasad et al. [10]. Prasad et al. synthesized similar 3,6-disubstituted 7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazine derivatives and screened their antibacterial, antifungal, and anti-inflammatory activities. They reported that among the compounds tested for anti-inflammatory activity, the compound having 6-(4-chlorophenyl)substituent on the condensed ring showed notable anti-inflammatory activity.

The fact that the compounds with low ulcerogenic risk do not cause any increase on stomach lipid peroxidation level leads us to think that the antioxidant properties of these compounds contributes to the anti-inflammatory activities. Further studies may reveal the exact mechanism responsible both for the analgesic/anti-inflammatory and gastric toxicity.

These types of compounds might lead to further studies for developing better candidates with potent analgesic and anti-inflammatory activities, together with less ulcerogenic activity and minimum peroxidative stomach injury. To probe structural requirements for optimal activity in this series, the research on modification of the title compounds is underway.

6. Experimental protocols

6.1. Chemistry

Melting points were detected with a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra (KBr) were recorded on a Perkin Elmer 1720X FT-IR spectrometer. Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were taken on a Varian Mercury 400, 400 MHz High Performance Digital FT-NMR instrument in DMSO-d_6 or CDCl3 using TMS as internal standard. All chemical shift values were recorded as δ (ppm). Splitting patterns are as follows: s, singlet; d, doublet; m, multiplet; b, broad; dd (doublet in doublet). Mass spectra were obtained with an

Table 4
Dose-dependent anti-inflammatory effects of the compounds against carrageenan-induced hind paw edema model in mice in different doses.

Compounds	Dose mg/kg (per os)	Swelling in thickness ($\times 10^{-2}$ mm) ± SEM (percent inhibitory activity)			
		90 min	180 min	270 min	360 min
Control		42.0 ± 5.1	65.0 ± 7.4	69.0 ± 7.6	48.0 ± 4.9
1	100	44.9 ± 5.2	53.0 ± 9.0	44.9 ± 8.7 (**34.9**)	32.0 ± 3.0* (**33.3**)
	50	47.0 ± 2.6	60.5 ± 4.1	58.7 ± 4.7	44.7 ± 2.5
	200	37.6 ± 3.6	49.7 ± 3.7 (**23.5**)	46.8 ± 4.2 (**32.2**)	32.0 ± 2.6* (**33.3**)
2	100	41.5 ± 6.0	55.1 ± 5.1	54.1 ± 3.4 (**21.6**)	30.5 ± 2.8* (**36.5**)
	50	43.0 ± 3.4	60.5 ± 2.1	63.9 ± 2.7	48.0 ± 4.7
	200	30.8 ± 1.7 (**26.6**)	47.2 ± 1.5 *(**27.4**)	45.6 ± 1.5* (**33.9**)	30.0 ± 1.6* (**37.5**)
3	100	42.0 ± 4.5	58.6 ± 3.2	55.4 ± 1.9	39.0 ± 2.9 (**18.7**)
	50	29.4 ± 4.4 (**30.0**)	44.0 ± 6.5 (**32.3**)	51.8 ± 4.7 (**24.9**)	24.4 ± 1.9** (**49.2**)
	200	39.9 ± 3.0	86.7 ± 8.7	59.3 ± 4.5	37.8 ± 1.7 (**21.2**)
4	100	39.5 ± 1.8	57.2 ± 3.2	44.3 ± 2.4* (**35.8**)	27.5 ± 4.1** (**42.7**)
	50	42.0 ± 3.4	61.6 ± 4.7	63.9 ± 2.7	38.2 ± 3.8 (**20.4**)
	200	39.8 ± 4.1	53.5 ± 5.2	41.9 ± 4.6* (**39.3**)	28.0 ± 3.4* (**41.7**)
1a	100	41.7 ± 1.7	61.7 ± 3.3	68.3 ± 3.3	46.7 ± 3.3
1b	100	40.1 ± 3.9	52.1 ± 9.7	56.7 ± 6.5	29.1 ± 4.8* (**39.4**)
	50	43.0 ± 2.6	60.5 ± 4.8	64.9 ± 2.7	42.5 ± 3.2
	200	35.4 ± 2.9	48.4 ± 3.3 (**25.5**)	51.8 ± 3.7 (**25.0**)	29.0 ± 2.9* (**38.9**)
1c	100	38.7 ± 3.2	42.7 ± 7.2 (**34.3**)	40.5 ± 3.9** (**41.3**)	38.6 ± 1.8
	50	42.0 ± 2.6	62.8 ± 3.3	60.8 ± 2.0	49.0 ± 2.4
	200	32.1 ± 3.2 (**23.6**)	31.9 ± 2.8** (**50.9**)	32.0 ± 3.6** (**53.6**)	28.0 ± 2.6** (**41.7**)
2a	100	39.7 ± 6.9	53.9 ± 5.4	43.1 ± 7.3* (**37.5**)	32.0 ± 3.0* (**33.3**)
	50	43.0 ± 2.6	59.4 ± 2.2	63.9 ± 4.7	41.4 ± 2.8
	200	34.3 ± 2.1	49.7 ± 4.2 (**23.5**)	34.5 ± 4.2** (**50.0**)	30.0 ± 4.2* (**37.5**)
2b	100	42.1 ± 3.7	45.9 ± 7.4 (**29.4**)	40.6 ± 5.2* (**41.2**)	23.4 ± 3.8** (**51.2**)
	50	44.0 ± 1.9	61.6 ± 2.5	56.6 ± 2.2	38.2 ± 3.5 (**20.4**)
	200	37.6 ± 3.6	45.9 ± 3.7* (**29.4**)	30.8 ± 4.3** (**55.4**)	24.0 ± 3.7** (**50.0**)
2c	100	31.1 ± 3.9 (**26.0**)	43.1 ± 2.7* (**33.7**)	54.2 ± 3.1 (**21.4**)	43.3 ± 2.7
	50	38.0 ± 2.6	55.0 ± 4.5	66.0 ± 3.8	48.0 ± 3.2
	200	34.3 ± 3.2	33.1 ± 3.7** (**49.0**)	45.6 ± 3.2* (**34.0**)	35.0 ± 2.2 (**27.1**)
3a	100	31.9 ± 3.6 (**24.0**)	42.3 ± 5.0* (**34.9**)	54.2 ± 4.3 (**21.4**)	38.8 ± 4.6
	50	37.0 ± 3.4	51.6 ± 5.5 (**20.6**)	58.7 ± 2.7	52.4 ± 2.8
	200	32.1 ± 4.1 (**23.6**)	32.1 ± 3.7** (**50.6**)	46.8 ± 4.2* (**32.2**)	32.0 ± 4.1* (**33.3**)
3b	100	38.8 ± 5.2	52.1 ± 4.9	46.1 ± 2.5* (**33.1**)	38.3 ± 2.7 (**20.2**)
	50	41.0 ± 1.9	62.8 ± 6.7	69.0 ± 2.0	48.0 ± 3.2
	200	36.5 ± 5.1	44.6 ± 5.9 (**31.4**)	40.7 ± 6.9 * (**41.0**)	36.0 ± 4.0 (**25.0**)
3c	100	41.4 ± 3.9	47.6 ± 5.6 (**26.8**)	58.3 ± 3.1	47.9 ± 2.2
	50	45.0 ± 4.2	65.0 ± 3.8	68.0 ± 3.0	50.2 ± 3.2
	200	35.4 ± 4.5	44.6 ± 4.1* (**31.4**)	51.8 ± 5.4 (**24.9**)	36.0 ± 3.7 (**25.0**)
4a	100	28.7 ± 3.4 (**31.7**)	40.0 ± 3.3* (**38.5**)	55.0 ± 2.2 (**20.3**)	44.2 ± 2.8
	50	38.0 ± 4.1	52.7 ± 4.1	66.9 ± 1.6	44.7 ± 3.2
	200	23.2 ± 4.8* (**44.8**)	33.1 ± 5.1** (**49.0**)	37.0 ± 4.3** (**46.4**)	34.0 ± 2.5* (**29.2**)
4b	100	29.7 ± 3.5 (**29.3**)	41.0 ± 4.1* (**36.9**)	51.7 ± 4.5 (**25.0**)	42.7 ± 3.2
	50	39.0 ± 3.3	57.1 ± 5.1	63.8 ± 3.5	43.6 ± 3.5
	200	27.6 ± 3.9 (**34.3**)	35.7 ± 5.6* (**45.1**)	42.0 ± 5.3* (**39.1**)	36.0 ± 2.9 (**25.0**)
4c	100	24.9 ± 2.2** (**40.7**)	39.7 ± 4.3* (**38.9**)	48.9 ± 2.8* (**29.1**)	41.2 ± 3.2
	50	33.0 ± 4.1 (**21.4**)	42.6 ± 2.2* (**34.5**)	62.8 ± 3.4	43.6 ± 2.4
	200	17.7 ± 3.2** (**57.9**)	31.9 ± 4.5** (**51.0**)	37.0 ± 5.2** (**46.4**)	37.0 ± 2.6 (**23.0**)
INDO	100	24.4 ± 1.1** (**41.9**)	37.0 ± 2.4*** (**43.1**)	37.1 ± 1.3** (**46.2**)	28.1 ± 1.0** (**41.5**)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; significant from control ($n = 4$–5).

electron impact technique using a Direct Insertion Probe and Agilent 5973-Network Mass Selective Dedector at 70 eV. The purity of the compounds was checked on silicagel-coated aliminium sheets (Merck, 1.005554, silicagel $HF_{254-361}$, Type 60, 0.25 mm, Darmstadt, Germany) by thin-layer chromatography. The elementary analysis of the result compounds was performed with a Leco CHNS 932 analyzer at the Scientific and Technical Research Council of Turkey, Instrumental Analysis Laboratory in Ankara. All chemicals were from Aldrich Chemical Co. (Steinheim, Germany).

6.1.1. Synthesis of 4-amino-3-substituted-1,2,4-triazole-5-thiones (**1**–**4**) [9,24]

A mixture of equimolar amount of thiocarbohydrazide and aralkyl carboxylic acid was heated in an oil bath at 160–170 °C for 30 min. The resulting melted mass was triturated with hot water to obtain the compounds. Two methods were used to purify the compounds. Compound **2** was purified with column chromatography ($CHCl_3$:CH_3OH); the others were purified through crystallization. Compound **1** was previously synthesized [29].

Table 5
Dose-dependent analgesic effects of the test compounds on acetic acid-induced writhing response, ulcer score and lipid peroxidation (as TBARS level) in mice.

Compounds	Dose (mg/kg) per os	TBARS nmol/g wet w	Ulcer score	Analgesic activity mean ± SEM (% inhibition)
Control		78.5 ± 5.0	0/4	88 ± 1.08
1	100			61.4 ± 0.8*** (**30.2**)
	50			67.4 ± 0.4* (**23.4**)
	200	93.0 ± 5.3	0/4	46.9 ± 1.7*** (**46.7**)
2	100			38.2 ± 0.8*** (**56.6**)
	50			79.6 ± 3.2 (**9.5**)
	200	105.9 ± 10.8	0/4	38.6 ± 1.4 *** (**56.1**)
3	100			12.5 ± 0.5*** (**85.8**)
	50			69 ± 11.4 (**21.6**)
	200	96.2 ± 2.0*	1/4	34.3 ± 1.08*** (**61.0**)
4	100			41.9 ± 2.3*** (**52.4**)
	50			67.7 ± 1.5* (**23.1**)
	200	104.3 ± 4.2**	2/4	40.3 ± 1.4*** (**54.2**)
1a	100			53.2 ± 2.2*** (**39.5**)
	50			75.4 ± 2.3 (**14.3**)
	200	112.3 ± 3.1**	1/4	47.2 ± 1.5*** (**46.4**)
1b	100			59 ± 0.8*** (**33.0**)
	50			79.3 ± 0.8 (**9.9**)
	200	97.9 ± 7.2	0/4	53.7 ± 3.2*** (**39.0**)
1c	100			64.9 ± 1.6** (**26.3**)
	50			70 ± 7.0* (**20.5**)
	200	95.4 ± 4.7*	1/4	60.3 ± 2.9** (**31.5**)
2a	100			50.3 ± 1.8*** (**42.8**)
	50			82.4 ± 3.2 (**6.4**)
	200	110.8 ± 2.0***	3/4	49.1 ± 3.2*** (**44.2**)
2b	100			25.1 ± 0.5*** (**71.5**)
	50			70.2 ± 0.6 (**20.2**)
	200	108.4 ± 5.7**	1/4	33.1 ± 1.5*** (**62.4**)
2c	100			62.3 ± 1.2*** (**29.2**)
	50			66 ± 3.4*** (**25.0**)
	200	103.5 ± 7.7*	2/4	55.4 ± 0.7*** (**37.0**)
3a	100			41.4 ± 1.0*** (**53.0**)
	50			59.5 ± 5.4** (**32.4**)
	200	92.2 ± 5.7	0/4	36.6 ± 1.3*** (**58.4**)
3b	100			59.3 ± 0.8*** (**32.6**)
	50			76.1 ± 2.4 (**13.5**)
	200	101.1 ± 6.2*	3/4	67.4 ± 4.5* (**23.4**)
3c	100			57.2 ± 0.4*** (**35.0**)
	50			68.5 ± 6.3* (**22.1**)
	200	107.6 ± 12.0	1/4	42.1 ± 1.9*** (**52.2**)
4a	100			73 ± 0.4** (**17.1**)
	50			59.5 ± 5.4** (**32.4**)
	200	100.3 ± 3.4*	2/4	63.8 ± 3.3** (**27.5**)
4b	100			43.8 ± 1.8*** (**50.2**)
	50			62.9 ± 2.5** (**28.5**)
	200	101.9 ± 9.2	1/4	39.8 ± 1.3*** (**54.8**)
4c	100			50.1 ± 1.4*** (**43.1**)
	50			57 ± 2.9*** (**35.2**)
	200	105.1 ± 6.7*	1/4	38.6 ± 0.9*** (**56.1**)
ASA	200	118.2 ± 2.1***	4/5	40.1 ± .9***(**54.4**)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; significant from control ($n = 4$–5).

*6.1.1.1. 4-Amino-3-(4-methoxyphenylmethyl)-1,2,4-triazole-5-thione (**1**).* Yield 79%; mp 203–204 °C (methanol) [(mp 164 °C) [29]; IR (KBr): 3292, 3203 (N–H), 1647 (C═N), 1315 (C–N), 1249 (C–O), 1180 (C═S) cm^{-1}; ^1H NMR δ (ppm): 3.72 (s, 3H, OCH_3), 3.95 (s, 2H, CH_2–), 5.55 (s, 2H, NH_2), 6.87 (d, 2H, arom. H-3, H-5), 7.20 (d, 2H, arom. H-2, H-6), 13.52 (s, 1H, N*H*); ^{13}C NMR δ (ppm): 30.03 (–CH_2–), 55.74 (–OCH_3), 114.57 (arom. C-3, C-5), 127.96 (arom. C-1), 130.61 (arom. C-2, C-6), 152.32 (arom. C-4), 158.83 (triazole *C*-3), 166.61 (triazole *C*-5); MS (70 eV, EI): *m*/*z* (%): 236 (M^+, 93%), 220 (M^+, –NH_2, 74%), 146 ($CH_3OC_6H_4CHCN$, 17%), 121 ($CH_3OC_6H_4CH_2$, 100%), 91 (C_7H_7, 12.50%), 77 (C_6H_5, 20.83%). Anal. Calcd. for $C_{10}H_{12}N_4OS$: C, 50.83; H, 5.12; N, 23.71; S, 13.57. Found: C, 50.76; H, 4.93; N, 23.64; S, 13.56.

*6.1.1.2. 4-Amino-3-[2-(4-methoxyphenyl)ethyl]-1,2,4-triazole-5-thione (**2**).* Yield 83%; mp 202–203 °C (chloroform-methanol); IR (KBr): 3250 (N-H), 1627 (C═N), 1307 (C–N), 1246 (C–O), 1176 (C═S) cm^{-1}; ^1H NMR δ (ppm): 2.87 (t, 4H, CH_2CH_2), 3.69 (s, 3H, OCH_3), 5.55 (s, 2H, NH_2), 6.82 (d, 2H, arom. H-3, H-5), 7.12 (d, 2H, arom.

H-2, H-6), 13.41 (s, 1H, N*H*); MS (70 eV, EI): *m*/*z* (%): 250 (M^+, 86.36%), 234 (M^+ –NH_2, 2.61%), 134 ($CH_3OC_6H_4CHCH_2$, 50.44%), 121 ($CH_3OC_6H_4CH_2$, 100%), 91 (C_7H_7, 17.39%), 77 (C_6H_5, 20.87%). Anal. Calcd. for $C_{11}H_{14}N_4OS$: C, 52.78; H, 5.64; N, 22.38; S, 12.81. Found: C, 52.46; H, 5.57; N, 22.38; S, 12.71.

*6.1.1.3. 4-Amino-3-(3,4,5-trimethoxyphenylmethyl)-1,2,4-triazole-5-thione (**3**).* Yield 47%; mp 173–175 °C (benzene-methanol); IR (KBr): 3300, 3234 (N–H), 1591 (C=N), 1311 (C–N), 1244 (C–O), 1182 (C=S) cm^{-1}; ^1H NMR δ (ppm): 3.83 (s, 3H, O*CH*$_3$), 3.85 (s, 6H, O*CH*$_3$), 4.02 (s, 2H, *CH*$_2$), 4.57 (s, 2H, N*H*$_2$), 6.52 (s, 2H, arom. H-2, H-6), 10.56 (s, 1H, N*H*); MS (70 eV, EI): *m*/*z* (%): 296 (M^+, 100%), 281 (M^+ –CH_3, 32,14%), 280 (M^+ –NH_2, 57.86%), 264 (M^+ –S, 4.29%), 192 ($(H_3CO)_3C_6H_2CH_2CN$–CH_3, 12.14%), 181 ($(CH_3O)_3C_6H_2CH_2$, 8.57%). Anal. Calcd. for $C_{12}H_{16}N_4O_3S$: C, 48.64; H, 5.44; N, 18.91; S, 10.82. Found: C, 48.60; H, 5.44; N, 18.72; S, 10.80.

*6.1.1.4. 4-Amino-3-[2-(3,4,5-trimethoxyphenyl)ethyl]-1,2,4-triazole-5-thione (**4**).* Yield 70%; m.p. 139–140 °C (chloroform); IR (KBr): 3294 (N–H), 1587 (C=N), 1311 (C–N), 1242 (C–O), 1188 (C=S) cm^{-1}; ^1H NMR δ (ppm): 2.93 (t, 4H, *CH*$_2$*CH*$_2$), 3.62 (s, 3H, O*CH*$_3$), 3.74 (s, 6H, O*CH*$_3$), 5.52 (s, 2H, N*H*$_2$), 6.52 (s, 2H, arom. H-2, H-6), 13.44 (s, 1H, N*H*); MS (70 eV, EI): *m*/*z* (%): 310 (M^+, 50.87%), 181 ($(CH_3O)_3C_6H_2CH_2$, 100%). Anal. Calcd. for $C_{13}H_{18}N_4O_3S$: C, 50.31; H, 5.85; N, 18.05; S, 10.33. Found: C, 49.96; H, 5.81; N, 17.88; S, 10.34.

*6.1.2. Synthesis of 3,6-disubstituted 7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (**1a**–**4c**) [8–10]*

A mixture of the corresponding triazoles **1**–**4** (1 mmol) and phenacyl halogenes (1 mmol) in anhydrous ethanol (20 mL) was heated under reflux. Reactions were ended under control of TLC. The mixture was then cooled to the room temperature and neutralized with 20% ammonium hydroxide solution. The separated precipitate was collected by filtration and purified with crystallization.

Compound **1a** [38] has already been reported. Since there are only NMR findings describing compound **1a** in literature, all spectral analysis was performed. Single crystal X-ray crystallographic and spectral data of the compounds **2a** and **4a** have been submitted elsewhere for publication. Compounds **1b** (886949-15-1) and **1c** (933228-88-7) are seen as commercial products in "Science Finder." Because there is no information in the literature for the preparation and spectral characteristics, these two compounds have been included in our research program and characterized by spectral data.

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

*6.1.2.1. 3-(4-Methoxyphenylmethyl)-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**1a**) [38].* Yield 54%; m.p. 153–154 °C (ethanol); IR (KBr): 1610 (C=N), 1301 (C–N), 1246 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.79 (s, 3H, O*CH*$_3$), 3.95 (s, 2H, *CH*$_2$), 4.25 (s, 2H, S*CH*$_2$), 6.85 (d, 2H, arom. H-3, H-5), 7.32 (d, 2H, arom. H-2, H-6), 7.52–7.61 (m, 3H, arom. H-3′, H-4′, H-5′), 7.87 (d, 2H, arom. H-2′, H-6′); ^{13}C NMR δ (ppm): 23.45 (–*CH*$_2$–), 29.85 (-S*CH*$_2$-), 55.71 (–O*CH*$_3$), 114.60 (arom. C-3, C-5), 128.12 (arom. C-3′, C-5′), 128.61 (arom. C-4′), 129.76 (arom. C-2′, C-6′), 130.54 (arom. C-2, C-6), 132.59 (arom. C-1), 134.18 (arom. C-1′), 140.93 (C-8a), 153.52 (arom. C-4), 155.36 (*C*-3), 158.75 (*C*-6); MS (70 eV, EI): *m*/*z* (%): 338 (M^+ +2, 7.90%), 336 (M^+, 100%), 321 (M^+ –CH_3, 14.91%), 304 (M^+ –S, 2.63%), 303 (M^+ –SH, 11.40%), 259 (M^+ –C_6H_5, 18.42%), 233 (M^+ –C_6H_5CN, 10.53%), 218 (M^+ –[(C_6H_5CN) + CH_3], 20.18%), 147 ($CH_3OC_6H_4CH_2CN$, 75.44%), 132 ([$CH_3OC_6H_4CH_2CN$-CH_3], 33.33%), 121 ($CH_3OC_6H_4CH_2$, 50.0%), 77 (C_6H_5, 61.40%). Anal. Calcd. for $C_{18}H_{16}N_4OS$: C, 64.27; H, 4.79; N, 16.65; S, 9.53. Found: C, 64.12; H, 4.89; N, 16.75; S, 9.45.

*6.1.2.2. 3-(4-Methoxyphenylmethyl)-6-(4-chlorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**1b**).* Yield 56%; m.p. 188–190 °C (It is precipitated with H_2NOH sol.); IR (KBr): 1610 (C=N), 1301 (C–N), 1249 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.72 (s, 3H, O*CH*$_3$), 3.95 (s, 2H, *CH*$_2$), 4.30 (s, 2H, S*CH*$_2$), 6.84 (d, 2H arom. H-3, H-5), 7.29 (d, 2H, arom. H-2, H-6), 7.52 (d, 2H, arom. H′-2, H′-6), 7.81 (d, 2H, arom. H-3′, H-5′); MS (70 eV, EI): *m*/*z* (%): 374 (M^+ +4, 2.42%), 372 (M^+ +2, 35.76%), 370 (M^+, 100%), 355 (M^+ –CH_3, 15.76%), 338 (M^+ –S, 12.73%), 337 (M^+ –SH, 10.30%), 335 (M^+ –Cl, 13.33%), 259 (M^+ –C_6H_4Cl, 26.67%), 233 (M^+ –ClC_6H_4CN, 7.27%), 218 ([M^+ –(ClC_6H_4CN + CH_3)], 27.88%), 147 ($CH_3OC_6H_4CH_2CN$, 75.44%), 132 ([$CH_3OC_6H_4CH_2CN$-CH_3], 48.49%), 121 ($CH_3OC_6H_4CH_2$, 79.39%), 111 (·C_6H_4Cl, 30.45%). Anal. Calcd. for $C_{18}H_{15}ClN_4OS$: C, 58.30; H, 4.08; N, 15.11; S, 8.64. Found: C, 57.98; H, 3.92; N, 14.99; S, 8.54.

*6.1.2.3. 3-(4-Methoxyphenylmethyl)-6-(4-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**1c**).* Yield 47%; m.p. 192–193 °C (ethanol); IR (KBr): 1600 (C=N), 1307 (C–N), 1249 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.78 (s, 3H, O*CH*$_3$), 3.94 (s, 2H, *CH*$_2$), 4.29 (s, 2H, S*CH*$_2$), 6.84 (d, 2H, arom. H-3, H-5), 7.20–7.22 (m, 2H, arom. H-3′, H-5′), 7.29 (d, 2H, arom. H-2, H-6) 7.86–7.91 (m, 2H, arom. H-2′, H-6′); MS (70 eV, EI): *m*/*z* (%): 356 (M^+ +2, 6.88%), 354 (M^+, 100%), 339 (M^+ –CH_3, 15.63%), 322 (M^+ –S, 1.88%), 321 (M^+ –SH, 10.00%), 259 (M^+ –C_6H_4F, 10.00%), 233 (M^+ –C_6H_5CN, 8.13%), 218 ([M^+ –(FC_6H_4CN + CH_3)], 21.88%), 147 ($CH_3OC_6H_4CH_2CN$, 63.13%), 132 ($CH_3OC_6H_4CH_2CN$–CH_3, 28.75%), 121 ($CH_3OC_6H_4CH_2$, 72.50%), 95 (C_6H_4F, 23.75%). Anal. Calcd. for $C_{18}H_{15}FN_4OS$: C, 61.00; H, 4.27; N, 15.81; S, 9.05. Found: C, 61.32; H, 4.30; N, 15.87; S, 9.08.

*6.1.2.4. 3-[2-(4-Methoxyphenyl)ethyl]-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**2a**).* Yield 64%; m.p. 142–143 °C (ethanol). All spectral data have been submitted for publication.

*6.1.2.5. 3-[2-(4-Methoxyphenyl)ethyl]-6-(4-chlorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**2b**).* Yield 53%; m.p. 141–142 °C (ethanol); IR (KBr): 1616 (C=N), 1300 (C–N), 1249 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.12 (t, 2H, *CH*$_2$CH$_2$), 3.31 (t, 2H, CH$_2$*CH*$_2$), 3.70 (s, 3H, O*CH*$_3$), 3.91 (s, 2H, S*CH*$_2$), 6.74 (d, 2H, arom. H-3, H-5), 7.09 (d, 2H, arom. H-2, H-6), 7.52 (d, 2H, arom. H-2′, H-6′), 7.78 (d, 2H, arom. H-3′, H-5′); MS (70 eV, EI): *m*/*z* (%): 388 (M^+ +4, 0.97%), 386 (M^+ +2, 17.10%), 384 (M^+ 45.81%), 369 (M^+ –CH_3, 1.61%), 351 (M^+ –SH, 1.29%), 247 (M^+, –ClC_6H_4CN, 2.58%), 232 ([M^+ –(ClC_6H_4CN + CH_3)], 36.13%), 134 ($CH_3OC_6H_4CHCH_2$, 9.68%), 121 ($CH_3OC_6H_4CH_2$, 100%), 111 (C_6H_4Cl, 6.45%). Anal. Calcd. for $C_{19}H_{17}ClN_4OS$: C, 59.29; H, 4.45; N, 14.56; S, 8.33. Found: C, 59.13; H, 4.44; N, 14.70; S, 8.37.

*6.1.2.6. 3-[2-(4-Methoxyphenyl)ethyl]-6-(4-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**2c**).* Yield 61%; m.p. 155–156 °C (ethanol); IR (KBr): 1598 (C=N), 1300 (C–N), 1247 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.14 (t, 2H, *CH*$_2$CH$_2$), 3.35 (t, 2H, CH$_2$*CH*$_2$), 3.70 (s, 3H, O*CH*$_3$), 3.95 (s, 2H, S*CH*$_2$), 6.74 (d, 2H, arom. H-3, H-5), 7.10 (d, 2H, arom. H-2, H-6), 7.22–7.26 (m, 2H, arom. H-3′, H-5′), 7.85–7.88 (m, 2H, arom. H-2′, H-6′); MS (70 eV, EI): *m*/*z* (%): 370 (M^+ +2, 6.00%), 368 (M^+, 79.33%), 353 (M^+ –CH_3, 3.00%), 335 (M^+ –SH, 1.67%), 247 (M^+ –FC_6H_4CN, 3.33%), 232 ([M^+ –(FC_6H_4CN + CH_3)], 41.33%), 134 ($CH_3OC_6H_4CHCH_2$, 10.67%), 121 ($CH_3OC_6H_4CH_2$, 100%), 95 (C_6H_4F, 12.00%). Anal. Calcd. for $C_{19}H_{17}FN_4OS$: C, 61.94; H, 4.65; N, 15.21; S, 8.70. Found: C, 61.82; H, 4.74; N, 15.08; S, 8.80.

*6.1.2.7. 3-(3,4,5-Trimethoxyphenylmethyl)-6-phenyl-7H-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazine (**3a**).* Yield 70%; m.p. 170–171.5 °C (ethanol); IR (KBr): 1587 (C=N), 1330 (C–N), 1240 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.77 (s, 6H, O*CH*$_3$), 3.81 (s, 3H, O*CH*$_3$), 3.98 (s, 2H,

CH_2), 4.30 (s, 2H, SCH_2), 6.65 (s, 2H, arom. H-2, H-6), 7.51–7.59 (m, 3H, arom. H-3′, H-4′, H-5′), 7.89 (d, 2H, arom. H-2′, H-6′); MS (70 eV, EI): *m/z* (%): 398 (M^+ +2, 7.86%), 396 (M^+, 100%), 381 (M^+ –CH_3, 12.14%), 365 (M^+ –OCH_3, 1.07%), 293 (M^+ –C_6H_5CN, 1.43%), 278 ([M^+ –($C_6H_5CN + CH_3$)], 38.57%), 207 (($(CH_3O)_3C_6H_2CH_2CN$, 11.43%), 192 (($(H_3CO)_3C_6H_2CH_2CN$–CH_3, 30.0%), 181 (($(CH_3O)_3C_6H_2CH_2$, 13.57%), 77 (C_6H_5, 27.14%). Anal. Calcd. for $C_{20}H_{20}N_4O_3S$: C, 60.59; H, 5.08; N, 14.13; S, 8.09. Found: C, 60.57; H, 5.22; N, 14.11; S, 8.11.

*6.1.2.8. 3-(3,4,5-Trimethoxyphenylmethyl)-6-(4-chlorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**3b**).* Yield 48%; m.p. 165–166 °C (ethanol); IR (KBr): 1589 (C═N), 1317 (C–N), 1246 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.57 (s, 3H, OCH_3), 3.66 (s, 6H, OCH_3), 4.19 (s, 2H, CH_2), 4.39 (s, 2H, SCH_2), 6.63 (s, 2H, arom. H-2, H-6), 7.63 (d, 2H, arom. H-2′, H-6′), 8.04 (d, 2H, arom. H-3′, H-5′); MS (70 eV, EI): *m/z* (%): 434 (M^+ +4, 2.76%), 432 (M^+ +2, 41.38%), 430 (M^+ 100%), 415 (M^+ –CH_3, 13.10%), 399 (M^+ –OCH_3, 1.72%), 293 (M^+ –ClC_6H_4CN, 2.07%), 278 ([M^+ –($ClC_6H_4CN + CH_3$)], 51.72%), 207 (($(CH_3O)_3C_6H_2CH_2CN$, 17.93%), 192 ((($(H_3CO)_3C_6H_2CH_2CN$)–CH_3, 40.69%), 181 (($(CH_3O)_3C_6H_2CH_2$, 21.38%), 111 (C_6H_4Cl, 6.25%). Anal. Calcd. for $C_{20}H_{19}ClN_4O_3S.H_2O$: C, 53.51; H, 4.72; N, 12.48; S, 7.14. Found: C, 53.42; H, 4.49; N, 12.62; S, 7.20.

*6.1.2.9. 3-(3,4,5-Trimethoxyphenylmethyl)-6-(4-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**3c**).* Yield 71%; m.p. 182–183 °C (it is precipitated with H_2NOH sol.); IR (KBr): 1598 (C═N), 1317 (C–N), 1236 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.57 (s, 3H, OCH_3), 3.66 (s, 6H, OCH_3), 4.19 (s, 2H, CH_2), 4.39 (s, 2H, SCH_2), 6.63 (s, 2H, arom. H-2, H-6), 7.39–7.43 (m, 2H, arom. H-3′, H-5′), 8.08–8.12 (m, 2H, arom. H-2′, H-6′); MS (70 eV, EI): *m/z* (%): 416 (M^+ +2, 6.92%), 414 (M^+, 100%), 399 (M^+ –CH_3, 12.31%), 383 (M^+ –OCH_3, 0.77%), 293 (M^+ –FC_6H_4CN, 1.54%), 278 ([M^+ –($FC_6H_4CN + CH_3$)], 33.85%), 207 (($(CH_3O)_3C_6H_2CH_2CN$, 12.31%), 192 ((($(H_3CO)_3C_6H_2CH_2CN$)–CH_3, 30.39%), 181 (($(H_3CO)_3C_6H_2CH_2$, 14.62%), 95 (C_6H_4F, 13.08%). Anal. Calcd. for $C_{20}H_{19}FN_4O_3S$: C, 57.96; H, 4.62; N, 13.52; S, 7.74. Found: C, 57.65; H, 4.44; N, 13.63; S, 7.78.

*6.1.2.10. 3-[2-(3,4,5-Trimethoxyphenyl)ethyl]-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**4a**).* Yield 39%; m.p. 158–159 °C (ethanol). All spectral data have been submitted for publication.

*6.1.2.11. 3-[2-(3,4,5-Trimethoxyphenyl)ethyl]-6-(4-chlorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**4b**).* Yield 68%; m.p. 168–169 °C (ethanol); IR (KBr): 1595 (C═N), 1309 (C–N), 1232 (C–O) cm^{-1}; ^1H NMR δ (ppm): 2.96 (t, 2H, CH_2CH_2), 3.15 (t, 2H, CH_2CH_2), 3.50 (s, 3H, OCH_3), 3.68 (s, 6H, OCH_3), 4.28 (s, 2H, SCH_2), 6.46 (s, 2H, arom. H-2, H-6), 7.61 (d, 2H, arom. H′-2, H′-6), 7.97 (d, 2H, arom. H-3′, H-5′); MS (70 eV, EI): *m/z* (%): 448 (M^+ +4, 0.69%), 446 (M^+ +2, 9.66%), 444 (M^+, 27.24%), 429 (M^+ –CH_3, 4.83%), 379 (M^+ –OCH_3, 1.38%), 292 ([M^+ –($ClC_6H_4CN + CH_3$)], 7.86%), 181 (($(CH_3O)_3C_6H_2CH_2$, 100%), 111 ($\cdot C_6H_4Cl$, 3.96%). Anal. Calcd. for $C_{21}H_{21}ClN_4O_3S$: C, 56.69; H, 4.76; N, 12.59; S, 7.21. Found: C, 56.46; H, 4.59; N, 12.69; S, 7.25.

*6.1.2.12. 3-[2-(3,4,5-Trimethoxyphenyl)ethyl]-6-(4-fluorophenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (**4c**).* Yield 56%; m.p. 183–184 °C (ethanol); IR (KBr): 1589 (C═N), 1309 (C–N), 1238 (C–O) cm^{-1}; ^1H NMR δ (ppm): 3.14 (t, 2H, CH_2CH_2), 3.39 (t, 2H, CH_2CH_2), 3.73 (s, 6H, OCH_3), 3.74 (s, 3H, OCH_3), 3.88 (s, 2H, SCH_2), 6.32 (s, 2H, arom. H-2, H-6), 7.20–7.24 (m, 2H, arom. H-3′, H-5′), 7.81–7.84 (m, 2H, arom. H-2′, H-6′); MS (70 eV, EI): *m/z* (%): 430 (M^+ +2, 2.50%), 428 (M^+, 31.43%), 413 (M^+ –CH_3, 6.07%), 397 (M^+ –OCH_3, 1.79%), 292 ([M^+ –($FC_6H_4CN + CH_3$)], 6.43%), 181 (($(CH_3O)_3C_6H_2CH_2$, 100%), 95 (C_6H_4F, 7.14%). Anal. Calcd. for $C_{21}H_{21}FN_4O_3S$: C, 58.87; H, 4.94; N, 13.08; S, 7.48. Found: C, 58.72; H, 4.91; N, 13.05; S, 7.58.

6.2. Single crystal X-ray crystallographic data of compounds **1** and **3a**

Single crystals suitable for X-ray diffraction of compounds **1** and **3a** were mounted on the ends of glass and used for the intensity data collection. The X-ray diffraction data on **1** and **3a** were collected using an Enraf-Nonius CAD-4 diffractometer employing graphite-monochromated MoK_α radiation ($\lambda = 0.71073$ Å). The datasets were corrected for Lorentz and polarization effect. An empirical ψ-scan absorption correction was applied.

Both structures were solved by the direct method programs SHELXS-97 [39] and refined by the full-matrix least squares method, based on F^2 using SHELXL-97 [40]. All non-hydrogen atoms were refined with anisotropic displacement factors. In compound **1**, H1 atom was found from difference map and all other hydrogen atoms were positioned geometrically and refined using a riding model. The geometric calculations were carried out with the Platon program [41].

Crystallographic data (excluding structure factors) for compounds **1** and **3a** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-700921, 700922. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

6.3. Pharmacology

6.3.1. Animals

Locally bred BALB/c mice of both sexes (30–35 g) were purchased from the animal breeding laboratories of Inonu University (Malatya, Turkey). The animals were fed a standard pellet diet and water *ad libitum* in a temperature-controlled room. On the day before the treatments, food was withdrawn, but the animals were allowed free access of water. The allocation of animals to different groups was randomized, and the experiments were carried out under blind conditions. Mice used in the present study were cared for in accordance with the directory of the Inonu University Animal Care Unit, which applies the guidelines of the National Institutes of Health on laboratory animal welfare. Procedures involving animals and their care were conducted in conformity with international laws and policies and animal studies accepted by Inonu University Ethical Council (2007/48).

To avoid wasting animals, groups composed of 3 or 4 mice were employed for preliminary testing using the carrageenan-induced paw edema model and writhing test, respectively. For preliminary activity screening, all test drugs were administered to mice at doses of 100 mg/kg (body weight). Test compounds that possessed more than a 20% inhibitory effect in any of the measurement ranges were selected for further evaluation of the activity–dose relationship in two different doses (50 mg/kg and 200 mg/kg) using groups consisting of 4–5 animals.

6.3.2. Preparation of test samples for bioassay

Test samples, suspended in a mixture of distilled water and 0.5% sodium carboxymethyl cellulose (CMC), were given orally to the animals. 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. Either indomethacin (10 mg/kg) or acetylsalicylic acid (ASA, 200 mg/kg) in 0.5% CMC was used as reference drug.

6.3.3. Anti-inflammatory activity

6.3.3.1. Carrageenan induced oedema [34]. For the determination of the effects on carrageenan-induced paw oedema, the modified

method of Kasahara et al. was employed [34]. One hour after the oral administration of either test sample or dosing vehicle, each mouse was injected with a freshly prepared (0.5 mg/25 μl) suspension of carrageenan (Sigma, St. Louis, Missouri, U.S.A.) in physiological saline (154 mM NaCl). The subplantar tissue of the right hind paw was the injection site for all mice. As the control, 25 μl saline solution was injected into the left hind paw. Paw oedema was measured every 90 min for 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. Percent inhibitory effects were estimated according to the following equation, where n was the average difference in thickness between the left and right hind paw of the control group and n' was that of test group of animals.

$$\text{Anti-inflammatory activity}(\%) = [(n - n')/n] \times 100$$

Indomethacin was used as a reference compound and administered at 10 mg/kg.

6.3.4. Analgesic activity

6.3.4.1. Koster test [35]. One hour after oral administration of test sample, each mouse was injected intraperitoneally with 3% (w/v) acetic acid solution (0.1 ml/10 g body weight). Starting 5 min after the acetic acid injection, the number of muscular contractions on mice was counted for a period of 10 min. A significant reduction in the number of writhings by any treatment as compared to the number of writhings in control animals was considered a positive analgesic response. The antinociceptive activity was expressed as a percentage change from writhing controls. Aspirin (ASA) was used as a reference compound and administered at 200 mg/kg.

6.3.5. Gastric ulceration studies

Only the animals that were administered 200 mg/kg (body weight) of test samples were subjected to this experimental process. Eight hours after the analgesic activity experiment, mice under deep ether anesthesia were killed, and their stomachs were removed. The abdomen of each mouse was opened through great curvature and, using a dissecting microscope was examined for lesions or bleeding.

6.3.6. Lipid peroxidation

All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation (LPO) by using the method of Ohkawa et al. [42] as modified by Jamall and Smith [43]. The LPO is measured as nmol of thiobarbituric acid (TBA)-reactive substances (TBARS)/g wet weight of tissue. ASA was used as a standard drug (200 mg/kg).

TBARS, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the oxidative stress. The tissue extract supernatant was obtained by two-step-centrifugation, first at 1000×g for 10 min and then at 2000×g for 30 min at 4 °C. Twenty millilitres of supernatant were transferred to a vial and was mixed with 0.20 ml sodium dodecyl sulfate (SDS; 8.1%) solution, 1.50 ml of acetic acid (CH_3COOH; 20% v/v, adjusted to pH 3.5 with NaOH) solution, and 1.50 ml of 0.8% w/v solution of TBA. The final volume was adjusted to 4.0 ml with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of blank tissue or test sample and 10% trichloroacetic acid (TCA) were centrifuged at 1000×g for 10 min. The absorbance of the supernatant was measured at 532 nm against Blank tissue. Blank tissue was processed using the same experimental procedure, except that the TBA solution was replaced with distilled water. 1,1,3,3-Tetraethoxypropane was used as a standard for the calibration curve.

6.3.7. Acute toxicity

Animals employed in the carrageenan-induced paw oedema experiment were observed for 72 h, and the mortality rate was recorded for each group at the end of the observation period.

6.3.8. Statistical analysis of data

Data obtained from animal experiments were expressed as means ± standard error (SEM). Statistical differences between the treatment and the control group of animals were evaluated by two-tailed Student's t test.

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