Med Chem Res (2012) 21:192–201
DOI 10.1007/s00044-010-9508-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of some thiazolotriazolyl esters as anti-inflammatory and analgesic agents

Birsen Tozkoparan · S. Peri Aytaç · Şule Gürsoy · Göknur Aktay

Received: 28 June 2010 / Accepted: 4 November 2010 / Published online: 12 December 2010

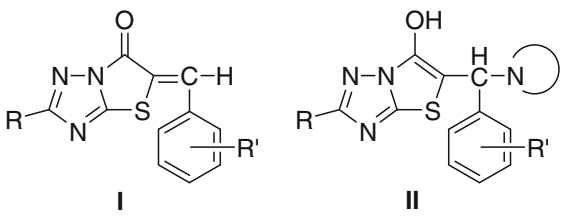
Abstract In order to develop potent analgesic/anti-inflammatory compounds with reduced ulcerogenic risk, a series of thiazolotriazolyl-carboxylic and acetic acid esters were synthesized and characterized by spectral and elementary analysis. All synthesized compounds were screened for in vivo anti-inflammatory activities in mice by carrageenan-induced paw edema model. The compounds showing 20% reduction in paw edema were also evaluated for their analgesic activities by acetic acid-induced writhing test and the gastric ulceration risk by determining the lipid peroxidation level in stomachs. Among the compounds tested, compounds **1**, **4**, **6**, **7**, **8**, **1a**, **2a**, **3a**, **4a**, **7a**, **2b**, and **8b** showed moderate-to-good anti-inflammatory activity at various doses in any of the measurement intervals. Compounds **7a**, **2b**, and **8b** were the most actives of the series in analgesic activity test. Moreover, compounds **1**, **4**, and **8** were found to be safe in stomach in respect of free radical production.

Keywords Thiazolotriazolyl-carboxylic acid esters · Thiazolotriazolyl acetic acid esters · Analgesic/antiinflammatory activity

Introduction

In recent decades, the literature has been enriched with progressive findings about the synthesis and pharmacological activities of various condensed heterocycles derived from symmetrical triazoles. The compounds bearing both a symmetrical triazole and 1,3-thiazole moieties represent an interesting class of compounds possessing a wide spectrum of biological activities such as anticancer, anti-inflammatory/analgesic, and antimicrobial properties (Barbuceanu *et al.*, 2009; El-Emam and Ibrahim, 1991; Karthikeyan *et al.*, 2008, 2009; Lesyk *et al.*, 2007; Pignatello *et al.*, 1991).

Thiazolo[3,2-*b*]-1,2,4-triazole fused ring system (**I** and **II**) having various substituents has become the subject of our recent attention as new analgesic/anti-inflammatory agent. We have prepared many compounds and screened them for their anti-inflammatory/analgesic properties in vivo. A considerable number of the prepared compounds have anti-inflammatory/analgesic activities comparable to or higher than the reference compounds besides lower ulcerogenic risks in the stomach (Dogdas *et al.*, 2007; Tozkoparan *et al.*, 1999, 2000, 2001, 2004, 2005). These results have further promoted our interest to design and synthesize some new products derived from thiazolo[3,2-*b*]-1,2,4-triazoles and evaluate their anti-inflammatory and analgesic activities.



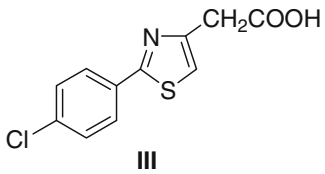
I II

Some thiazole-4(5)acetic acid derivatives are also known to possess anti-inflammatory activity (Brown *et al.*, 1968,

B. Tozkoparan (✉) · S. P. Aytaç
Department of Pharmaceutical Chemistry, Hacettepe University, Faculty of Pharmacy, 06100 Sıhhıye, Ankara, Turkey
e-mail: tbirsen@hacettepe.edu.tr

Ş. Gürsoy · G. Aktay
Department of Pharmacology, Inonu University, Faculty of Pharmacy, 44280 Malatya, Turkey

1974; Sharma and Sawhney, 1997). For example, fenclozic acid (**III**, I.C.I. 54,450; Myalex) is a synthetic thiazol-4-yl-acetic acid derivative, which has been reported to have potent anti-inflammatory, analgesic, and antipyretic properties (Hepworth *et al.*, 1969).



III

Besides, the literature survey revealed that derivatization (esterification or amidation) of carboxylic acid function of representative NSAIDs, resulted in an increased anti-inflammatory activity with reduced ulcerogenic effect (Galanakis *et al.*, 2004; Kalgutkar *et al.*, 2000, 2002, 2005; Khanna *et al.*, 2006; Zhong *et al.*, 2009).

Based on these findings and as part of our continuous efforts in this area, it was thought to be interesting to combine thiazolo[3,2-*b*]-1,2,4-triazole condensed ring with an ester side chain to elucidate the essential structure requirements in relation to activity. On this basis, herein we report the synthesis and preliminary pharmacological evaluation of a series of novel esters of thiazolotriazolyl carboxylic and acetic acids in this study.

Results and discussion

Chemistry

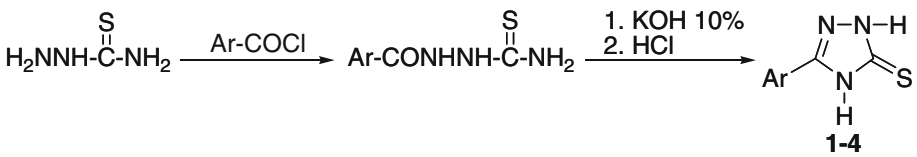
The synthesis of starting compounds, 3-aryl-/3-arylalkyl-1,2,4-triazole-5-thiones was, accomplished by various methods as outlined in Schemes 1 and 2. 3-Aryl substituted derivatives (**1**–**4**) of series were prepared by the reaction of benzoyl chlorides with the thiosemicarbazide, while the 3-arylalkyl-1,2,4-triazole-5-thiones (**5**–**8**) were obtained via dicyclohexylcarbodiimide (DCC)-promoted amide formation reaction starting from a carboxylic acid to the method reported earlier (Dogdas *et al.*, 2007; Tozkoparan *et al.*, 1999, 2000).

Scheme 3 reports the reactions which led to thiazolotriazole derivatives. It has been reported that thiazolo[3,2-*b*]-1,2,4-triazole derivatives could be obtained either by a direct reaction between mercaptotriazoles and appropriate halogeno-β-ketoesters (**A** route) or through alternative pathway involving the formation of *S*-alkyl products, which were then cyclized by an acid-catalyzed reaction ($POCl_3$, HCl, H_2SO_4) (**B** route) (Mazzone *et al.*, 1981; Moskowitz *et al.*, 1980; Pignatello *et al.*, 1991; Simiti *et al.*, 1991). In our study, title compounds, thiazolo[3,2-*b*]-1,2,4-triazole derivatives, were obtained by refluxing 3-substituted-1,2,4-triazole-5-thiones with either ethyl 4-chloro-3-oxobutyrate (**a**) or ethyl 2-chloroacetoacetate (**b**) in anhydrous ethanol in one-step.

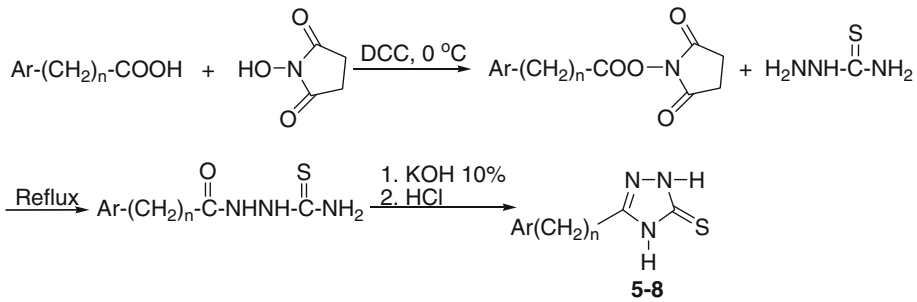
Assignment of new compounds was based on their elemental analysis and spectroscopic data. Characterization data of all new compounds are summarized in monographs.

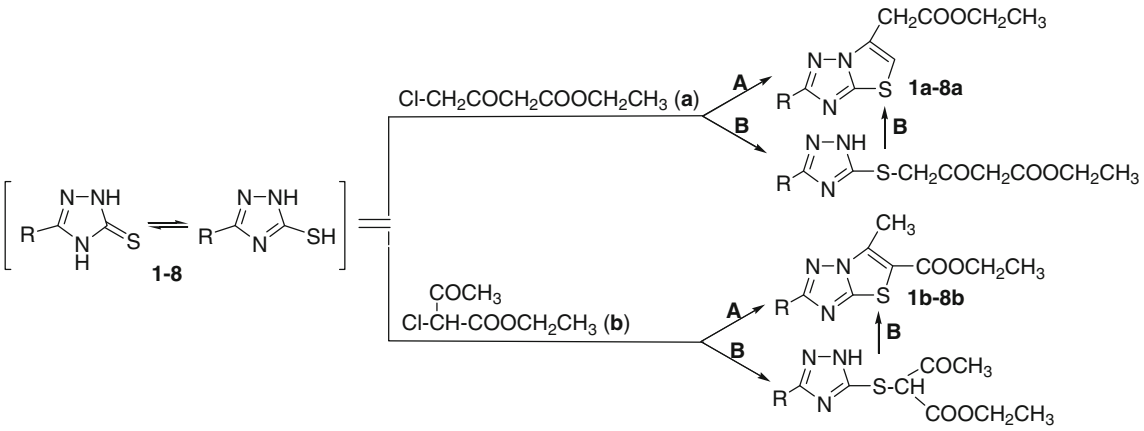
The IR spectra of the compounds **1a**–**8a** and **1b**–**8b** showed only one C=O stretching bands at 1737–1691 cm^{-1} because of ring closure. In 400 MHz ^1H NMR spectrum of compounds **1a**–**8a**, the CH (6) proton of the thiazolotriazole ring resonated as a singlet at 6.90–7.02 ppm, in agreement with data reported for analogous compounds (Pignatello *et al.*, 1991). The ^1H NMR spectra of compounds **1b**–**8b** showed a particular singlet due to the methyl group 1.28–1.42 ppm. The protons belonging to the aromatic ring and the other aliphatic groups were observed with the expected chemical shifts and integral values. All compounds gave satisfactory elemental analysis.

Scheme 1 Synthesis of 3-aryl-1,2,4-triazole-5-thiones (**1**–**4**)



Scheme 2 Synthesis of 3-arylalkyl-1,2,4-triazole-5-thiones (**5**–**8**)





Scheme 3 The reactions leading to thiazolo[3,2-*b*]-1,2,4-triazole derivatives

Pharmacological studies

Both starting compounds **1**–**8** and their corresponding condensed derivatives (**1a**–**8a** and **1b**–**8b**) were subjected to preliminary testing for anti-inflammatory and analgesic activity as well as the ulcerogenic risk on acute administration. At first, the substances were screened for anti-inflammatory activity at 100 mg/kg, p.o. dose. The compounds having more than 20% effectiveness in relieving symptoms, even some of them are not significant statistically, were considered both for further evaluation of anti-inflammatory activity in two different dose levels (50 and 200 mg/kg) and for screening their analgesic activities at the dose of 100 mg/kg. Besides, these compounds were also evaluated for their gastric toxicity by determining the lipid peroxidation level in stomachs. All pharmacological activity results have been summarized in Table 1.

Anti-inflammatory activity

The anti-inflammatory activity of derivatives was assessed from their ability to inhibit the paw edema induced by carrageenan in mice, and 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 (Kasahara *et al.*, 1985). As seen in Table 1, the compounds **1**, **4**, **6**, **7**, **8**, **1a**–**4a**, **7a**, **2b**, and **8b** possessed moderate-to-good anti-inflammatory activity at 100 mg/kg dose/p.o. in any of the measurement intervals. Especially, the compounds **6** and **8** having 3-[2-(4-methoxyphenyl)ethyl] and 3-[2-(3,4,5-trimethoxyphenyl)ethyl] substituent, respectively, showed the most remarkable activity at this dose. It was interesting to note that these two compounds when condensed with the thiazole ring, the activity was lost except **8b**. However, the selected compounds when administered increasing dose in a twofold (200 mg/kg) showed no activity (**1**, **4**, **6**, **7**, **8**, **1a**, **4a**, **7a**, and **2b**) or decreased activity (**2a**, **3a**, and **8b**). These results may suggest that the compounds **1**, **4**, **6**, **7**, **8**, **1a**, **4a**, **7a**, and **2b** reach their maximum effects at 100 mg/kg. There was a noticeable anti-inflammatory activity at 50 mg/kg dose only in compounds **1**, **6**, **7**, **8a**, and **8b**. Moreover, the activity of the compound **8b** at 50 mg/kg dose was more prominent than the ones seen with other doses.

In case of anti-inflammatory activity, many of the compounds carrying acetic acid ester at the 6th position of the fused ring (**1a**, **2a**, **3a**, **4a**, and **7a**) exhibited some degree of anti-inflammatory activity in any of the measurement intervals when compared to their carboxylic acid esters counterparts. It was observed that among the derivatives with acetic acid ester (**1a**–**8a**) compound **3a**, having a 2,4-dichlorophenyl at the 2nd position of the fused ring possess highest activity (42.8%). Among the compounds carrying a carboxylic acid ester (**1b**–**8b**), compound **8b** having a (3,4,5-trimethoxyphenyl)ethyl group at the 2nd position have attracted attention with almost equivalent activity to indomethacin at 50 mg/kg dose.

It is known that an edema produced by carrageenan is a biphasic event and if the inhibition is more effective in the first phase of carrageenan-induced edema, it means that the reply is histamin and serotonin mediated. In addition, if the inhibition is more effective in the second phase of carrageenan-induced edema, it means that the reply is prostaglandin mediated (Holsapple *et al.*, 1980). According to this literature information, it might be stated that our compounds show their anti-inflammatory effect through the PG-mediated mechanism (Table 1). In order to enlighten the mechanism in completely, further studies made with serotonin, histamine, and PG are also needed.

Analgesic activity

The analgesic activity of the compounds was studied using the acetic acid-induced writhing test in mice and expressed

Table 1 Preliminary anti-inflammatory and analgesic effects of the test compounds against carrageenan-induced hind paw edema model and acetic acid-induced abdominal constriction test in mice, respectively, and effects of selected compounds on the TBARS level in stomach tissue ($n = 4$–5)

Compounds	Dose (mg/kg)	TBARS (nmol/g wet weight)	Writhing reflex ± SEM (Inhibition %)	Swelling in thickness ($\times 10^{-2}$ mm) ± SEM (percent inhibitory activity)			
				90 min	180 min	270 min	360 min
Control ($n = 5$)	100	80.4 ± 5.4	15.6 ± 2.6	39.0 ± 5.6	52.0 ± 4.6	78.0 ± 8.4	105.0 ± 4.5
1	100	88.4 ± 2.	10.0 ± 0.8 (**35.9**)	44.3 ± 2.8	36.9 ± 3.6* (**27.1**)	111.0 ± 12.1	126.0 ± 8.6
	200			45.8. ± 1.4	51.7 ± 1.9	74.3 ± 2.4	105.4 ± 3.0
	50			39.8 ± 5.4	34.9 ± 5.2* (**32.9**)	61.3 ± 6.3 (**21.4**)	89.1 ± 5.4 (15.1)
4	100	73.3 ± 4.1	19.4 ± 2.5	33.7 ± 3.3	32.3 ± 2.8* (**37.9**)	79.5 ± 6.6	133.0 ± 13.1
	200			32.4 ± 2.7 (16.9)	53.2 ± 0.9	75.0 ± 2.8	104.5 ± 3.6
	50			59.3 ± 7.2	49.7 ± 7.3	64.8 ± 5.9 (17.9)	100.0 ± 8.8
6	100	116.2 ± 7.7**	11.8 ± 1.7 (**24.3**)	36.3 ± 5.3	25.3 ± 2.8** (**51.3**)	54.0 ± 6.0 (**30.8**)	80.5 ± 4.2** (**23.3**)
	200			42.1 ± 2.6	46.4 ± 3.9	66.3 ± 2.9 (15.0)	87.6 ± 5.7 (16.6)
	50			50.0 ± 4.9	46.8 ± 2.2	73.6 ± 5.1	78.2 ± 8.3* (**25.5**)
7	100	115.1 ± 10.4*	24.4 ± 3.6	36.2 ± 6.2	42.5 ± 7.8 (18.3)	60.0 ± 8.2 (**23.1**)	65.0 ± 8.7* (**38.1**)
	200			45.6 ± 3.6	49.8 ± 3.8	77.0 ± 3.8	104.5 ± 5.9
	50			35.6 ± 7.1	46.0 ± 6.9	57.8 ± 5.8 (**25.9**)	77.3 ± 7.4* (**26.4**)
8	100	79.1 ± 0.9	23.0 ± 1.4	28.0 ± 3.2 (**28.2**)	30.8 ± 6.7* (**40.8**)	37.3 ± 6.7** (**52.2**)	65.1 ± 0.8*** (**38.0**)
	200			39.7 ± 2.7	50.5 ± 2.8	70.3 ± 3.1	106.7 ± 7.6
	50			52.6 ± 6.8	48.2 ± 4.4	74.5 ± 4.1	84.1 ± 5.1* (**19.9**)
1a	100	174.1 ± 13.8***	18.2 ± 3.8	45.0 ± 5.0	40.0 ± 9.1 (**23.1**)	53.7 ± 13.1 (**31.1**)	87.5 ± 4.1* (16.7)
	200			35.2 ± 3.2	48.1 ± 1.9	75.0 ± 2.5	105.4 ± 5.1
	50			76.3 ± 6.4	53.5 ± 5.4	79.7 ± 7.6	102.0 ± 10.5
2a	100	157.2 ± 2.9***	9.6 ± 2.9 (**38.5**)	43.7 ± 8.5	52.5 ± 18.9	82.5 ± 10.3	70.0 ± 10.6* (**33.3**)
	200			29.1 ± 3.8 (**25.4**)	37.2 ± 2.4* (**28.5**)	58.6 ± 6.5 (**24.9**)	86.7 ± 3.9* (17.4)
	50			61.0 ± 6.8	66.1 ± 2.4	88.5 ± 5.9	105.0 ± 6.1
3a	100	200.1 ± 7.9***	10.0 ± 2.1 (**35.9**)	40.0 ± 15.8	66.2 ± 24.7	65.0 ± 25.0 (16.7)	60.0 ± 11.8* (**42.8**)
	200			41.6 ± 3.2	43.0 ± 2.5 (17.3)	61.3 ± 4.9 (**21.4**)	83.1 ± 6.2* (**20.8**)
	50			70.4 ± 8.8	49.0 ± 4.6	78.0 ± 8.7	96.0 ± 9.3
4a	100	128.7 ± 11.5**	11.8 ± 2.6 (**24.3**)	47.9 ± 4.5	38.3 ± 3.0* (**24.4**)	90.0 ± 12.5	122.5 ± 11.0
	200			43.7 ± 1.3	52.2 ± 1.9	79.0 ± 3.	110.8 ± 2.9
	50			71.2 ± 5.6	65.4 ± 3.6	76.2 ± 8.5	96.1 ± 8.2
7a	100	157.1 ± 8.6***	5.8 ± 1.9* (**62.8**)	40.8 ± 4.5	32.3 ± 4.4* (**37.9**)	105.0 ± 9.4	129.5 ± 16.1
	200			38.0 ± 2.3	42.5 ± 3.5 (18.3)	70.3 ± 1.7	93.4 ± 4.5
	50			55.1 ± 5.7	46.0 ± 3.4	61.3 ± 6.0 (**21.4**)	87.2 ± 7.7 (16.9)
2b	100	116.9 ± 6.3**	1.6 ± 0.2*** (**89.7**)	45.5 ± 2.0	42.8 ± 1.9 (17.7)	69.9 ± 7.2	75.4 ± 8.3* (**28.2**)
	200			35.7 ± 3.3	45.7 ± 1.4	69.6 ± 2.1	98.3 ± 5.2

Table 1 continued

Compounds	Dose (mg/kg)	TBARS (nmol/g wet weight)	Writhing reflex ± SEM (Inhibition %)	Swelling in thickness ($\times 10^{-2}$ mm) ± SEM (percent inhibitory activity)			
				90 min	180 min	270 min	360 min
	50			66.1 ± 4.7	70.0 ± 6.9	91.1 ± 9.5	117.8 ± 5.5
8b	100	109.1 ± 10.1*	2.2 ± 0.6*** (**85.9**)	34.5 ± 11.6	37.0 ± 3.8* (**28.8**)	63.4 ± 21.6 (18.7)	83.8 ± 13.8 (**20.2**)
	200			32.4 ± 1.9 (16.9)	38.9 ± 2.6* (**25.2**)	60.9 ± 3.4 (**21.9**)	92.9 ± 7.6
	50			19.5 ± 3.4* (**50.0**)	29.0 ± 9.5 (**44.2**)	73.8 ± 5.9	101.4 ± 8.3
INDO	10	–	–	36.3 ± 5.3 (6.9)	22.5 ± 1.4** (**56.7**)	51.0 ± 3.6* (**34.6**)	77.0 ± 5.5** (**26.7**)
ASA	200	121.0 ± 2.1***	7.8 ± 1.7* (**50.0**)				

* $p < 0.05$ (significant); ** $p < 0.01$ (very significant); *** $p < 0.001$ (extremely significant)

as "mean increase in latency after drug administration ± SEM" relative to control and percentage inhibition in writhing reflex (Koster *et al.*, 1959). Although the compounds **1**, **6**, **2a**, **3a**, and **4a** showed some activity, it is not significant statistically. Among the compounds, the compounds **7a**, **2b**, and **8b** attracted attention with higher analgesic activity than aspirin with percentage inhibition values 62.8, 89.7, and 85.9%, respectively, at 100 mg/kg dose level.

Antioxidant activity, lipid peroxidation

It is well established that reactive oxygen species have a decisive role in inflammatory conditions (Halliwell and Gutteridge, 1999). It has also been reported that oxidative stress is an important component of gastrointestinal ulceration (Bragt *et al.*, 1980; Halliwell, 1991). The compounds having antioxidant activity besides anti-inflammatory/analgesic activity may offer a viable route safer anti-inflammatory/analgesic agents. Therefore, all compounds having more or less analgesic activity were also analyzed for their antioxidant properties by determining lipid peroxidation level. The lipid peroxidation is measured as nmol of TBARS/g wet weight of tissue. The obtained lipid peroxidation values revealed that the ulcerogenic effect of only the compounds **1**, **4**, and **8** was appreciably less than aspirin. The compound **8b**, exhibited both noteworthy anti-inflammatory and analgesic activities, was found relatively safer for ulcerogenic risk than the other compounds.

Acute toxicity

None of the test compounds used in the pharmacologic study produced lethal effect and did not induce any significant behavioral modification at the employed doses during observation period.

Conclusions

The research study reports the synthesis and anti-inflammatory/analgesic activity and ulcerogenic risk of new triazolothiazolyl ester derivatives. Several derivatives have been found to possess analgesic and anti-inflammatory activities. Few derivatives have been evaluated for remarkable anti-inflammatory activity devoid of ulcerogenic risk. Although findings of our pharmacological tests did not allow to delineate an exact relationship between the structure of the synthesized thiazolotriazole derivatives and their analgesic/anti-inflammatory activity, the results are conclusive in showing that there is no noticeable increase in analgesic and anti-inflammatory activity when 3-aryl-/5-arylalkyl-1,2,4-triazole-5-thiones are condensed with the thiazole ring.

According to the results of the present study, among the synthesized compounds, a carboxylic acid ester derivative **8b**, having a (3,4,5-trimethoxyphenyl)ethyl group at the 2nd position of the fused ring, was found to have both significant analgesic and consistent anti-inflammatory activity with relatively reduced lipid peroxidation and would deserve further attention to develop new leadings.

Experimental part

Chemistry

Melting points (mp) were detected with a Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA) and were uncorrected. Infrared (IR) spectra were recorded on Perkin Elmer 1720X FT-IR spectrometer (Beaconsfield, UK) using potassium bromide pellets, and the result was expressed in wave number (cm^{-1}). ^1H nuclear magnetic resonance (^1H NMR) spectra were taken on Varian Mercury 400, 400 MHz High Performance Digital FT-NMR instrument (Palo Alto, CA, USA) in $CDCl_3$ using TMS as internal standard. All chemical shifts were recorded as δ (ppm) values. Splitting patterns are as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; m, multiplet. The purity of the compounds was checked on silicagel-coated aliminium sheets (Merck, 1.005554, silicagel $HF_{254-361}$, Type 60, 0.25 mm) by thin layer chromatography. The elementary analysis of the new compounds were performed with Leco CHNS 932 analyzer at Ankara University, Faculty of Pharmacy Central II Laboratory. Elementary analysis for C, H, N, and S were within ±0.4% of theoretical values. All chemicals were from Aldrich Chemical Co. (Steinheim, Germany).

Synthesis of 3-aryl-1,2,4-triazole-5-thiones (**1**–**4**)

The synthesis of the compounds 3-phenyl-5-mercapto-1,2,4-triazole (**1**), 3-(4-chlorophenyl)-5-mercapto-1,2,4-triazole (**2**), 3-(3,5-dichlorophenyl)-5-mercapto-1,2,4-triazole (**3**), and 3-(4-methoxyphenyl)-5-mercapto-1,2,4-triazole (**4**) were performed according to the reported literature procedure (Tozkoparan *et al.*, 1999). The compounds had already been reported previously (Diaz *et al.*, 2006; Malbec *et al.*, 1984).

Synthesis of 3-arylalkyl-1,2,4-triazole-5-thiones (**5**–**8**)

The compounds **5**–**8** were prepared to the method reported earlier (Tozkoparan *et al.*, 2000; Doğdaş *et al.*, 2007). Compound **5**, 3-[(4-methoxyphenyl)methyl]-1,2,4-triazole-5-thione, has been reported before (Ovsepyan *et al.*, 1977). Furthermore, compound **6** [*1097795-00-0*] is seemed as commercial product in "Science Finder." Since there is no information in literature for the preparation and spectral characteristics, the compound has been included in our research program and characterized by spectral data.

3-[2-(4-Methoxyphenyl)ethyl]-1,2,4-triazole-5-thione (**6**)

Yield 63%. mp 211–212°C. ^1H NMR ($CDCl_3$-d): δ 2.71 (2H, t, $-CH_2-$), 2.81 (2H, t, $-CH_2-$), 3.63 (3H, s, $-OCH_3$), 6.66 (2H, d, arom.H), 6.95 (2H, d, arom.H), 12.73 (1H, s, NH). IR ν_{max} cm^{-1} (KBr): 3352 (N–H), 1598 (C=N), 1252 (C=S).

3-[(3,4,5-Trimethoxyphenyl)methyl]-1,2,4-triazole-5-thione (**7**)

Yield 70%. mp 235–237°C. ^1H NMR ($CDCl_3$-d): δ 3.66 (2H, s, $-CH_2-$), 3.73 (9H, s, $3OCH_3$), 6.46 (2H, s, arom.H), 12.99 (1H, bs, NH). IR ν_{max} cm^{-1} (KBr): 3361 (N–H), 1590 (C=N), 1241 (C=S).

3-[2-(3,4,5-Trimethoxyphenyl)ethyl]-1,2,4-triazole-5-thione (**8**)

Yield 70%. mp 228–229°C. ^1H NMR ($CDCl_3$-d): δ 2.47 (2H, t, $-CH_2-$), 2.55 (2H, t, $-CH_2-$), 3.38 (3H, s, $-OCH_3$), 3.43 (6H, s, $2OCH_3$), 6.01 (2H, s, arom.H), 12.60 (1H, bs, NH). IR ν_{max} cm^{-1} (KBr): 3355 (N–H), 1590 (C=N), 1242 (C=S).

Thiazolo[3,2-*b*]-1,2,4-triazoles, 2,6-disubstituted and 2,5,6-trisubstituted (**1a**–**8a** and **1b**–**8b**)

A solution of 0.01 mol 5-mercapto-1,2,4-triazole and 0.01 mol ethyl 4-chloro-3-oxobutyrate or 2-chloroacetoacetate in 8 ml of absolute etanol was refluxed over a period of 4 days. After cooling, the reaction mixture was filtered, and the resulting solid was recrystallized from appropriate solvents. Compounds **1a**, 2-phenyl-thiazolo[3,2-*b*]-1,2,4-triazole-6-acetic acid ethyl ester, and **2a**, 2-(4-chlorophenyl)-thiazolo[3,2-*b*]-1,2,4-triazole-6-acetic acid ethyl ester, **1b** 6-methyl-2-phenyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester, and **2b**, 2-(4-chlorophenyl)-6-methyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester, and **4b**, 2-(4-methoxyphenyl)-6-methyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester, have been reported previously (Moskowitz *et al.*, 1980; Simiti *et al.*, 1991; Zaharia *et al.*, 2000).

Since we are not able to reach literature belonging Zaharia *et al.* (2000) in where compound **4b** is reported, the compound has been characterized by spectral and elemental analysis.

2-(3,5-Dichlorophenyl)-thiazolo[3,2-*b*]-1,2,4-triazole-6-acetic acid ethyl ester (**3a**)

Yield 64%. mp 144–145°C. ^{1}H NMR ($CDCl_3$-d): δ 1.32 (3H, t, $–CH_3$), 4.01 (2H, s, $–CH_2C=O$), 4.27 (2H, q, $–CH_2–$), 7.02 (1H, s, –CH=), 7.40 (1H, s, arom.H-4), 8.06 (2H, s, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1715 (C=O), 1594 (C=N). Anal. Calcd. for $C_{14}H_{11}Cl_2N_3O_2S$: C, 47.20; H, 5.54; N, 12.17; S, 9.28. Found: C, 46.66; H, 5.19; N, 12.07; S, 9.28.

2-(4-Methoxyphenyl)-thiazolo[3,2-*b*]-1,2,4-triazole-6-acetic acid ethyl ester (**4a**)

Yield 81%. mp 115–116°C. ^{1}H NMR (400 MHz, $CDCl_3$-d): δ 1.31 (3H, t, $–CH_3$), 3.87 (3H, s, $–OCH_3$), 4.00 (2H, s, $–CH_2C=O$), 4.26 (2H, q, $–CH_2–$), 6.92 (1H, s, –CH=), 6.98 (2H, d, arom.H-3 and H-5), 8.09 (2H, d, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1732 (C=O), 1616 (C=N). Anal. Calcd. for $C_{15}H_{15}N_3O_3S$: C, 53.72; H, 5.11; N, 12.53; S, 9.56. Found: C, 54.08; H, 4.77; N, 12.66; S, 9.66.

2-[(4-Methoxyphenyl)methyl]-thiazolo[3,2-*b*]-1,2,4-triazole-6-acetic acid ethyl ester (**5a**)

Yield 62%. mp 59–60°C. ^{1}H NMR ($CDCl_3$-d): δ 1.28 (3H, t, $–CH_3$), 3.77 (3H, s, OCH_3), 3.94 (2H, s, $–CH_2–$), 4.11 (2H, s, $–CH_2C=O$), 4.23 (2H, q, $–CH_2–$), 6.85 (2H, d, arom.H-3 and H-5), 6.88 (1H, s, –CH=), 7.29 (2H, d, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1719 (C=O), 1611 (C=N). Anal. Calcd. for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68; S, 9.67. Found: C, 57.90; H, 5.16; N, 12.74; S, 9.80.

2-[2-(4-Methoxyphenyl)ethyl]-thiazolo[3,2-*b*]-1,2,4-triazole-6-acetic acid ethyl ester (**6a**)

Yield 53%. mp 61–62°C. ^{1}H NMR ($CDCl_3$-d): δ 1.31 (3H, t, $–CH_3$), 3.08–3.12 (4H, m, $–CH_2CH_2–$), 3.79 (3H, s, OCH_3), 4.09 (2H, s, $–CH_2C=O$), 4.21 (2H, q, $–CH_2–$), 6.85 (2H, d, arom.H-3 and H-5), 6.90 (1H, s, –CH=), 7.31 (2H, d, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1720 (C=O), 1612 (C=N). Anal. Calcd. for $C_{17}H_{19}N_3O_3S$: C, 59.11; H, 5.54; N, 12.17; S, 9.28. Found: C, 59.46; H, 5.91; N, 12.30; S, 9.19.

2-[(3,4,5-Trimethoxyphenyl)methyl]-thiazolo[3,2-*b*]-1,2,4-triazole-6-acetic acid ethyl ester (**7a**)

Yield 73%. mp 81–82°C. ^{1}H NMR ($CDCl_3$-d): δ 1.29 (3H, t, $–CH_3$), 3.80 (3H, s, OCH_3), 3.84 (6H, s, $–OCH_3$), 3.96 (2H, s, $–CH_2–$), 4.11 (2H, s, $–CH_2C=O$), 4.24 (2H, q, $–CH_2–$), 6.61 (2H, s, arom.H-2 and H-6), 6.91 (1H, s, –fCH=). IR ν_{max} cm^{-1} (KBr): 1720 (C=O), 1593 (C=N). Anal. Calcd. for $C_{18}H_{21}N_3O_5S$: C, 55.23; H, 5.41; N, 10.73; S, 8.19. Found: C, 54.90; H: 5.35; N, 10.77; S, 8.29.

2-[2-(3,4,5-Trimethoxyphenyl)ethyl]-thiazolo[3,2-*b*]-1,2,4-triazole-6-acetic acid ethyl ester (**8a**)

Yield 45%. mp 99–100°C. ^{1}H NMR ($CDCl_3$-d): δ 1.29 (3H, t, $–CH_3$), 3.07–3.16 (4H, m, $–CH_2CH_2–$), 3.83 (9H, s, $3OCH_3$), 3.94 (2H, s, $–CH_2C=O$), 4.24 (2H, q, $–CH_2–$), 6.49 (2H, s, arom.H-2 and H-6), 6.91 (1H, s, –CH=). IR ν_{max} cm^{-1} (KBr): 1720 (C=O), 1591 (C=N). Anal. Calcd. for $C_{19}H_{23}N_3O_5S$: C, 56.28; H, 5.72; N, 10.36; S, 7.91. Found: C, 56.14; H, 5.50; N, 10.52; S, 8.05.

2-(3,5-Dichlorophenyl)-6-methyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester (**3b**)

Yield 53%. mp 213–215°C. ^{1}H NMR ($CDCl_3$-d): δ 1.42 (3H, t, $–CH_3$), 2.94 (3H, s, $–CH_3$), 4.43 (2H, q, $–CH_2–$), 7.43 (1H, t, arom.H-4), 8.09 (2H, d, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1711 (C=O), 1584 (C=N). Anal. Calcd. for $C_{14}H_{11}Cl_2N_3O_2S$: C, 47.20; H, 5.54; N, 12.17; S, 9.28. Found: C, 46.23; H, 5.19; N, 11.63; S, 8.97.

2-(4-Methoxyphenyl)-6-methyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester (**4b**)

Yield 80%. mp 147–148°C. ^{1}H NMR ($CDCl_3$-d): δ 1.42 (3H, t, $–CH_3$), 2.93 (3H, s, $–CH_3$), 3.88 (3H, s, $–OCH_3$), 4.41 (2H, q, $–CH_2–$), 7.00 (2H, d, arom.H-3 and H-5), 8.13 (2H, d, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1704 (C=O), 1612 (C=N). Anal. Calcd. for $C_{15}H_{15}N_3O_3S$: C, 53.72; H, 5.11; N, 12.53; S, 9.56. Found: C, 56.45; H, 4.63; N, 13.18; S, 10.16.

2-[(4-Methoxyphenyl)methyl]-6-methyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester (**5b**)

Yield 77%. mp 135–136°C. ^{1}H NMR ($CDCl_3$-d): δ 1.28 (3H, t, $–CH_3$), 2.87 (3H, s, CH_3), 3.78 (3H, s, OCH_3), 4.11 (2H, s, $–CH_2–$), 4.38 (2H, q, $–CH_2–$), 6.86 (2H, d, arom.H-3 and H-5), 7.30 (2H, d, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1695 (C=O), 1606 (C=N). Anal. Calcd. for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68; S,9.67. Found: C, 57.88; H, 5.19; N, 12.71; S, 9.78.

2-[2-(4-Methoxyphenyl)ethyl]-6-methyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester (**6b**)

Yield 69%. mp 91–92°C. ^{1}H NMR ($CDCl_3$-d): δ 1.40 (3H, t, $–CH_3$), 2.87 (3H, s, $–CH_3$), 3.10–3.14 (4H, m, $–CH_2CH_2–$), 3.79 (3H, s, $–OCH_3$), 4.39 (2H, s, $–CH_2–$),

6.84 (2H, d, arom.H-3 and H-5), 7.18 (2H, d, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1709 (C=O), 1613 (C=N). Anal. Calcd. for $C_{17}H_{19}N_3O_3S$: C, 59.11; H, 5.54; N, 12.17; S, 9.28. Found: C, 58.70; H, 5.79; N, 12.05; S, 9.23.

2-[(3,4,5-Trimethoxyphenyl)methyl]-6-methyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester (**7b**)

Yield 47%. mp 114–115°C. ^{1}H NMR ($CDCl_3$-d): δ 1.39 (3H, t, $-CH_3$), 2.88 (3H, s, $-CH_3$), 3.78 (3H, s, OCH_3), 3.82 (6H, s, $2OCH_3$), 3.96 (2H, s, $-CH_2-$), 4.26 (2H, q, $-CH_2-$), 6.90 (2H, s, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1700 (C=O), 1591 (C=N). Anal. Calcd. for $C_{18}H_{21}N_3O_5S$: C, 55.23; H, 5.41; N, 10.73; S, 8.19. Found: C, 54.95; H, 5.27; N, 10.69; S, 8.26.

2-[2-(3,4,5-Trimethoxyphenyl)ethyl]-6-methyl-thiazolo[3,2-*b*]-1,2,4-triazole-5-carboxylic acid ethyl ester (**8b**)

Yield 32%. mp 103–104°C. ^{1}H NMR (400 MHz, $CDCl_3$-d): δ 1.41 (3H, t, $-CH_3$), 2.88 (3H, s, $-CH_3$), 3.09–3.14 (4H, m, $-CH_2CH_2-$), 3.81 (3H, s, OCH_3), 3.85 (6H, s, $2OCH_3$), 4.25 (2H, q, $-CH_2-$), 6.89 (2H, s, arom.H-2 and H-6). IR ν_{max} cm^{-1} (KBr): 1691 (C=O), 1588 (C=N). Anal. Calcd for $C_{19}H_{23}N_3O_5S$: C, 56.28; H, 5.72; N, 10.36; S, 7.91. Found: C, 55.85; H, 6.06; N, 10.17; S, 7.95.

Pharmacology

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).

All of the experimental groups composed of five 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 and 200 mg/kg). Because of the daily changing circumstance, we used another control group for each experiment. In the final stage of the experiment, all the results were prepared and showed on Table 1, considering only one control group.

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.

Anti-inflammatory activity, carrageenan-induced edema

For the determination of the effects on carrageenan-induced paw edema, the modified method of Kasahara *et al.* was employed (Kasahara *et al.*, 1985). 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, MO, USA) in physiological saline (154 mM NaCl). The subplantar tissue of the right hind paw was the injection site for all mice. The contralateral paw (left hind paw) was injected with 25 µl saline solution as the internal control. Paw edema was measured every 90 min for 6 h after induction of inflammation with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). The difference in footpad thickness between the right and left foot indicated the degree of inflammation for each mouse. The change in paw volume, either increase or decrease, was calculated and compared with the control group (dosing vehicle) 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.

Analgesic activity, Koster test (Koster *et al.*, 1959)

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 were 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 analgesic activity was expressed as a percentage change from writhing controls. Aspirin (ASA) was used as a reference compound and administered at 200 mg/kg.

Antioxidant activity, lipid peroxidation

Only the animals who were administered 100 mg/kg (body weight) of the 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 stomach of each mouse was opened through great curvature and was examined for antioxidant activity. Lipid peroxidation was assessed by using the method of Ohkawa *et al.* (1979) as modified by Jammal and Smith (1985) and was expressed 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 $1000\times g$ for 10 min and then at $2000\times g$ for 30 min at 4°C. Twenty millilitre of supernatant was 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 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 $1000\times g$ 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.

Acute toxicity

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

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.

Acknowledgments We are grateful Hacettepe University Research Center for its financial supports (P. N.: 0701301001).

Conflict of interest The authors have declared no conflict of interest.

References

Barbuceanu SF, Almajan GL, Saramet I, Draghici C, Tarcomnicu AI, Bancescu G (2009) Synthesis, characterization and evaluation of antibacterial activity of some thiazolo[3,2-*b*][1,2,4]triazole incorporating diphenylsulfone moieties. Eur J Med Chem 44(11): 4752–4757

Bragt PC, Bansberg JI, Bonta IL (1980) Antiinflammatory effects of free radical scavengers and antioxidants: further support for proinflammatory roles of endogenous hydrogen peroxide and lipid peroxides. Inflammation 4(3):289–299

Brown K, Cavalla JF, Green D, Wilson AB (1968) Diaryloxazole and diarylthiazolealkanoic acids: two novel series of non-steroidal anti-inflammatory agents. Nature 219:164

Brown K, Cater DP, Cavalla JF, Green D, Newberry RA, Wilson AB (1974) Nonsteroidal antiinflammatory agents. 1,2,4-Diphenylthiazole-5-acetic acid and related compounds. J Med Chem 17:1177–1181

Diaz P, Raffin C, Pelisson I (2006) Tyrosinase inhibitors and their use for treating hyperpigmentary disorders. PCT Int. Appl., WO 2006103345, 28 pp.

Doğdaş E, Tozkoparan B, Kaynak FB, Eriksson L, Küpeli E, Yeşilada E, Ertan M (2007) Design and synthesis of some new thiazolo[3,2-*b*]-1,2,4-triazole-5(6H)-ones substituted with flurbiprofen as antiinflammatory-analgesic agents. Arzneim-Forsch/Drug Res 57(4):196–202

El-Emam AA, Ibrahim TM (1991) Synthesis, anti-inflammatory and analgesic activity of certain 3-(1-adamantyl)-4-substituted-5-mercapto-1,2,4-triazole derivatives. Arzneim Forsch/Drug Res 41(II):1260–1264

Galanakis D, Kourounakis AP, Tsiakitzis KC, Doulgkeris C, Rekka EA, Gavalas A, Kravaritou C, Charitos C, Kourounakis PN (2004) Synthesis and pharmacological evaluation of amide conjugates of NSAIDs with L-cysteine ethyl ester, combining potent antiinflammatory and antioxidant properties with significantly reduced gastrointestinal toxicity. Bioorg Med Chem Lett 14(14):3639–3643

Halliwell B (1991) Drug antioxidant effects. A basis for drug selection? Drugs 42(4):569–605

Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine, 3rd edn. Oxford University Press, Oxford

Hepworth W, Newbould BB, Platt DS, Stacey GJ (1969) 2-(4-Chlorophenyl)thiazol-4-ylacetic acid ('Myalex'): a new compound with anti-inflammatory, analgesic and antipyretic activity. Nature 221:582–583

Holsapple MP, Schnur M, Yim GK (1980) Pharmacological modulation of edema mediated by prostaglandin, serotonin and histamine. Agents Actions 10(4):368–373

Jammal S, Smith JC (1985) Effects of cadmium on glutathione peroxidase, superoxide dismutase and lipid peroxidation in the rat heart: a possible mechanism of cadmium cardiotoxicity. Toxicol Appl Pharmacol 80:33–42

Kalgutkar AS, Marnett AB, Crews BC, Remmel RP, Marnett LJ (2000) Ester and amide derivatives of the nonsteroidal antiinflammatory drug, indomethacin, as selective cyclooxygenase-2 inhibitors. J Med Chem 43(15):2860–2870

Kalgutkar AS, Rowlinson SW, Crews BC, Marnett LJ (2002) Amide derivatives of meclofenamic acid as selective cyclooxygenase-2 inhibitors. Bioorg Med Chem Lett 12(4):521–524

Kalgutkar AS, Crews BC, Saleh S, Prudhomme D, Marnett LJ (2005) Indolyl esters and amides related to indomethacin are selective COX-2 inhibitors. Bioorg Med Chem 13(24):6810–6822

Karthikeyan MS (2009) Synthesis, analgesic, anti-inflammatory and antimicrobial studies of 2,4-dichloro-5-fluorophenyl containing thiazolotriazoles. Eur J Med Chem 44(2):827–833

Karthikeyan MS, Holla BS (2008) Synthesis, antiinflammatory and antimicrobial activities of some 2,4-dichloro-5-fluorophenyl substituted arylidenetriazolo thiazolidinones. Monatsh Chem 139:691–696

Kasahara Y, Hikino H, Tsurufuji S, Watanabe M, Ohuchi K (1985) Antiinflammation actions of ephedrines in acute inflammations. Planta Med 51(4):325–331

Khanna S, Madan M, Vangoori A, Banerjee R, Thaimattam R, Jafar Sadik Basha SK, Ramesh M, Casturi M, Pal M (2006) Evaluation of glycolamide esters of indomethacin as potential cyclooxygenase-2 (COX-2) inhibitors. Bioorg Med Chem 14(14): 4820–4833

Koster R, Anderson R, De Beer EJ (1959) Acetic acid for analgesic screening. Fed Proc 18:412–418

Lesyk R, Vladzimirska O, Holota S, Zaprutko L, Gzella A (2007) New 5-substituted thiazolo[3,2-*b*][1,2,4]triazol-6-ones: synthesis and anticancer evaluation. Eur J Med Chem 42(5):641–648

Malbec F, Milcent R, Barbier G (1984) Dérivés de la dihydro-2,4 triazole-1,2,4 thione-3 et de l'amino-2 thiadiazole-1,3,4 à partir de nouvelles thiosemicarbazones d'esters. J Het Chem 21: 1689–1698

Mazzone G, Bonina F, Arrigo Reina R, Blandino G (1981) 3-Methylthio-,3-(amino-substituted)ethylthio-,3-carboxymethylthio-,3,3′-(dithiobis)-5-aryl-4H(R)-1,2,4-triazoles and 2-aminomethyl-N-substituted derivatives of 5-aryl-4H(R)-1,2,4-triazolin-3-thione: synthesis and biological activity. Il Farmaco 36: 181–196

Moskowitz H, Mignot A, Miocque M (1980) Synthesis of thiazolotriazolylacetic acids. J Het Chem 17(6):1321–1323

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Ovsepyan TR, Avetisyan AKh, Aroyan AA, Paronikyan RV (1977) Synthesis of substituted 1,2,4-triazoles and 1,3,4-thiadiazoles. Arm Khim Z 30(1):70–73

Pignatello R, Mazzone S, Panico AM, Mazzone G, Pennisi G, Castana R, Matera M, Blandino G (1991) Synthesis and biological evaluation of thiazolo-triazole derivatives. Eur J Med Chem 26:929–938

Sharma PK, Sawhney SN (1997) Potent antiinflammatory 3-thiazole-4(5)-acetic acids of 1,2-benzisothiazole. Bioorg Med Chem Lett 7:2427–2430

Simiti I, Zaharia V, Coman M, Demian H (1991) Heterocycles. LXV: synthesis and chemical characterization of some 2-aryl-6-ethoxycarbonyl-5-methyl-thiazolo[3,2-*b*]-1,2,4-triazoles. Arch Pharm 324:49–51

Tozkoparan B, Ayhan-Kilcigil G, Ertan R, Ertan M, Kelicen P, Demirdamar R (1999) Synthesis and evaluation of antiinflammatory activity of some thiazolo[3,2-*b*]-1,2,4-triazole-5(6H)-ones and their Michael addition products. Arzneim-Forsch/Drug Res 49(2):1006–1011

Tozkoparan B, Gökhan N, Aktay G, Yeşilada E, Ertan M (2000) 6-Benzylidene thiazolo[3,2-*b*]-1,2,4-triazole-5(6H)-ones substituted with ibuprofen: synthesis, characterization and evaluation of antiinflammatory activity. Eur J Med Chem 35(7–8):743–750

Tozkoparan B, Aktay G, Yesilada E, Ertan M (2001) Synthesis of some thiazolo[3,2-*b*]-1,2,4-triazole-5(6H)-ones and their novel Michael addition products with antiinflammatory activity. Arzneim-Forsch/Drug Res 51(6):470–477

Tozkoparan B, Gökhan N, Küpeli E, Yeşilada E, Ertan M (2004) Synthesis, characterization and antiinflammatory-analgesic properties of 6-(α-amino-4-chlorobenzyl)thiazolo[3,2-b]-1,2,4-triazol-5-ols. Arzneim-Forsch/Drug Res 54(1):35–41

Tozkoparan B, Küpeli E, Yeşilada E, Işik S, Ozalp M, Ertan M (2005) Synthesis and evaluation of analgesic/antiinflammatory and antimicrobial activities of 3-substituted-1,2,4-triazole-5-thiones. Arzneim-Forsch/Drug Res 55(9):533–540

Zaharia V, Chirtoc I, Simiti I (2000) Chemical behavior of some 2-aryl-5R1–6R2-thiazolo[3,2-*b*][1,2,4]triazoles. Farmacia 48(5):71–78

Zhong GX, Hu JQ, Zhao K, Chen LL, Hu WX, Qiu MY (2009) Synthesis and biological evaluation of amide derivatives of diflunisal as potential anti-inflammatory agents. Bioorg Med Chem Lett 19(2):516–519