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# Synthesis of some 1,2,4-triazolo[3,2-*b*]-1,3-thiazine-7-ones with potential analgesic and antiinflammatory activities☆

Birsen Tozkoparan [a,*], Göknur Aktay [b], Erdem Yeşilada [c]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, Sıhhiye, Turkey*
[b] *Institute of Forensic Medicine, Toxicology Unit, Ankara University, Dikimevi, Turkey*
[c] *Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey*

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**Abstract**

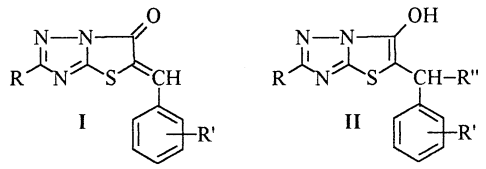
Starting from 3-substituted-1,2,4-triazole-5-thiones (**1a**–**h**), eight new 5-carbomethoxy-2-substituted-7*H*-1,2,4-triazolo[3,2-*b*]-1,3-thiazine-7-ones (**2a**–**h**) were synthesized and characterized by spectral and elementary analysis. The obtained compounds were submitted to preliminary pharmacological assay to evaluate their antiinflammatory and analgesic activities as well as gastrointestinal irritation liability and acute toxicity. Among the compounds studied, compounds **2c**, **2d**, **2e** and **2h** showed most remarkable antiinflammatory activity in the carrageenan and serotonin induced edema and in the inhibition of castor oil-induced diarrhea tests. The analgesic activity of these active compounds correlated with their antiinflammatory activities in the inhibition of acetic acid-induced writhing test. In gastric ulceration studies, the compounds were found safety at low dose levels (10 and 20 mg/kg).


*Keywords:* 3-Substituted-1,2,4-triazole-5-thiones; 5-Carbomethoxy-2-substituted-7*H*-1,2,4-triazolo[3,2-*b*]-1,3-thiazine-7-ones; Synthesis; Pharmacological properties

## 1. Introduction

Commercially available nonsteroidal antiinflammatory drugs (NSAIDs) are widely used for reducing the pain and swelling associated with inflammation [1]. The antiinflammatory mechanism of NSAIDs is due to a reduction of prostaglandin synthesis by the inhibition of cyclooxygenase enzyme (COX) in arachidonic acid metabolism [2]. The disruption of cytoprotective prostaglandins by all currently used NSAIDs results in a mechanism-based toxicity mainly in the gastrointestinal tract and kidney and thus limits their therapeutic usefulness when long-term treatment is necessary [3,4]. These current therapeutic deficiencies provide the need to develop safer drugs.

So far, various substituted 1,2,4-triazole-5-thiones and some of their condensed derivatives have been paid attention as antiinflammatory agents [5–12]. We also described the synthesis of some bicyclic 1,2,4-triazoles (**I** and **II**) and tested their antiinflammatory activities [13–15]. Hopeful antiinflammatory activities together with low ulcerogenic properties of some derivatives prompted us to investigate them further. In continuation of our earlier studies, we attempted to expand our series of compounds from 5-membered condensed 1,2,4-triazole heterocycles to 6-membered condensed derivatives. We have investigated a series of 5-carbomethoxy-2-substituted-7*H*-1,2,4-triazolo[3,2-*b*]-1,3-thiazine-7-ones (**2a**–**h**) and now report our preliminary results.

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* Corresponding author.
*E-mail address:* tbirsen@hacettepe.edu.tr (B. Tozkoparan).

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## 2. Experimental section

### 2.1. Chemistry

Ibuprofen and Naproxen were kindly supplied by Atabay and Syntex Pharmaceuticals (İstanbul, Turkey). All chemicals were from Aldrich Chemical Co. (Steinheim, Germany). Melting points were determined with a Thomas Hoover capillary melting point apparatus (Philadelphia, PA, USA) and are uncorrected. IR spectra (KBr) were recorded on a Perkin Elmer 1720X FT–IR spectrometer (Beaconsfield, UK) as potassium bromide pellets. $^1$H NMR spectra were obtained by a Bruker 200 MHz FT NMR instrument (Karlsruhe, Germany) using $CDCl_3$. All chemical shift values were recorded as $\delta$ (ppm). Mass spectra were taken on a Finnigan MAT GCQ mass spectrometer with electron ionization (EI) (Westfälische Wilhelms, University of Münster, Germany). The $[\alpha]_D^{20}$ value was determined and calculated using a Rudolph Autopol IV Automatic Polarimeter (USA). The purity of the compounds was controlled by thin layer chromatography (Merck, silica gel, $HF_{254-361}$, Type 60, 0.25 mm, Darmstadt, Germany). The elementary analyses were performed by the Scientific and Technical Research Council of Turkey (Ankara, Turkey). Elementary analyses for H, N, S were within $\pm 0.4\%$ of theoretical value.

#### 2.1.1. 3-Substituted-5-mercapto-1,2,4-triazoles (**1a–h**)

3-Substituted-5-mercapto-1,2,4-triazoles were prepared to the methods reported earlier [13,14,16].

#### 2.1.2. 5-Carbomethoxy-2-substituted-7H-1,2,4-triazolo[3,2-b]-1,3-thiazine-7-ones (**2a–h**)

A solution of 0.002 mole 3-substituted-5-mercapto-1,2,4-triazoles (**1a–h**) and 0.0025 mole dimethyl acetylenedicarboxylate in 100 ml of toluene was refluxed for 4 h. After cooling, the reaction mixture was filtered and the resulting solid was recrystallized from appropriate solvents.

### 2.2. Pharmacology

Locally bred Swiss Albino mice of both sexes (Refik Saydam Hıfzısıhha Institute, Animal Care Unit, Ankara, Turkey) weighing approximately 20–25 g were used. All the animals were left 2 days in the laboratory conditions for acclimatization and maintained on standard pellet diet and water ad libitum before the day of the experiment. The last day food was withdrawn and they were given water only. Test samples and reference compounds were suspended in 0.5% carboxymethylcellulose and administered to each mouse by using gastric gavage needle. The control group animals, however, received the same volume of dosing vehicle. To avoid wasting animals, groups composed of four mice were employed for the preliminary testing using carrageenan-induced paw edema model. For the other in vivo experiments, however, each group consisted of six mice.

#### 2.2.1. Carrageenan-induced paw edema model [17]

The method of Kasahara et al. was employed for antiinflammatory activity testing with some modifications. Naproxen was used as reference compound. Test samples were administered orally 60 min before the injection of 25 μl of freshly prepared solution of carrageenan (0.5 mg/ml) in physiological saline (154 mM NaCl) into subplantar tissue of the right hind paw of each mouse. The same volume of saline solution was injected into that of the left hind paw as internal control. The differences in foot pad thickness between the right and left foot were measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo) in a different pattern of intervals than that described by Kasahara et al. Foot thickness of each mouse was measured four times with 90 min intervals up to 360 min. 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 control group and $n'$ was that of the test group of animals.

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

Statistical differences between the treatment and the control group of animals were evaluated by two-tailed Student's $t$-test.

#### 2.2.2. Serotonin-induced hind paw edema [17]

The method described by Kasahara et al. was employed. Each mouse was injected with serotonin in Tyrode's solution (0.5 μg/5 μl) into subplanar tissue of the right hind paw. As the control Tyrode's solution (5 μl) was injected into that of left hind paw. The difference in foot pad thickness between the right and left foot was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan) every 6 min. 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 control group and $n'$ was that of test group of animals.

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

Results were evaluated by two tailed Student's $t$ test.

#### 2.2.3. Effect on castor oil-induced diarrhea [18]

The method described by Awouters et al. was employed with some modifications. Instead of rats, mice were used according to lit [19,20]. One hour after the oral administration of test sample, each mouse treated with 0.1 ml/10 g body weight of castor oil and housed in individual cages with white filter paper on the bottom in equal size and weight [21]. 1, 2, 3 and 4 h after the castor oil challenging, each filter paper was

weighted with its feces and urine content and replaced with a new paper. Net weight of excretion for each animal was statistically evaluated at 1-h intervals (see 'weight' in Table 5). The density of feces was also examined in order to evaluate the induction of diarrhea (excretion of wet or shapeless feces). In order to evaluate the results, Awouters et al. [18] employed scoring parameters as (+) or (−). However, for the quantitative evaluation of the feces density following relative scores, according to the hardness of feces, were established: 0 for no feces, 1 for hard, and 4 for gooey feces. Results were then statistically analyzed by Dunnette test.

*2.2.4. Koster Test [22]*

The method described by Koster et al. was employed. One hour after the 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 were counted for a period of 10 min. A significant reduction in the number of writhings by any treatment as compared to vehicle-treated animals was considered as a positive analgesic response. Aspirin (ASA) (200 mg/kg, p.o.) was used as a reference drug. For the evaluation of results, two-tailed 'Student's *t*' test was employed.

*2.2.5. Acute toxicity*

Single oral doses of test compounds, suspended in 0.5% carboxymethylcellulose were administered to groups, in increasing doses, i.e. 10, 20, 40, 80 and 160 mg/kg, after a 24 h fast. Control group animals received only 0.5% carboxy methylcellulose. One hour after dosing, food and water were allowed ad libitum. The animals were observed 1 h after drug administration and then once daily for 72 h for signs of toxicity and mortality.

*2.2.6. Gastric ulceration studies*

All the animals were subjected to this experimental process. They were sacrificed immediately after the last measurement, i.e. 7 h after the application of each test drug, under ether anesthesia and stomachs were removed, opened through the greater curvature, washed and fixed in 5% formaline solution. The stomachs were then examined for lesions under a dissecting microscope. Stomachs exhibiting one or more ulcers were considered positive.

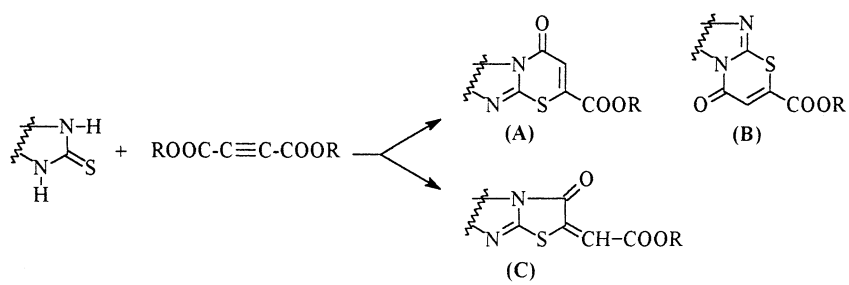
## 3. Results and discussion

The starting materials 3-substituted-1,2,4-triazole-5-thiones (**1a**–**h**) were obtained by the reaction of acylthiosemicarbazides, synthesized to the method reported earlier, with KOH 10% under reflux, followed by the acidification with concentrated hydrochloric acid [13,14,16].

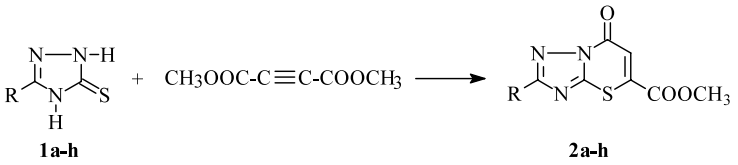
The condensation of 1,2,4-triazole-5-thiones (**1a**–**h**) with dimethyl acetylenedicarboxylate (DMAD) in toluene gave 5-carbomethoxy-7*H*-1,2,4-triazolo[3,2-*b*]-1,3-thiazine-7-ones (**2a**–**h**) in 17–50% yields (Scheme 1). Some physical properties of compounds **2a**–**h** are given in Table 1.

The structures of the isolated compounds were characterized by spectral methods, crystallographic and elementary analysis.

The reaction of DMAD with 3-substituted-1,2,4-triazol-5-thione may be expected to give either 7-oxo-1,2,4-triazolo[3,2-*b*]-1,3-thiazine-5-carboxylate (**A**) or 5-oxo-1,2,4-triazolo[3,4-*b*]-1,3-thiazine-7-carboxylate (**B**). For both pathways examples are known, deriving from other 1,2,4-triazole-5-thiones [23–26]. It is pertinent to mention here that, when similar cyclization has been applied to the compounds having thiourea moiety in the ring in different reaction conditions, a five-membered cyclization product (**C**) has been obtained [27,28]. Usual spectroscopic methods are not sufficient to distinguish between these constitutional isomers.




The cyclization of 3-methyl-1,2,4-triazol-5-thione (**1a**) with DMAD in methanol was reported in 1991 by Giammonia et al. and the compound obtained was assigned as 3-methyl-5-oxo-1,2,4-triazolo[3,4-*b*]-1,3-thiazine-7-carboxylate (**B**) [25]. To clarify the situation, an X-ray structure determination of **2a**, which gave suitable quality crystal, was carried out. Although $^{1}$H NMR assignments and melting point of **2a** are very similar to those of a compound reported by Giammonia et al., we identified it as 2-methyl-7-oxo-1,2,4-




1a-h 2a-h

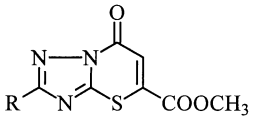
Scheme 1.

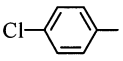
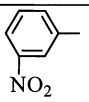
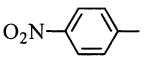
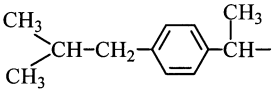
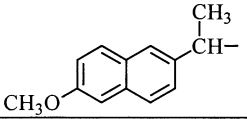
triazolo[3,2-*b*]-1,3-thiazine-5-carboxylate (**A**). X-ray crystallographic analysis data will be published soon elsewhere.

The IR spectra of compounds showed two carbonyl absorption bands about 1707–1720 $cm^{-1}$ and 1726–1750 $cm^{-1}$. In the $^1H$ NMR spectra of the compounds, methoxy protons and ethylenic proton appeared as two singlets at about 3.90–4.10 and 7.60–7.66 ppm, respectively (Table 2). The other protons were seen at the expected chemical shifts and integral values. The mass spectroscopic fragmentation of the compounds **2a**–**2c**, **2e**, and **2f** was studied under electron ionization. Molecular ion peaks, which appeared corresponding to the expected values, were the base peaks. The cleavage of C–C bond adjacent to heteroatom (O/S) gave $M^{+\bullet} - COOCH_3$ ion peak. Another fragment corresponding to $M^{+\bullet} - CO$ ion was observed in all compounds.

In the pharmacological study, we have investigated the antiinflammatory and analgesic activity as well as the ulcerogenic potential and acute toxicity. In order to

Table 1
The compounds synthesized and their melting points, percentage yields and formulas



| Compd. | R | Mp °C * | Yield % | Formula |
|---|---|---|---|---|
| **2a** | -$CH_3$ | 154-5 [a] | 32 | $C_8H_7N_3O_3S$ |
| **2b** |  | 198-9 [b] (a) | 49 | $C_{13}H_9N_3O_3S$ |
| **2c** |  | 191-2 [b] | 34 | $C_{13}H_8ClN_3O_3S$ |
| **2d** |  | 218-9 [b] | 50 | $C_{13}H_8ClN_3O_3S$ |
| **2e** |  | 197-8 [a] | 41 | $C_{13}H_8N_4O_5S$ |
| **2f** |  | 207-8 [c] | 31 | $C_{13}H_8N_4O_5S$ |
| **2g** (b) |  | 87-8 [d] | 17 | $C_{19}H_{21}N_3O_3S$ |
| **2h** (c) |  | 162-3 [a] | 41 | $C_{20}H_{17}N_3O_4S$ |

Recrystallization solvents: [a] ethyl acetate–*n*-hexane; [b] toluene; [c] ethyl acetate; [d] methanol.
(a) Lit. m.p. 196 °C (MeOH), Ref. [23].
(b) **2g** is a racemic mixture.
(c) **2h** is optically active, $[\alpha]_D^{20} = +47.5$ (c, 1 g/100 ml in $CHCl_3$).

Table 2
$^1$H NMR data of the compounds of **1–8**

| Comp. no | $^1$H NMR ($CDCl_3$): $\delta$ (ppm) |
|---|---|
| **2a** | 2.60 (s, 3H, $-CH_3$), 4.00 (s, 3H, $-OCH_3$), 7.60 (s, 1H, =C–H). |
| **2b** | 4.00 (s, 3H, $-OCH_3$), 7.40–7.50 (m, 3H, arom.H), 7.60 (s, 1H, =C–H), 8.20–8.30 (m, 2H, arom.H). |
| **2c** | 4.00 (s, 3H, $-OCH_3$), 7.32–7.56 (m, 3H, arom.H), 7.66 (s, 1H, =C–H), 7.94–8.20 (m, 1H, arom.H). |
| **2d** | 4.00 (s, 3H, $-OCH_3$), 7.40 (d, 2H, arom.H), 7.60 (s, 1H, =C–H), 8.20 (d, 2H, arom.H). |
| **2e** | 4.10 (s, 3H, $-OCH_3$), 7.60–7.80 (m, 2H, arom.H and =C–H), 8.29 (d, 1H, arom.H), 8.62 (d, 1H, arom.H), 9.70 (s, 1H, arom.H). |
| **2f** | 4.00 (s, 3H, $-OCH_3$), 7.60 (s, 1H, =C–H), 8.28 (d, 2H, arom.H), 8.39 (d, 2H, arom.H). |
| **2g** | 0.95 (d, 6H, $2\times-CH_3$), 1.80 (d, 3H, $-CH_3$), 1.80–1.90 (m, 1H, $(CH_3)_2CH-$), 2.45 (d, 2H, $-CH_2-$), 4.10 (s, 3H, $-OCH_3$), 4.40 (q, 1H, CH), 7.05 (d, 2H, arom.H), 7.30 (d, 2H, arom.H), 7.60 (s, 1H, =C–H). |
| **2h** | 1.90 (d, 3H, $-CH_3$), 3.90 (s, 3H, $-OCH_3$), 4.00 (s, 3H, $-OCH_3$), 4.60 (q, 1H, CH), 7.00–7.20 (s, 2H, arom.H), 7.40–7.80 (m, 5H, arom.H and =C–H). |

screen the antiinflammatory activity of the synthesized compounds, carrageenan-induced hind paw edema model was used as the first step. In preliminary testing, compounds were administered in 20 and 80 mg/kg (per os) doses and those possessing more than 20% inhibitory effect, **2c**, **2d**, **2e** and **2h**, were further tested in 10 and 40 mg/kg doses as well (Table 3). The synthesized compounds were then studied for their effects on serotonin-induced hind paw edema model in mice at 20 mg/kg dose. As shown in Table 4, the antiinflammatory activity of **2d**, **2e** and **2h** were significant and more consistent. The results of both tests were in good agreement.

Awouters et al. [18] reported a relationship between the delaying effect of nonsteroidal antiinflammatory compounds on castor oil-induced diarrhea and prostaglandin biosynthesis. In order to determine the effects of active compounds on prostaglandin biosynthesis, a modified technique of Awouters was employed. The results were also found in good agreement with those of the ulcer scoring. As shown in Table 3, **2c**, **2d** and **2h** were found to induce gastric lesions in some mice, suggesting that these compounds possibly possess an inhibitory effect on prostaglandin biosynthesis. This conclusion was further supported by using castor oil-induced diarrhea test, where **2d** and **2h** remarkably delayed the castor-oil induced diarrhea in mice (Table 5). On the other hand, since **2c** induced gastric lesions, only in 80 mg/kg dose, we did not observe any significant inhibitory effect on castor-oil induced diarrhea in 20 mg/kg dose. Since **2e** did not induce any delay in diarrhea, the antiinflammatory effect might be through another pathway, which is not based on the inhibition of prostaglandin biosynthesis.

The analgesic activity of these active compounds was also studied by using the acetic acid induced writhing test in mice. The compounds which were given at 20

Table 3
Carrageenan-induced hind paw edema in mice ($n = 4$–6)

| Comp. | Dose (per os) mg/kg | Ulcer score | Swelling in thickness ($10^{-2}$ mm) $\pm$ standard error (% inhibition) | | | |
|---|---|---|---|---|---|---|
| | | | 90 min | 180 min | 270 min | 360 min |
| Control | | 0/6 | 46.3 $\pm$ 7.2 | 84.2 $\pm$ 12.0 | 83.3 $\pm$ 7.4 | 62.0 $\pm$ 4.7 |
| **2c** | 10 | 0/5 | 121.6 $\pm$ 28.6 | 94.7 $\pm$ 20.8 | 82.8 $\pm$ 17.8 | 64.1 $\pm$ 13.8 |
| | 20 | 0/4 | 28.0 $\pm$ 9.2 (39.5) | 54.0 $\pm$ 18.8 (35.9) | 46.2 $\pm$ 14.2 * (44.5) | 36.5 $\pm$ 11.7 * (41.1) |
| | 40 | 0/6 | 139.3 $\pm$ 17.2 | 102.0 $\pm$ 11.1 | 75.3 $\pm$ 11.1 (9.6) | 50.7 $\pm$ 11.4 (18.3) |
| | 80 | 1/4 | 52.5 $\pm$ 13.4 | 73.0 $\pm$ 7.5 (13.3) | 62.0 $\pm$ 5.8 (25.6) | 48.5 $\pm$ 3.0 * (21.8) |
| **2d** | 10 | 0/5 | 39.1 $\pm$ 16.4 (15.4) | 96.1 $\pm$ 14.9 | 67.3 $\pm$ 13.8 (19.2) | 39.9 $\pm$ 8.3 * (35.6) |
| | 20 | 0/4 | 35.8 $\pm$ 8.7 (22.7) | 79.0 $\pm$ 15.3 (1.2) | 67.2 $\pm$ 13.8 (19.3) | 43.0 $\pm$ 14.8 (30.6) |
| | 40 | 2/6 | 54.9 $\pm$ 9.2 | 107.0 $\pm$ 4.2 | 72.9 $\pm$ 8.5 (12.4) | 35.2 $\pm$ 5.6 ** (43.2) |
| | 80 | 0/4 | 41.5 $\pm$ 7.9 (10.4) | 84.5 $\pm$ 8.5 | 81.2 $\pm$ 12.5 (2.5) | 54.0 $\pm$ 10.6 (12.9) |
| **2e** | 10 | 0/6 | 91.7 $\pm$ 13.1 | 86.3 $\pm$ 3.8 | 56.5 $\pm$ 8.5 * (32.1) | 33.0 $\pm$ 5.8 ** (46.7) |
| | 20 | 0/4 | 31.3 $\pm$ 4.5 (32.4) | 65.0 $\pm$ 13.5 (22.8) | 57.0 $\pm$ 7.5 * (31.6) | 39.7 $\pm$ 2.5 ** (36.0) |
| | 40 | 0/6 | 97.6 $\pm$ 6.3 | 84.2 $\pm$ 9.9 | 65.7 $\pm$ 9.0 (21.2) | 36.6 $\pm$ 5.0 ** (40.9) |
| | 80 | 0/4 | 29.7 $\pm$ 6.6 (35.8) | 71.7 $\pm$ 13.9 (14.8) | 76.7 $\pm$ 8.3 (7.9) | 81.2 $\pm$ 10.4 |
| **2h** | 10 | 0/6 | 67.7 $\pm$ 10.3 | 111.2 $\pm$ 8.0 | 98.7 $\pm$ 18.0 | 66.9 $\pm$ 12.3 |
| | 20 | 0/4 | 33.3 $\pm$ 6.9 (28.1) | 83.0 $\pm$ 11.7 | 64.9 $\pm$ 16.8 (22.1) | 42.9 $\pm$ 11.5 (31) |
| | 40 | 1/6 | 43.5 $\pm$ 10.9 | 85.6 $\pm$ 23.1 | 71.5 $\pm$ 14.4 (14) | 36.2 $\pm$ 7.3 * (41.6) |
| | 80 | 1/4 | 56.5 $\pm$ 5.1 | 81.0 $\pm$ 4.6 (3.8) | 75.4 $\pm$ 3.9 (9.5) | 67.3 $\pm$ 4.2 |
| Naproxen | 200 | 2/6 | 47.6 $\pm$ 5.4 | 29.4 $\pm$ 3.2 ** (65.1) | 49.9 $\pm$ 8.6 * (40.1) | 37.0 $\pm$ 6.0 ** (40.3) |

** $P < 0.01$.
* $P < 0.05$ significant from control.

mg/kg dose level as orally showed almost an equal degree of analgesic activity (Table 6). When the test compounds, **2c**, **2d**, **2e** and **2h** are evaluated in order to examine the acute toxicity, no mortality or toxic effects are observed at doses of 10, 20, 40, 80 and 160 mg/kg administered in first 72 h.

Although it is not possible to draw an exact conclusion according to the results of in vivo experiments whether triazoles **I**, **II** or **2a**–**h** are more active, when we compare the results of this study with those previously obtained [13–16], it could be said that the condensation of 3-substituted-1,2,4-triazol-5-thiones with thiazine ring (six-membered ring) in place of the thiazole (five-membered ring) does not provide any superiority in the antiinflammatory activity. In order to examine the effects of the various substituents on the biological activity, it has been planned to continue studies on this ring system in the future.

As a conclusion, among the synthesized compounds, **2e** showed a significant and consistent antiinflammatory effect in mice at lower doses and did not induce any gastric lesions or death during the observation period.

Table 4
Serotonin-induced hind paw edema ($n = 6$)

| Comp. | Dose (per os) mg/kg | Swelling in thickness ($10^{-2}$ mm) ± standard error (% inhibition) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 min | 6 min | 12 min | 18 min | 24 min | 30 min |
| Control | | 8.3 ± 3.6 | 10.0 ± 2.4 | 28.0 ± 8.9 | 40.8 ± 13.9 | 38.8 ± 14.5 | 43.5 ± 17.5 |
| **2a** | 20 | 11.8 ± 3.4 | 6.8 ± 2.5 * (31.8) | 38.6 ± 15.1 | 44.8 ± 21.4 | 39.7 ± 10.2 | 40.3 ± 18.9 (7.3) |
| **2b** | 20 | 9.9 ± 2.1 | 8.5 ± 1.2 (14.4) | 73.5 ± 5.6 | 67.9 ± 9.8 | 55.4 ± 8.9 | 52.2 ± 11.1 |
| **2c** | 20 | 4.3 ± 1.6 (48.2) | 43.3 ± 11.0 | 47.0 ± 6.5 | 37.0 ± 7.1 | 35.7 ± 5.9 (8.1) | 32.3 ± 6.0 (25.7) |
| **2d** | 20 | 2.5 ± 0.9 (69.8) | 12.0 ± 4.5 | 30.3 ± 7.5 | 32.3 ± 8.4 (20.8) | 26.7 ± 8.7 (26.1) | 26.3 ± 6.5 (39.5) |
| **2e** | 20 | 4.2 ± 1.6 * (49.3) | 4.0 ± 1.0 * (59.8) | 42.7 ± 12.5 | 47.6 ± 8.8 | 44.8 ± 5.8 | 39.2 ± 8.7 (9.7) |
| **2f** | 20 | 7.0 ± 1.6 (15.7) | 8.8 ± 1.4 (12.0) | 39.1 ± 5.5 | 58.1 ± 8.4 | 57.6 ± 8.6 | 81.3 ± 10.3 |
| **2g** | 20 | 4.0 ± 1.1 (51.8) | 10.5 ± 3.7 | 26.3 ± 6.4 (6.1) | 40.0 ± 8.8 | 34.3 ± 7.6 (11.6) | 31.6 ± 6.4 (27.2) |
| **2h** | 20 | 3.2 ± 0.7 (61.4) | 20.5 ± 5.8 | 25.0 ± 8.1 (10.7) | 31.7 ± 7.9 (22.4) | 33.3 ± 7.1 (14.2) | 28.2 ± 12.5 (35.3) |
| ASA | 200 | 14.7 ± 3.8 | 7.1 ± 1.0 (29.0) | 20.6 ± 1.9 (26.4) | 26.1 ± 3.3 (36.0) | 47.3 ± 2.0 | 26.4 ± 3.8 (39.3) |
| Naproxen | 200 | 27.8 ± 7.0 | 4.4 ± 0.9 * (56.0) | 13.1 ± 2.2 (53.2) | 13.8 ± 2.9 (66.2) | 14.9 ± 4.3 (61.6) | 17.2 ± 5.4 (60.4) |

* $P < 0.05$ significant from control.

Table 5
Inhibition of castor oil-induced diarrhea ($n = 6$–10)

| Comp. | Dose per os mg/kg | Feces weight (g) and relative density (% inhibition) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 60 min | | 120 min | | 180 min | | 240 min | |
| | | Weight (g) | Density | Weight (g) | Density | Weight (g) | Density | Weight (g) | Density |
| Control | | 0.39 ± 0.06 | 1.5 ± 0.3 | 0.35 ± 0.05 | 2.5 ± 0.2 | 0.23 ± 0.04 | 3.4 ± 0.2 | 0.12 ± 0.04 | 2.2 ± 0.5 |
| **2c** | 20 | 0.13 ± 0.08 * (67.0) | 1.0 ± 0.6 | 0.26 ± 0.09 (26.0) | 2.0 ± 0.7 | 0.25 ± 0.09 | 3.2 ± 0.6 | 0.24 ± 0.11 | 3.0 ± 0.6 |
| **2d** | 20 | 0.12 ± 0.03 ** (69.2) | 0.67 ± 0.2 * | 0.09 ± 0.03 ** (74.2) | 1.5 ± 0.5 * | 0.11 ± 0.05 * (52.2) | 1.2 ± 0.5 *** | 0.06 ± 0.02 (50) | 0.2 ± 0.2 ** |
| **2e** | 20 | 0.15 ± 0.04 * (61.5) | 1 ± 0.2 | 0.26 ± 0.08 (25.7) | 2.5 ± 0.2 | 0.22 ± 0.07 (4.3) | 3.2 ± 0.4 | 0.20 ± 0.06 | 2.3 ± 0.4 |
| **2h** | 20 | 0.14 ± 0.08 * (64.1) | 0.5 ± 0.2 * | 0.20 ± 0.06 * (42.8) | 0.7 ± 0.3 *** | 0.14 ± 0.02 (39.1) | 2.5 ± 0.6 | 0.0 ± 0.0 *** (100.0) | 0.05 ± 0.05 *** |
| Naproxen | 200 | 0.29 ± 0.12 (25.6) | 1.2 ± 0.5 | 0.18 ± 0.05 * (48.6) | 1.2 ± 0.5 * | 0.05 ± 0.02 * (78.3) | 0.7 ± 0.5 *** | 0.11 ± 0.6 (8.3) | 1.5 ± 0.7 |

*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$ significant from the control.

Table 6
Analgesic activity (Koster test) ($n = 8$)

| Comp. | Dose (mg/kg) | Analgesic activity [a] | Inhibitory ratio (%) |
|---|---|---|---|
| Control | | $29.75 \pm 2.30$ | |
| **2c** | 20 | $17.13 \pm 1.23$ *** | 42.2 |
| **2d** | 20 | $16.62 \pm 1.58$ *** | 44.1 |
| **2e** | 20 | $17.75 \pm 1.25$ *** | 40.3 |
| **2h** | 20 | $16.5 \pm 2.36$ *** | 44.5 |
| ASA | 200 | $9.87 \pm 0.77$ *** | 66.8 |

[a] Number of writhing reflex induced by acetic acid (i.p.).
*** $P < 0.001$.

Thus, following detailed studies should be more focused on this compound.

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## References

[1] G. Lombardino, Nonsteroidal Antiinflammatory Drugs, Wiley Interscience, John Wiley and Sons, New York, 1985.
[2] S.H. Ferreira, S. Moncada, J.R. Vane, Indomethacin and aspirin abolish prostaglandin release from the spleen, Nature (New. Biol.) 231 (1971) 237.
[3] A.D. McMahon, Observation and experiment with the efficacy of drugs: A warning example from a cohort of nonsteroidal antiinflammatory and ulcer-healing drug users, Am. J. Epidemiol. 154 (2001) 557.
[4] G.M. Rocha, L.F. Michea, E.M. Petey, M. Kirby, Y. Xu, D.R. Ferguson, M.B. Burg, Direct toxicity of nonsteroidal antiinflammatory drugs for renal medullary cells, Proc. Natl. Acad. Sci. 98 (2001) 5317.
[5] J.R. Maxwell, D.A. Wasdahl, A.C. Wolfson, V.I. Stenberg, Synthesis of 5-aryl-2H-tetrazoles, 5-aryl-2H-tetrazole-2-acetic acids, and [(4-phenyl-5-aryl-4H-1,2,4-triazol-3-yl)thio]acetic acids as possible superoxide scavengers and antiinflammatory agents, J. Med. Chem. 27 (1984) 1565.
[6] A.A. El-Emam, T.M. Ibrahim, Synthesis and antiinflammatory and analgesic activity of some 3-(1-adamantyl)-4-substituted-5-mercapto-1,2,4-triazoles, Arzneim.-Forsch./Drug Res. (II) 41 (1991) 1260.
[7] R. Pignatello, S. Mazzone, A.M. Panico, G. Mazzone, G. Pennisi, R. Castana, M. Matera, G. Blandino, Synthesis and biological evaluation of thiazolo-triazole derivatives, Eur. J. Med. Chem. 26 (1991) 929.
[8] G. Mazzone, R. Pignatello, S. Mazzone, A. Panico, F. Barbera, T. Catti, S. Chiechio, R. Arrigo-Reina, C. Castorina, A. Russo, Synthesis of carboxyalkylthiotriazoles and bicyclic derivatives with antiinflammatory and analgesic activity, Farmaco 47 (1992) 149.
[9] M.D. Mullican, M.W. Wilson, D.T. Connor, C.R. Kostlan, D.J. Schrier, R.D. Dyer, Design of 5-(3,5-di-tert-butyl-4-hydroxyphenyl)-1,3,4-thiadiazoles, 1,3,4-oxadiazoles, and 1,2,4-triazoles as orally active, nonulcerogenic antiinflammatory agents, J. Med. Chem. 36 (1993) 1090.
[10] D.H. Boschelli, D.T. Conner, D.A. Bornemeier, R.D. Dyer, J.A. Kennedy, P.J. Kuipers, G.C. Okonkwo, D.J. Schrier, C.D. Wright, 1,3,4-Oxadiazole, 1,3,4-thiadiazole, and 1,2,4-triazole analogs of fenamates: in vitro inhibition of cyclooxygenase and 5-lipoxygenase activities, J. Med. Chem. 36 (1993) 1802.
[11] G. Mekuskýene, P. Gaidelis, P. Vainilavicius, Synthese und Eigenschaften von 5-(4,6-Diphenyl-pyrimidin-2-yl)-1,2,4-triazolin-3-thion und seinen Derivaten, Pharmazie 53 (1998) 94.
[12] L. Labanauskas, V. Kalcas, E. Udrenaite, P. Gaidelis, A. Brukštus, V. Daukšas, Synthesis of 3-(3,4-dimethoxyphenyl)-1H-1,2,4-triazole-5-thiol and 2-amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole derivatives exhibiting antiinflammatory activity, Pharmazie 56 (2001) 617.
[13] B. Tozkoparan, G. Ayhan Kılcıgıl, R. Ertan, M. Ertan, P. Kelicen, R. Demirdamar, Synthesis and evaluation of antiinflammatory activity of the thiazolo[3,2-b]-1,2,4-triazole-5(6H)-ones and their Michael addition products, Arzneim. Forsch./Drug Res. (II) 49 (1999) 1006.
[14] B. Tozkoparan, N. Gökhan, G. Aktay, E. Yeşilada, M. Ertan, 6-Benzylidenethiazolo[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 (2000) 743.
[15] B. Tozkoparan, G. Aktay, E. Yesilada, M. Ertan, 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. (I) 51 (2001) 470.
[16] B. Berk, G. Aktay, E. Yesilada, M. Ertan, Synthesis and pharmacological activities of some new 2-[1-(6-methoxy-2-naphtyl)ethyl] - 6 - (substituted)benzylidenethiazolo[3,2 - b] - 1,2,4-triazole-5(6H)-one derivatives, Pharmazie 56 (2001) 8.
[17] Y. Kasahara, H. Hikino, S. Tsurufuji, M. Watanabe, M. Ohuchi, Oriental medicines. Part 75. Studies on the constituents of ephedra. 18. Antiinflammatory actions of ephedrines in acute inflammations, Planta Med. 51 (1985) 325.
[18] F. Awouters, C.J.E. Niemegeers, F.M. Lenaerts, A.J. Janssen, Delay of castor-oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis, J. Pharm. Pharmac. 30 (1978) 41.
[19] C.J. Niemegeers, F.M. Lenaerts, F. Awouters, P.A. Janssen, Gastrointestinal effects and acute toxicity of suprofen, Arzneim. Forsch./Drug Res. 25 (1975) 1537.
[20] M.V. Diurno, O. Mazzoni, F. Capasso, A.A. Izzo, A. Bolognese, Synthesis and pharmacological activity of 2-(substituted phenyl)-3-[2- or 3-[(4-substituted phenyl-4-hydroxy)piperidino]ethyl or propyl]-1,3-thiazolidin-4-ones, Farmaco 52 (1997) 237.
[21] E. Yesilada, E. Küpeli, *Berberis crataegina* root exhibits potent antiinflammatory, analgesic and febrifuse effects in mice and rats, J. Ethnopharmacol. 79 (2002) 237.
[22] R. Koster, Method of writing test, Fed. Proc. 18 (1959) 412.
[23] V.P. Upadhyaya, V.R. Srinivasan, Synthesis of s-triazolo[3,2-b]-1,3-thiazin-5-one and s-triazolo[3,4-b]-1,3,4-thiadiazin-6-one through the use of dimethylacetylene dicarboxylate, Indian J. Chem. Sect. B. 16 (1978) 737.
[24] P.C. Gogoi, C.S. Kataky, Bridgehead nitrogen heterocycles. Part III. Synthesis of fused heterocycles from 3-(2,4-dichlorophenyl)-5-mercapto-1,2,4-s-triazoles, Heterocycles 32 (1991) 231.
[25] G. Giammonia, M. Neri, B. Carlisi, A. Palazzo, C. Rosa, Reactions of azoesters and dimethyl acetylenedicarboxylate with 3-methyl-1,2,4-triazole-5-thione, J. Heterocyclic Chem. 28 (1991) 325.

[26] M.M. Heravi, Y.Sh. Beheshtiha, H.A. Oskooie, N. Nami, Synthesis and characterization of novel heterocyclic system 1,2,4-triazolo[3,4-b]-1,3-thiazin-4-ones, Indian J. Heterocyclic Chem. 8 (1999) 245.
[27] K. Liu, J. Chern, M. Yen, Y. Lin, Synthese und Antihypertensive Aktivitaet Einiger 2,3-Dihydro-5H-thiazolo[2,3-b]chinazolin-Derivative, Arch. Pharm (Weinheim) 316 (1983) 569.
[28] V.S. Georgiev, G.A. Bennett, L.A. Radov, D.K. Kamp, L.A. Trusso, 2-Substituted 2,3-dihydro-5H-thiazolo[2,3-b]quinazoline derivatives, J. Heterocyclic Chem. 23 (1986) 1359.