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Short communication

6-Benzylidenethiazolo[3,2-b]-1,2,4-triazole-5(6H)-ones substituted with ibuprofen: synthesis, characterization and evaluation of anti-inflammatory activity

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Abstract – In this study, the synthesis of 3-[1-(4-(2-methylpropyl)phenyl)ethyl]-1,2,4-triazole-5-thione (**2**) and its condensed derivatives 6-benzylidenethiazolo[3,2-b]-1,2,4-triazole-5(6H)-ones (**2a–u**) are described. The structures of the compounds were elucidated by spectral and elemental analysis. In the pharmacological studies, anti-inflammatory activities of these compounds have been screened. Among the compounds examined, the compounds **2** and **2g** possessed the most prominent and consistent activity. In gastric ulceration studies the synthesized compounds were generally found to be safe at a 200 mg/kg dose level. © 2000 Éditions scientifiques et médicales Elsevier SAS

3-[1-(4-(2-methylpropyl)phenyl)ethyl]-1,2,4-triazole-5-thione / 6-benzylidenethiazolo[3,2-b]-1,2,4-triazole-5(6H)-ones / synthesis / anti-inflammatory activity

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of a number of arthritic diseases such as rheumatoid arthritis and osteoarthritis. However, their therapeutic use is often limited by common side effects, such as gastrointestinal haemorrhage and ulceration [1]. In spite of abundance of NSAIDs in the market, the search continues to develop new drugs that have potent anti-inflammatory activity with minimum side effects.

Recent studies revealed that the compounds containing a 1,2,4-triazole skeleton have shown moderate anti-inflammatory activity [2–8]. Recently, some 3-(3,5-di-tert-butyl-4-hydroxybenzylidene)pyrrolidin-2-ones (**I**) [9], α-(3,5-di-tert-butyl-4-hydroxybenzylidene)-γ-butyro lactone (**II**) (KME-4) [10, 11] and 5-(3,5-di-tert-butyl-4-hydroxybenzylidene)thiazol-4-one (**III**) [12] derivatives have attracted widespread attention as potent anti-inflammatory compounds. In the light of these studies, in a previous study, we synthesized some thiazolo[3,2-b]-1,2,4-triazole derivatives (**IV**) (*figure 1*) containing a benzylidenethiazolidin-4-one structure in the condensed ring and evaluated their anti-inflammatory activities [13].

In continuation of our interest towards the chemical and pharmacological properties of thiazolo[3,2-b]-1,2,4-triazoles, with the aim of obtaining the compounds with not only improved activity but also with reduced side effects, we synthesized new compounds by combining a thiazolo[3,2-b]-1,2,4-triazole ring with a non-steroidal anti-inflammatory compound, ibuprofen.

2. Chemistry

The key intermediate in the preparation of 1,2,4-triazol-5-thione is 4-acylthiosemicarbazide (**1b**). 4-Acylthiosemicarbazides could be synthesized either by the reaction of acylchlorides, acid anhydrides and esters with thiosemicarbazide [8, 14–16], or the reaction of carboxylic acid hydrazides with potassium thiocyanate [3, 5]. In our first attempt, ibuprofen was converted to the acid chloride then treated with thiosemicarbazide to yield intermediate **1b**. But there were too many spots in its thin

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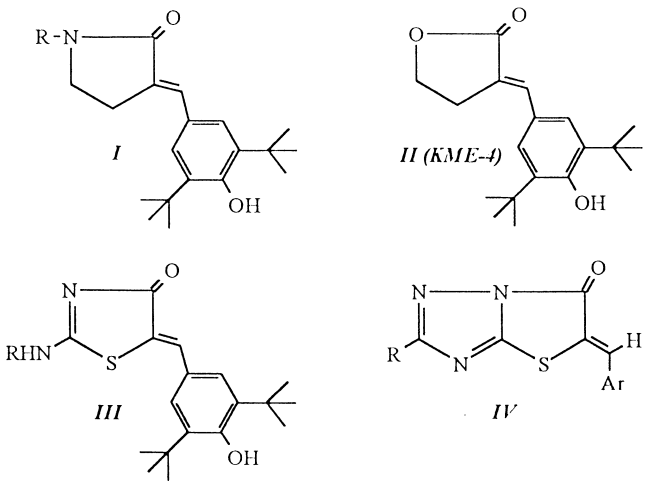

Figure 1. Compounds **I–IV**.

layer chromatogram and the yield was very low. **Ib** was prepared as outlined in *figure 2* by the reaction of ibuprofen with N-hydroxysuccinimide in the presence of DCC. The ester thus obtained was reacted with thiosemicarbazide to give the desired 4-acylthiosemicarbazide **1b**. 1,2,4-Triazole-5-thione (**2**) was prepared by cyclization of **1b** with 10% KOH.

The treatment of **2** with chloroacetic acid and substituted or non-substituted benzaldehydes in the presence of sodium acetate, acetic acid and acetic anhydride gave the fused ring products (**2a–u**) (*figure 3*) [13, 16]. However, the reaction of 3-hydroxybenzaldehyde resulted in formation of the O-acetylated derivative (**2s**) in this reaction condition.

3. Results and discussion

During the reaction of 1,2,4-triazole-5-thione with various dielectrophiles, two isomeric cyclization products such as thiazolo[3,2-b]-1,2,4-triazole or thiazolo[2,3-c]-1,2,4-triazole can be obtained. The examples derived from various 3-aryl/-alkyl substituted 1,2,4-triazole-5-thiones are known for both pathways [2, 17–20]. The mode of cyclization was studied earlier and confirmed the formation of thiazolo[3,2-b]-1,2,4-triazoles [13, 16]. In addition, the configuration around the C=C double bond of the compounds was assigned as *cis* (Z) on the basis of X-ray crystallographic analysis [21].

The melting points, % yields and formulae of the synthesized compounds were given in *table I*. The structures of the compounds were proved by IR, ^{1}H-NMR, mass spectra and elemental analysis. All spectral data were in accordance with the assigned structures (*table II*).

In the IR spectra, all compounds displayed strong absorption bands in the ranges 1 758–1 729 cm^{-1} (C=O) and 1 619–1 594 cm^{-1} (C=N), respectively. The absorption bands associated with other functional groups appeared in the expected regions.

In the ^{1}H-NMR spectra of the compounds, methyl protons appeared as two doublets at about 0.81–0.90 and

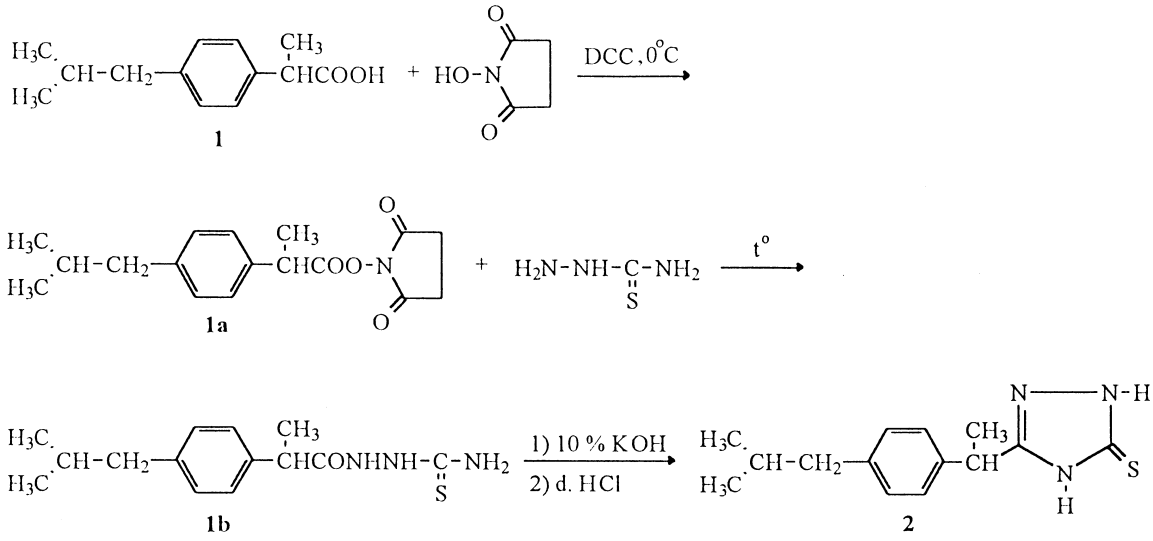

Figure 2. Synthesis of **2**.

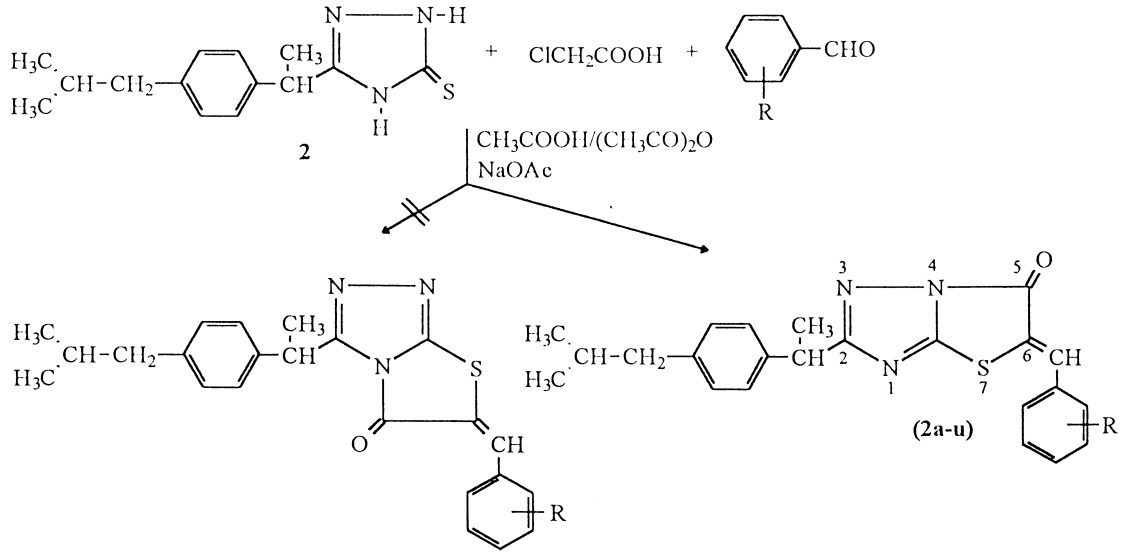

Figure 3. Synthesis of fused ring products.

1.68–1.76 ppm. The methyne protons were observed around 1.71–1.99, 4.24–4.35 and 8.05–8.55 ppm as multiplet, quartet and singlet, respectively. Methylene protons were seen as a doublet approximately at 2.36–2.44 ppm. The phenyl protons were seen at the expected chemical shifts and integral values. The ^{13}C-NMR spectra of the compounds **2c** and **2f** showed characteristic signals at 140.6 ppm (CH=) and 176.9 ppm (C=O) (*figure 4*).

Table I. Some physico-chemical data of the compounds **2a**–**u**.

Compound	R	M.p. (°C)	Yield (%)	Analysis
2a	-H	179–181	46	$C_{23}H_{23}N_3OS$
2b	2-F	120–122	34	$C_{23}H_{22}FN_3OS$
2c	3-F	178–180	62	$C_{23}H_{22}FN_3OS$
2d	4-F	161–163	37	$C_{23}H_{22}FN_3OS$
2e	2-Cl	126–128	48	$C_{23}H_{22}ClN_3OS$
2f	3-Cl	166–168	54	$C_{23}H_{22}ClN_3OS$
2g	4-Cl	200–201	65	$C_{23}H_{22}ClN_3OS$
2h	2-Br	126–127	44	$C_{23}H_{22}BrN_3OS$
2i	3-Br	150–151	54	$C_{23}H_{22}BrN_3OS$
2j	4-Br	209–211	62	$C_{23}H_{22}BrN_3OS$
2k	2-CH_3	113–114	41	$C_{24}H_{25}N_3OS$
2l	3-CH_3	149–150	44	$C_{24}H_{25}N_3OS$
2m	4-CH_3	174–175	44	$C_{24}H_{25}N_3OS$
2n	2-OCH_3	145–147	57	$C_{24}H_{25}N_3O_2S$
2o	3-OCH_3	139–141	62	$C_{24}H_{25}N_3O_2S$
2p	4-OCH_3	169–170	38	$C_{24}H_{25}N_3O_2S$
2r	3,4,5-t-OCH_3	173–174	86	$C_{26}H_{29}N_3O_4S$
2s	3-$OCOCH_3$	149–151	81	$C_{25}H_{25}N_3O_3S$
2t	4-CF_3	166–168	56	$C_{24}H_{22}F_3N_3OS$
2u	3-NO_2	148–150	68	$C_{23}H_{22}N_4O_3S$

In the EI-MS spectra, molecular ion ($M^{+\cdot}$) peaks which appeared at different intensities confirmed the molecular weights of the examined compounds (**2b**, **2c**, **2e**, **2f**, **2h**, **2**i, **2j**, **2k**, **2l** and **2m**). Molecular ion peaks were the base peak for the compounds **2b**, **2c**, **2e**, **2k**, **2l** and **2m**. The fragments resulting due to loss of $CH_3^{\cdot}$, $(CH_3)_2CH^{\cdot}$ and $C_4H_7O^{\cdot}$ [$(CH_3)_2CH^{\cdot}$ + $CO^{\cdot}$] ions from the molecular ion were observed for all compounds. The $M^{+\cdot}$ -$(CH_3)_2CH^{\cdot}$ peak was also the base peak for the compounds **2f** and **2h**. The condensed ring showed a fragmentation pattern giving rise to an $RC_6H_4CH{=}C{=}S^{+\cdot}$ type of ion. In the mass spectra of the compounds **2h**, **2**i, **2j**, appearance of an isotope peak (M + 2) as intense as the molecular ion peak confirmed the presence of the one bromine atom. A predominant M + 2 peak is characteristic of the compounds **2e** and **2f** having a chlorine atom.

In the pharmacological studies, in order to avoid wasting mice for inactive compounds, we employed a two-step activity screening model. For the preliminary activity screening, each test group was composed of three mice. The animals were first administered in 100 mg/kg (body weight) dose of the test drugs. Test compounds which possessed more than a 20% inhibitory effect in any of the measurement intervals (**2**, **2b**, **2c**, **2f**, **2g**, **2h**, **2i**, **2k**, **2l**, **2m**, **2n**, **2o**, **2r** and **2t**) were selected for the secondary evaluation using the same dose level and groups con-

Table II. IR and ^{1}H-NMR spectral data of the compounds **2a**–**u**.

Compound	R	IR (KBr): ν (cm^{-1})	^{1}H-NMR δ (ppm)
2a	-H	1 737 (C=O), 1 614 (C=N)	0.82 (d, 6H, 2xCH_3), 1.68 (d, 3H, CH_3), 1.71–1.99 (m, 1H, CH), 2.36 (d, 2H, CH_2), 4.24 (q, 1H, CH), 7.00 (d, 2H, ArH), 7.21 (d, 2H, ArH), 7.44-7.53 (m, 5H, ArH), 8.12 (s, 1H, CH=).
2b	2-F	1 746 (C=O), 1 608 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.44 (d, 2H, CH_2), 4.30 (q, 1H, CH), 7.09–7.65 (m, 8H, ArH), 8.42 (s, 1H, CH=).
2c	3-F	1 735 (C=O), 1 619 (C=N)	0.88 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.44 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.09–7.57 (m, 8H, ArH), 8.13 (s, 1H, CH=).
2d	4-F	1 734 (C=O), 1 599 (C=N)	0.81 (d, 6H, 2xCH_3), 1.68 (d, 3H, CH_3), 1.71–1.80 (m, 1H, CH), 2.36 (d, 2H, CH_2), 4.24 (q, 1H, CH), 7.00–7.54 (m, 8H, ArH), 8.08 (s, 1H, CH=).
2e	2-Cl	1 752 (C=O), 1 601 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.43 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.04–7.62 (m, 8H, ArH), 8.54 (s, 1H, CH=).
2f	3-Cl	1 737 (C=O), 1 613 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.44 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.11 (d, 2H, ArH), 7.29 (d, 2H, ArH), 7.46–7.54 (m, 4H, ArH), 8.10 (s, 1H, CH=).
2g	4-Cl	1 735 (C=O), 1 614 (C=N)	0.81 (d, 6H, 2xCH_3), 1.68 (d, 3H, CH_3), 1.71–1.80 (m, 1H, CH), 2.36 (d, 2H, CH_2), 4.24 (q, 1H, CH), 7.00 (d, 2H, ArH), 7.21 (d, 2H, ArH), 7.44 (m, 4H, ArH), 8.05 (s, 1H, CH=).
2h	2-Br	1 738 (C=O), 1 605 (C=N)	0.88 (d, 6H, 2xCH_3), 1.75 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.44 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.11 (d, 2H, ArH), 7.30 (d, 2H, ArH), 7.34-7.75 (m, 4H, ArH), 8.48 (s, 1H, CH=).
2i	3-Br	1 750 (C=O), 1 616 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.44 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.11 (d, 2H, ArH), 7.30 (d, 2H, ArH), 7.36-7.71 (m, 4H, ArH), 8.10 (s, 1H, CH=).
2j	4-Br	1 736 (C=O), 1 613 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.44 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.11 (d, 2H, ArH), 7.29 (d, 2H, ArH), 7.44 (d, 2H, ArH), 7.67 (d, 2H, ArH), 8.11 (s, 1H, CH=).
2k	2-CH_3	1 737 (C=O), 1 613 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.44 (d, 2H, CH_2), 2.48 (s, 3H, CH_3), 4.32 (q, 1H, CH), 7.09–7.55 (m, 8H, ArH), 8.42 (s, 1H, CH=).
2l	3-CH_3	1 741 (C=O), 1 612 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.43 (s, 3H, CH_3), 2.44 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.09–7.46 (m, 8H, ArH), 8.16 (s, 1H, CH=).
2m	4-CH_3	1 735 (C=O), 1 602 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.43 (s, 3H, CH_3), 2.44 (d, 2H, CH_2), 4.30 (q, 1H, CH), 7.11 (d, 2H, ArH), 7.25–7.37 (m, 4H, ArH), 7.48 (d, 2H, ArH), 8.17 (s, 1H, CH=).
2n	2-OCH_3	1 737 (C=O), 1 594 (C=N)	0.88 (d, 6H, 2xCH_3), 1.75 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.43 (d, 2H, CH_2), 3.90 (s, 3H, OCH_3), 4.30 (q, 1H, CH), 6.90–7.50 (m, 8H, ArH), 8.55 (s, 1H, CH=).
2o	3-OCH_3	1 744 (C=O), 1 597 (C=N)	0.88 (d, 6H, 2xCH_3), 1.75 (d, 3H, CH_3), 1.80–1.92 (m, 1H, CH), 2.43 (d, 2H, CH_2), 3.90 (s, 3H, OCH_3), 4.30 (q, 1H, CH), 7.01–7.61 (m, 8H, ArH), 8.14 (s, 1H, CH=).
2p	4-OCH_3	1 729 (C=O), 1 598 (C=N)	0.88 (d, 6H, 2xCH_3), 1.75 (d, 3H, CH_3), 1.80–1.92 (m, 1H, CH), 2.43 (d, 2H, CH_2), 3.88 (s, 3H, OCH_3), 4.30 (q, 1H, CH), 7.01 (d, 2H, ArH), 7.10 (d, 2H, ArH), 7.28 (d, 2H, ArH), 7.54 (d, 2H, ArH), 8.14 (s, 1H, CH=).
2r	3,4,5-t-OCH_3	1 742 (C=O), 1 607 (C=N)	0.88 (d, 6H, 2xCH_3), 1.75 (d, 3H, CH_3), 1.80–1.89 (m, 1H, CH), 2.43 (d, 2H, CH_2), 3.92 (s, 9H, 3xOCH_3), 4.35 (q, 1H, CH), 6.80 (s, 2H, ArH), 7.10 (d, 2H, ArH), 7.30 (d, 2H, ArH), 8.10 (s, 1H, CH=).
2s	3-$OCOCH_3$	1 766 (C=O), 1 735 (C=O), 1 614 (C=N)	0.90 (d, 6H, 2xCH_3), 1.75 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.35 (s, 3H, $COCH_3$), 2.44 (d, 2H, CH_2), 4.30 (q, 1H, CH), 7.10–7.57 (m, 8H, ArH), 8.15 (s, 1H, CH=).
2t	4-CF_3	1 745 (C=O), 1 609 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.80–1.90 (m, 1H, CH), 2.44 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.10 (d, 2H, ArH), 7.28 (d, 2H, ArH), 7.68 (d, 2H, ArH), 7.78 (d, 2H, ArH), 8.20 (s, 1H, CH=).
2u	3-NO_2	1 758 (C=O), 1 608 (C=N)	0.89 (d, 6H, 2xCH_3), 1.76 (d, 3H, CH_3), 1.81–1.91 (m, 1H, CH), 2.44 (d, 2H, CH_2), 4.32 (q, 1H, CH), 7.10 (d, 2H, ArH), 7.28 (d, 2H, ArH), 7.70–8.40 (m, 4H, ArH), 8.45 (s, 1H, CH=).

sisted of three mice (*table III*). Since the effect of the compounds **2c**, **2f**, **2**i were found to be inconsistent in secondary evaluation, they were not considered for further evaluation. The experiment was repeated for the selected compounds in two different dose levels (50 and 200 mg/kg) (*tables IV* and *V*). The stomachs of the animals administered with higher doses were also examined for gastric ulcerations. As shown in *table IV*, in spite of the high gastric ulcer incidence in the reference compound, ibuprofen, the synthesized compounds were generally found to be safer from the view point of ulcer induction, except for the compounds **2b**, **2o** and **2r**. It should be pointed out that compounds **2o** and **2r** were substituted with methoxy group in benzene ring.

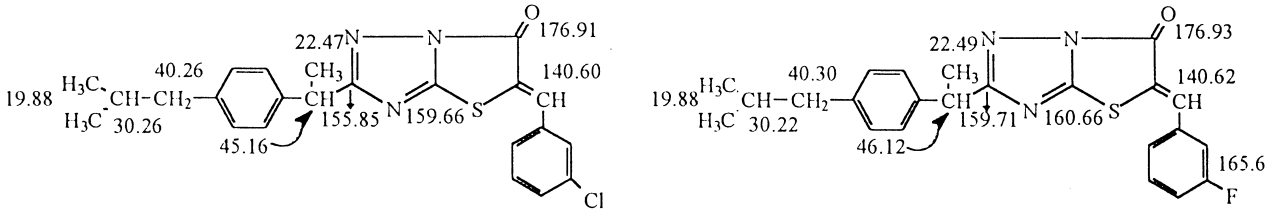

Figure 4. ^{13}C-NMR assignments of some carbon atoms of compounds **2c** and **2f**.

Among the compounds examined in this study, the compounds **2** and **2g** possessed the most prominent and consistent activity, which increased dose-dependently. For these two compounds ED_{50} values were calculated by using the Litchfield-Wilcoxon method and were found to be 243.78 mg and 192.75 mg, respectively (for the 180 min values). Although an exact dose–response relationship could not be established, compounds **2l**, **2m**, **2n**, **2r** and **2t** with decreasing order of importance, deserve attention and may be considered for further evaluation.

4. Experimental protocol

4.1. Chemistry

Melting points were detected with a Thomas Hoover capillary melting point apparatus and are uncorrected. The IR spectra (KBr) were recorded on a Perkin Elmer 1720X FT-IR spectrometer. The ^{1}H-NMR spectra were obtained by Bruker AC 250 MHz and Bruker DPX Analytic GMBH 400 MHz FT NMR instruments using $CDCl_3$ or DMSO-d_6 and tetramethylsilane as internal standard. All chemical shift values were recorded as δ (ppm). Mass spectra were taken on a Finnigan MAT GCQ mass spectrometer with electron ionization (EI) (Westfälische Wilhelms-University of Münster-Germany). The purity of the compounds were controlled by thin layer chromatography (Merck, silica gel, $HF_{254-366}$, 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 N were within ± 0.4% of theoretical value. All chemicals were from Aldrich Chemical Co. (Steinheim, Germany).

Table III. Secondary evaluation; effects of the synthesized compounds in 100 mg/kg dose, against carrageenan-induced hind paw oedema model in mice.

Test compounds	Dose (p.o) mg/kg	Swelling in thickness ($\times 10^{-2}$ mm) (percent inhibitory effect)			
		90 min	180 min	270 min	360 min
Control		76.3 ± 4.4	81.3 ± 4.7	74.7 ± 5.4	60.3 ± 5.6
2	100	66.8 ± 9.3 (12.4)	46.6 ± 5.9 (42.6)	44.9 ± 8.7 (39.9)	47.1 ± 1.9 (21.9)
2b	100	70.7 ± 10.3 (7.3)	84.0 ± 11.5	67.3 ± 12.2 (9.7)	67.3 ± 8.3
2c	100	52.3 ± 10.4 (31.5)	87.7 ± 11.9	92.0 ± 13.9	74.3 ± 6.8
2f	100	63.5 ± 19.5 (16.8)	80.6 ± 13.8 (0.9)	66.4 ± 14.3 (11.1)	58.6 ± 7.1 (2.8)
2g	100	38.2 ± 7.1 (49.9)	44.7 ± 4.5 (45.0)	48.9 ± 12.9 (34.5)	38.5 ± 7.5 (36.1)
2h	100	53.9 ± 13.3 (29.3)	48.5 ± 11.6 (40.3)	42.4 ± 8.7 (43.2)	35.6 ± 7.5 (41.0)
2i	100	64.7 ± 16.8 (13.1)	88.0 ± 21.2	80.3 ± 29.1	80.7 ± 22.0
2k	100	77.2 ± 9.6	68.6 ± 3.1 (15.6)	70.1 ± 12.3 (6.1)	61.4 ± 12.3
2l	100	56.7 ± 3.5 (25.7)	58.7 ± 4.3 (27.7)	66.0 ± 12.2 (11.6)	50.0 ± 4.6 (17.1)
2m	100	58.7 ± 5.9 (23.1)	57.3 ± 7.4 (29.5)	42.7 ± 2.2 (42.8)	34.0 ± 4.0 (43.6)
2n	100	36.6 ± 11.8 (52.0)	71.0 ± 16.1 (12.7)	88.6 ± 12.2	68.7 ± 11.9
2o	100	57.0 ± 8.5 (23.1)	70.7 ± 10.7 (13.0)	65.3 ± 13.8 (12.6)	55.7 ± 4.8 (7.7)
2r	100	44.4 ± 7.7 (41.8)	49.4 ± 10.7 (39.2)	46.7 ± 9.6 (37.4)	42.8 ± 10.2 (29.0)
2t	100	55.6 ± 15.0 (27.1)	62.1 ± 9.4 (23.6)	50.9 ± 2.2 (31.8)	47.6 ± 3.0 (21.1)

Table IV. The anti-inflammatory activity of the selected compounds in 50 mg/kg dose, against carrageenan-induced hind paw oedema model in mice.

Test compounds	Dose (p.o) mg/kg	Swelling in thickness ($\times 10^{-2}$ mm) (percent inhibitory effect)			
		90 min	180 min	270 min	360 min
Control		66.9 ± 9.8	72.6 ± 10.2	67.7 ± 13.3	50.7 ± 9.6
2	50	45.0 ± 6.3 * (32.7)	47.5 ± 7.9 * (34.6)	46.1 ± 5.4 * (32.0)	42.5 ± 5.4 (16.2)
2b	50	111.9 ± 7.3	82.7 ± 6.7	70.6 ± 8.1	51.6 ± 6.8
2g	50	64.5 ± 3.6 (3.6)	61.5 ± 5.5 (15.3)	59.5 ± 5.8 (12.1)	44.3 ± 4.1 (12.6)
2h	50	66.0 ± 3.1 (1.3)	62.9 ± 5.8 (13.4)	61.1 ± 4.4 (9.7)	54.7 ± 6.7
2k	50	100.9 ± 8.5	77.4 ± 6.9	70.1 ± 7.7	52.4 ± 6.7
2l	50	43.3 ± 8.8 (35.3)	58.0 ± 11.8 (20.1)	55.8 ± 10.6 (17.6)	50.8 ± 11.2
2m	50	50.7 ± 10.0 (24.2)	66.1 ± 8.6 (8.9)	65.1 ± 8.0 (3.8)	49.3 ± 7.0 (2.8)
2n	50	37.0 ± 5.8 * (44.7)	55.2 ± 6.0 (24.0)	49.7 ± 4.7 (27.2)	48.5 ± 6.4 (4.3)
2o	50	56.6 ± 6.2 (15.4)	65.7 ± 3.5 (9.5)	64.8 ± 3.0 (4.3)	56.3 ± 3.5
2r	50	65.4 ± 4.1 (2.2)	64.1 ± 11.1 (11.7)	67.5 ± 8.3	54.2 ± 5.6
2t	50	87.7 ± 6.0	102.3 ± 5.3	89.5 ± 5.7	68.3 ± 5.9

* $P < 0.05$; significant from the control.

4.1.1. General procedures

4.1.1.1. 3-[1-(4-(2-Methylpropyl)-phenyl)ethyl]-1,2,4-triazole-5-thione **2**

N,N′-Dicyclohexylcarbodiimide (DCC) (12.51 g, 59 mmol) was slowly added to a solution of 9.28 g (45 mmol) of ibuprofen **1** and 6.43 g (59 mmol) of N-hydroxysuccinimide (HOSu) in 200 mL of tetrahydrofuran (THF) at 0 °C. The mixture was kept at 5 °C overnight. The precipitate was removed by filtration and washed with THF. The filtrate was concentrated under vacuum. The oily residue was solidified with ether to afford the compound **1a** (yield: 70.19%). Compound **1a** (14.55 g, 51 mmol) was dissolved in 50 mL of THF. Thiosemicarbazide (5.01 g, 55 mmol) in 30 mL of DMSO was added to this solution and the solution was heated under reflux for 12 h. THF was removed under vacuum and then cold water was added. The resulting precipitate was collected by filtration, washed with water to yield product **1b** (yield: 79.42%). A solution of 11.30 g (39 mmol) **1b** in 50 mL of 10% aqueous KOH was heated

Table V. The anti-inflammatory activity and gastric ulceration effect of the selected compounds in 200 mg/kg dose, against carrageenan-induced hind paw oedema model in mice.

Test compounds	Dose (p.o) mg/kg	Ulcer incidence	Swelling in thickness ($\times 10^{-2}$ mm) (percent inhibitory effect)			
			90 min	180 min	270 min	360 min
Control		0/6	63.3 ± 8.7	91.3 ± 12.1	78.8 ± 7.5	70.5 ± 8.2
2	200	0/6	31.3 ± 3.8 * (50.6)	47.8 ± 6.2 *** (47.6)	51.0 ± 5.9 * (35.3)	50.9 ± 7.1 * (27.8)
2b	200	1/6	37.9 ± 7.4 * (40.1)	72.1 ± 13.7 (21.0)	74.2 ± 10.0 (5.8)	76.1 ± 7.6
2g	200	0/6	23.3 ± 3.2 *** (63.2)	43.8 ± 7.4 ** (52.0)	38.8 ± 5.1 *** (50.8)	32.8 ± 6.5 ** (53.5)
2h	200	0/6	51.7 ± 5.4 (18.3)	91.6 ± 9.4	76.8 ± 11.4 (2.5)	61.0 ± 9.0 (13.5)
2k	200	0/6	40.0 ± 7.0 * (36.8)	92.8 ± 12.2	64.5 ± 11.5 (18.1)	68.1 ± 11.4 (3.4)
2l	200	0/6	42.8 ± 1.8 * (32.4)	60.3 ± 2.4* (33.9)	56.3 ± 5.9 * (28.6)	45.8 ± 7.3 * (35.0)
2m	200	0/6	47.1 ± 5.7 (25.5)	66.6 ± 6.5 * (27.1)	63.4 ± 7.5 (19.5)	53.0 ± 8.0 (24.8)
2n	200	0/6	37.0 ± 7.1 * (41.5)	60.0 ± 7.5 (34.3)	64.2 ± 3.4 (18.5)	68.4 ± 8.1 (2.9)
2o	200	2/6	55.5 ± 6.9 (12.3)	94.4 ± 10.9	91.1 ± 10.9	85.4 ± 10.8
2r	200	1/6	44.7 ± 2.6 * (29.4)	71.1 ± 3.4 (22.1)	53.1 ± 4.3 * (32.6)	47.1 ± 5.1* (33.1)
2t	200	0/6	42.7 ± 2.5 * (32.5)	62.5 ± 3.3 * (31.5)	49.2 ± 4.6 ** (37.6)	33.2 ± 5.8 ** (52.9)
Ibuprofen	200	6/6	29.9 ± 4.2 * (52.8)	48.8 ± 10.2 ** (46.5)	25.6 ± 4.2 *** (67.5)	38.6 ± 6.3 * (45.2)
Phenyl butazon	100	1/6	28.3 ± 2.2 ** (55.3)	48.8 ± 2.9 ** (46.5)	38.4 ± 4.1 *** (51.3)	37.8 ± 7.0 * (46.4)

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

for 3 h, cooled, filtered and the filtrate was acidified with 37.5% HCl. The precipitated solid was filtered and washed with water. Yield: 7.8 g (74%). M.p.: 198–200 °C, IR (KBr) cm^{-1}: 3 100 (NH), 1 211 (C=S). ^{1}H-NMR (DMSO-d_6) ppm: 0.84 (d, 6H, 2xCH_3), 1.52 (d, 3H, CH_3), 1.76–1.82 (m, 1H, CH), 2.48 (d, 2H, CH_2), 4.00–4.10 (q, 1H, CH), 7.00–7.20 (m, 2H, ArH), 13.20 (s, 1H, NH), 13.30 (s, 1H, NH).

4.1.1.2. 6-Benzylidenethiazolo-[3,2-b]-1,2,4-triazol-5(6H)-ones **2a–u**

A mixture of 1.04 g (4 mmol) 5-mercapto-1,2,4-triazole **2**, 0.57 g (6 mmol) chloroacetic acid, 4 mmol various aromatic aldehyde and 0.54 g of anhydrous sodium acetate was refluxed in 8 mL of acetic anhydride and 10 mL of acetic acid for 6 h. The mixture was poured into ice-water. The precipitate was separated by filtration and dissolved in dichloromethane. The organic layer was washed using 6% $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude products were recrystallized from appropriate solvents. Melting points, yields and formulae of the synthesized compounds are shown in *table I*.

4.2. Pharmacology

Local breed albino mice of both sexes (Refiksaydam Hıfzıssıhha Institute, Animal Care Unit, Ankara, Turkey) weighing approximately 20–25 g were used. All the animals were left for 2 days under laboratory conditions for acclimatization and maintained on a standard pellet diet and water ad libidum before the day of the experiment. On the last day food was withdrawn and they were given water only. Test samples and reference compounds were suspended in 0.5% carboxymethyl cellulose and administered to each mouse by using a gastric gavage needle. The control group animals, however, received the same volume of the dosing vehicle. A minimum of six animals was used in each group. Mice used in the present study were cared for in accordance with the directory of Refiksaydam Hıfzıssıhha Institute's Animal Care Unit, which applies the guidelines of the National Institutes of Health on laboratory animal welfare.

4.2.1. Anti-inflammatory activity

The carrageenan induced hind paw oedema model according to the method reported by Kasahara et al. [22] was employed for anti-inflammatory activity testing with some modifications.

Phenylbutazone (100 mg/kg) and ibuprofen (200 mg/kg) were used as reference compounds. Test samples were administered orally 60 min before the injection of 0.5 mg/25μL of freshly prepared solution of carrageenan in physiological saline (154 mM NaCl) into the 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 the internal control. The difference in foot pad thickness between the right and left foot were measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo) in a different pattern of intervals than that described by Kasahara et al. [22]. Foot thickness of each mouse was measured four times within 90 min intervals up to 360 min. Statistical differences between the treatments and the control group of animals were evaluated by a two-tailed Student's *t*-test. 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:

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

4.2.2. Gastric ulceration studies

Only the animals who were administered 200 mg/kg (body weight) of test samples 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 anaesthesia and stomachs were removed, opened through the greater curvature, washed under running water and fixed in 5% formalin solution. The stomachs were then examined for lesions under a dissecting microscope.

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