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Synthesis and anti-inflammatory activity of 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones

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Abstract

Sixteen 1-(2-naphthyloxyacetyl)-4-substituted-3-thiosemicarbazide, 2-(2-naphthyloxymethyl)-5-substitutedamino-1,3,4-oxadiazole, 2-(2-naphthyloxymethyl)-5-substitutedamino-1,3,4-thiadiazole and 5-(2-naphthyloxymethyl)-4-substituted-1,2,4-triazole-3-thione derivatives have been prepared and evaluated as orally active anti-inflammatory agents with reduced side-effects. The structures of the compounds were confirmed by IR and ^{1}H NMR spectral data and microanalysis. The anti-inflammatory and ulcerogenic activities of the compounds were compared with naproxen, indomethacin and phenylbutazone. In carrageenan-induced foot pad edema assay, 2-(2-naphthyloxymethyl)-5-methylamino-1,3,4-oxadiazole, 5-(2-naphthyloxymethyl)-4-methyl-1,2,4-triazole-3-thione and 5-(2-naphthyloxymethyl)-4-ethyl-1,2,4-triazole-3-thione showed an interesting anti-inflammatory activity. In the air-pouch test, 1,3,4-oxadiazole and 1,2,4-triazole-3-thione derivatives reduced total number of leukocytes of the exudate that indicates excellent inhibition of prostaglandin production. Side effects of the compounds were examined on gastric mucosa, liver and stomach and none of the compounds showed significant side effects compared with reference nonsteroidal anti-inflammatory drugs (NSAIDs).

Keywords: Thiosemicarbazide; 1,3,4-Oxadiazole; 1,3,4-Thiadiazole; 1,2,4-Triazole-3-thione; Anti-inflammatory activity; Side effects

1. Introduction

Prostaglandins (PGs) are well known to be mediators of inflammation, pain and swelling. They are produced by the action of cyclooxygenase (COX) enzyme on arachidonic acid. Metabolites of the COX pathway are widely accepted as mediators of the inflammatory response. COX is known to be the principal target of nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs, block the formation of PGs and have analgesic, antipyretic and anti-inflammatory activity. However, PGs are produced by most cells, and their presence in tissues elicits a broad array of biological responses. Most notably, some PGs are cytoprotective in the gastrointestinal tract, responsible for normal renal function in the kidney and they allow platelet aggregation. In addition, PGs sensitize peripheral nerve endings to transmit pain signals to the brain and spinal cord. Recent studies have shown that COX exists in two isoforms which differ in their basal expression, tissue localization, and induction during inflammation. Differences in the pharmacological profiles of various NSAIDs might be accounted for by varying degrees of selectivity for COX-1 and COX-2. The potency and selectivity of NSAIDs appear to be directly related to their gastric, renal and hepatotoxicity.

A number of 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones were identified as potent anti-inflammatory compounds. Carrageenan-induced foot paw edema (CPE) inhibitory

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activity of 1-aryloxyacetyl-4-alkyl/aryl-3-thiosemicarbazide and their corresponding cyclized 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole-3-thione derivatives were shown equipotent with naproxen, phenylbutazone, hydrocortisone and other NSAIDs [1–9]. As a part of our continuing efforts in this area, a series of 1-(2-naphthyloxyacetyl)-4-substituted thiosemicarbazides **1a–d** and corresponding 2-(2-naphthyloxymethyl)-5-substitutedamino-1,3,4-oxadiazoles **2a–d**, 2-(2-naphthyloxymethyl)-5-substitutedamino-1,3,4-thiadiazoles **3a–d** and 5-(2-naphthyloxymethyl)-4-substituted-1,2,4-triazole-3-thiones **4a–d** were synthesized, and evaluated for their anti-inflammatory activities and side effects.

2. Experimental

2.1. Chemistry

All chemicals used in this study were supplied from Aldrich, Merck and Fluka. Melting points were taken in a Thomas Hoover capillary melting point apparatus and are uncorrected. UV and IR spectra were recorded in Shimadzu UV-160A UV–Vis spectrophotometer (2.5×10^{-5} mol/l, ethanol) and Perkin–Elmer FT-IR 1720x spectrophotometer (KBr disc) respectively. ^{1}H NMR spectra were run on a Bruker AC 80 MHz FT-NMR spectrometer (DMSO-d_6, TMS) and all chemical shifts were reported as δ (ppm) values. Elemental analysis was provided by the Scientific and Technical Research Council of Turkey. All new compounds yielded spectral data consistent with the proposed structure and microanalysis within $\pm 0.4\%$ of the theoretical values.

2.1.1. 1-(2-Naphthyloxyacetyl)hydrazine

2-Naphthol (1.44 g, 10 mmol), anhydrous potassium carbonate and 1.67 g ethyl bromoacetate (10 mmol) in 50 ml anhydrous acetone were refluxed on oil bath for 6 h. The reaction mixture was filtered and the excess solvent was removed by distillation under reduced pressure. The residue and 1.00 g hydrazine monohydrate (20 mmol) were dissolved in 50 ml absolute ethanol and refluxed on a steam bath for 1 h. The solid mass was filtered, dried and crystallized from ethanol.

2.1.2. 1-(2-Naphthyloxyacetyl)-4-substituted thiosemicarbazides **1a–d**

The mixture of 1-(2-naphthyloxyacetyl)hydrazine (10 mmol) and 10 mmol appropriate substituted isothiocyanate derivatives was dissolved in 25 ml of EtOH and refluxed 4 h. The crude product which precipitated on cooling was filtered, washed with diethyl ether, dried and crystallized from dioxane–water.

2.1.3. 2-(2-Naphthyloxymethyl)-5-substitutedamino-1,3,4-oxadiazoles **2a–d**

The appropriate 1-(2-naphthyloxyacetyl)-4-substituted thiosemicarbazides (1 mmol) were suspended in ethanol, 5 ml of 4N sodium hydroxide and iodine in potassium iodide solution (5%) were added gradually with stirring and cooling until the color of iodine persisted. The precipitated product was filtered, dried and crystallized from suitable solvents.

2.1.4. 2-(2-Naphthyloxymethyl)-5-substitutedamino-1,3,4-thiadiazoles **3a–d**

The appropriate 1-(2-naphthyloxyacetyl)-4-substituted thiosemicarbazide derivatives (1 mmol) were dissolved in 10 ml toluene and methanesulfonic acid (15 mmol) was added dropwise and refluxed 45 min. The reaction mixture was neutralized with 10% ammonium hydroxide and the precipitated product was filtered and crystallized from suitable solvents.

2.1.5. 5-(2-Naphthyloxymethyl)-4-substituted-1,2,4-triazole-3-thiones **4a–d**

1-(2-Naphthyloxyacetyl)-4-substitutedthiosemicarbazides (10 mmol) were refluxed for 8 h in 40 ml 1 N aquoeus sodium hydroxide. The mixture was acidified to pH 2 and the precipitated product was filtered off, washed with water and crystallized from suitable solvents.

2.2. Pharmacology

Mice used in the present study were housed and cared in accordance with the Hacettepe University—Animal Care Unit, which applies the guidelines of National Institutes of Health (NIH) on Laboratory Animal Welfare. Male albino mice (Hacettepe University, Animal House, Ankara, Turkey), weighing 22 ± 2 g, were used (local breed). The animals were housed in groups of six and acclimatized to room conditions for at least 2 days before the experiments, with food and water at libitum. The food was withdrawn on the day before the experiment, but free access to water was allowed.

All the compounds (100 mg/kg) and reference NSAIDs (phenylbutazone (100 mg/kg), indomethacin (10 mg/kg) and naproxen (30 mg/kg)) were suspended in 0.5% carboxymethylcellulose (CMC) and administered orally by animal feeding needle. The control groups received appropriate volumes of the vehicle (0.5% CMC, oral) only.

2.2.1. Anti-inflammatory activity

2.2.1.1. Carrageenan Paw Edema Test (CPE) [10,11]. One hour after oral administration of the compounds, the thickness of right hind paw was measured by a

peacok dial thickess gauge and 0.01 ml 2% carrageenan was injected subcutaneously into the plantar surface of the right hind paw. Two h later the volume of the edema was measured again and the antiedematous effects of the drugs were estimated in terms of percent inhibition. Percent inhibition effects of drugs was calculated according to the following equation:

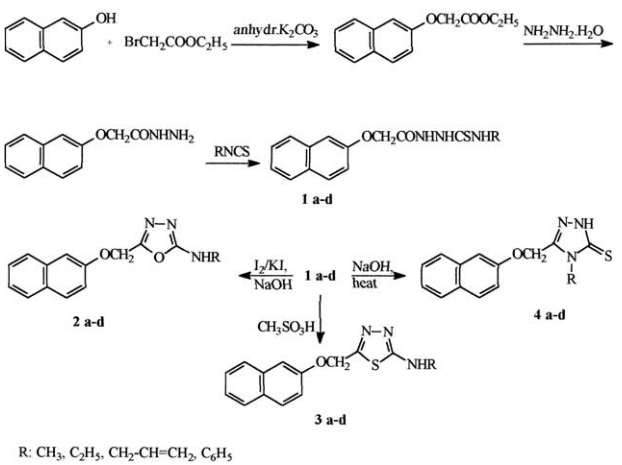
$$\text{Anti-inflammatory activity } (\%) = [(n - n')/n] \times 100$$

n and n' indicate the difference in thickness between first and second measurements of hind paws in control and test groups, respectively.

2.2.1.2. Air-pouch test [12–14]. The carrageenan powder was dissolved in 0.9% saline to a concentration of 10 mg/ml. The solution was sterilized and homogenized by storing in an oven at 90 °C for about 1 h and maintained at 37 °C. Air-pouches were formed by subcutaneous (s.c.) injection of 1 ml of air for 3 days. On the third day, after initial injection of air, 1 ml of carrageenan solution was injected into the air-pouch to induce inflammation. Compounds were administered orally 1 h before injection of the carrageenan into the pouch. Four h later, mice were killed by ether exposure and pouches washed thoroughly with 3 ml of phosphate buffer solution (PBS) containing 50 u/ml heparin. Lavage fluids were centrifugated at 2000 rpm for 15 min at 4 °C and pellet was resuspended in 1 ml of PBS–heparin. The total number of polymorphonuclear leukocytes (PMNL) infiltration was measured using a Coulter Counter (Model S-Plus VI. Coulter Electronics, Inc. Hielah, Florida).

2.2.2. Hystopathological examination

Mice were sacrified 4 h after the paw edema experiments and their liver, stomachs and kidneys were removed and put into 10% formalin solution. The sections taken from these specimens were stained with hematoxilen eosine and examined under the light microscope.



Scheme 1. Synthesis of compounds.

2.2.3. Statistical analysis

All data are expressed as mean value ± SEM. The data obtained from the control group were compared with pretreated groups, by analysis of variance (ANOVA) followed by post-hoc Bonferroni test. A $P < 0.05$ was considered significant.

3. Results and discussion

The synthesis of thiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles are outlined in Scheme 1. 2-Naphthyloxyacetyl-4-substituted thiosemicarbazides **1a**–**d** were prepared by the condensation of 1-naphthyloxyacetylhydrazine with alkyl or aryl isothiocyanates in yields varying from 45.8 to 75.4%. Cyclization of **1a**–**d** using iodine and alkali gave the desired products 2-(2-naphthyloxymethyl)-5-substitutedamino-1,3,4-oxadiazoles **2a**–**d** in 24.0–60.6% yields. 2 - (2 - Naphthyloxymethyl) - 5 - substitutedamino - 1,3,4-thiadiazoles **3a**–**d** were obtained in 15.2–60.4% yields by cyclization of **1a**–**d** by heating with methanesulfonic acid in toluene. Ring closure of acylthiosemicarbazides in the alkaline medium is a well known method for the synthesis of 1,2,4-triazoles. 5-(2-Naphthyloxymethyl)-4-substituted-1,2,4-triazole-3-thiones **4a**–**d** were obtained in 19.8–43.8% yields from the respective **1a**–**d** with this method (Scheme 1).

The structure of the compounds was elucidated by UV, IR, ^{1}H NMR and microanalyses. Crystallization solvents, melting points, yields%, spectral data and microanalyses of the compounds are given in Table 1. All spectral data were in accordance with the assumed structures. In UV spectra all compounds have two strong absorption bands at 218.8–228.8 and 254.5–272.6 nm. The IR spectra of the acylthiosemicarbazide derivatives **1a**–**d** have C=O stretching bands at 1703–1677 cm^{-1}. In 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole-3-thione derivatives, the disappearance of C=O stretching bands of the acylthiosemicarbazides and detecting of strong C=N stretching band at 1666–1598 cm^{-1} is an evidence for ring closure. The oxadiazoles **2a**–**d** and thiadiazoles **3a**–**d** showed N–H stretching bands at 3362–3236 and 3467–3106 cm^{-1}, respectively. 1,2,4-Triazole-5-thiones **4a**–**d** may exist in thiole and thione forms [15]. The IR spectra of **4a**–**d** showed N–H bands in the region of 3106–3030 cm^{-1} and C=S absorbtion bands at 1258–1249 cm^{-1} instead of S–H bands at around 2600–2550 cm^{-1}. In the ^{1}H NMR spectra, all protons were seen accordingly to the expected chemical shift and integral values. Methylenic and aromatic protons of 2-naphthyloxymethyl groups were seen at 4.65–5.80 and 6.80–8.75 ppm. The N–*H*

Table 1
Crystallization solvents, melting points, % yields, formula and microanalyses of the synthesized compounds

Comp.	R	Cryst. Solvent [a]	Melting point (°C)	Yield (%)	Formula (molecular weight)	Analysis
1a[18]	CH_3	Dioxane–Water	194–195	46	$C_{14}H_{15}N_3O_2S$ (289.36)	(C,H,N)
1b	C_2H_5	Dioxane–Water	148	54	$C_{15}H_{17}N_3O_2S$ (303.39)	(C,H,N)
1c[18]	C_3H_5	Dioxane–Water	157	75	$C_{16}H_{17}N_3O_2S$ (315.40)	(C,H,N)
1d[18]	C_6H_5	Dioxane–Water	149	60	$C_{19}H_{17}N_3O_2S$ (351.43)	(C,H,N)
2a	CH_3	Ethanol	139–141	54	$C_{14}H_{13}N_3O_2$ (255.28)	(C,H,N)
2b	C_2H_5	Acetone	140–141	50	$C_{15}H_{15}N_3O_2$ (269.31)	(C,H,N)
2c	C_3H_5	EtOAc–Ethanol	134–135	61	$C_{16}H_{15}N_3O_2$ (281.32)	(C,H,N)
2d[19]	C_6H_5	Ethanol	141–142	24	$C_{19}H_{15}N_3O_2$ (317.34)	(C,H,N)
3a	CH_3	EtOAc	188	25	$C_{14}H_{13}N_3OS$ (271.34)	(C,H,N)
3b	C_2H_5	EtOAc–Ethanol	161	31	$C_{15}H_{15}N_3OS$ (285.37)	(C,H,N)
3c	C_3H_5	EtOAc–Ethanol	166	15	$C_{16}H_{15}N_3OS$ (297.38)	(C,H,N)
3d[19]	C_6H_5	EtOAc	209–210	61	$C_{19}H_{15}N_3OS$ (333.41)	(C,H,N)
4a[18]	CH_3	Ethanol	184	35	$C_{14}H_{13}N_3OS$ (271.34)	(C,H,N)
4b	C_2H_5	Ethanol	215–216	42	$C_{15}H_{15}N_3OS$ (285.37)	(C,H,N)
4c[18]	C_3H_5	Ethanol	135–136	44	$C_{16}H_{15}N_3OS$ (297.38)	(C,H,N)
4d[18,20]	C_6H_5	EtOAc	156–158	20	$C_{19}H_{15}N_3OS$ (333.41)	(C,H,N)

[a] EtOAc, ethylacetate.

protons of acylthiosemicarbazides **1a**–**d** were observed at 8.05–8.20, 9.15–9.70 and 10.00–10.35 ppm. N–*H* protons of the compounds having oxadiazole **2a**–**d**, thiadiazole **3a**–**d** and triazole-3-thione **4a**–**d** ring were seen at about 7.55–10.55, 7.90–10.45 and 13.90–15.30 ppm, respectively (Table 2). The results of microanalyses were within $\pm$ 0.4% theoretical values.

All the synthesized compounds and reference NSAIDs were screened for anti-inflammatory activities by carrageenan hind-paw edema test (CPE) and by the air-pouch test. The obtained pharmacological results (Table 3) indicate that some of the title compounds possess notable anti-inflammatory properties. According to the results of the in vivo experiments, it is difficult to extract a definite structure–activity relationship between the compounds and anti-inflammatory properties. In CPE assay, 1,3,4-oxadiazole derivatives **2a**, **2c** and **2d** showed notable anti-inflammatory activity. Most effective compound was 2-(2-naphthyloxymethyl)-5-methylamino-1,3,4-oxadiazole **2a** (67.32 ± 9.26% inh.). All 1,3,4-thiazole derivatives **3a**–**d** showed weak anti-inflammatory activity whereas 5-(2-naphthyloxymethyl)-4-methyl-1,2,4-triazole-3-thione **4a** and 5-(2-naphthyloxymethyl)-4-ethyl-1,2,4-triazole-3-thione **4b** showed promising activity with the 60.62 ± 8.55 and 51.41 ± 3.98% CPE inhibition, respectively. Additionally, allyl **4c** (45.18 ± 9.89%) and phenyl analogs **4d** (42.50 ± 3.82% inh.) significantly inhibited edema when compared with phenylbutazone (53.27 ± 1.83% inh.). For the compounds having 1,3,4-oxadiazole, 1,3,4-thiadizole and 1,2,4-triazole-3-thione ring, methyl substituted derivatives showed equal or higher anti-inflammatory activity compared with their allyl and phenyl analogs.

Recent studies have clearly shown that, the anti-inflammatory effect of carragenan causes a massive influx of predominantly neutrophilic leucocytes (predominantly PMNL) from blood circulation into the cavity. Besides, leucocyte migration is induced locally in the inflammatory process and leucocytes also intensify inflammation by releasing several inflammatory mediators. The method is presented for measuring the edema induced by injection of carrageenan into the pouches in mice back. Air-pouches were highly reactive to inflammatory stimulus. The enhanced inflammatory reactions in the sites correlated with formation of lining tissue, the type of cells constituting the inflammatory sites, the activation of cells, and/or the reactivity of newly formed blood cells [12,13,16,17]. Mean leukocyte numbers per ml of exudate for each drug compared with control values obtained from similar group of animals receiving vehicle alone and the degree of inflammatory response produced in the air-pouch cavity was assessed by measuring total cell number of the exudate. 1,3,4-Oxadiazole **2a**–**d** (except **2b**) and 1,2,4-triazole-3-thione **4a**–**d** derivatives reduced total number of leukocytes of the exudate. These findings indicate a good inhibition of prostaglandin production. 5-Methylamino analogue of 1,3,4-oxadiazole **2a** ($30.75 \pm 0.75 \times 10^5/cm^3$) and 4-methyl substituted-1,2,4-triazole-3-thione **4a** ($32.43 \pm 3.15 \times 10^5/cm^3$) were extremely potent in inhibiting PMNL production compared with control and reference compounds (control: 122.50 ± 4.10, phenylbutazone: 53.27 ± 1.71, indomethacin: $68.80 \pm 1.80 \times 10^5/cm^3$) (Table 3).

Ulcerogenic potential of anti-inflammatory compounds can be demonstrated in animal models using positive (naproxen, indomethacin) and negative (phenylbutazone) controls. On microscopic examina-

tion, lesions seen in stomach, kidney and liver tissues are graded according to their severity. The grade of the scale is designed as (+) for mild, (+ +) for moderate, and (+ + +) for severe changes. Stomachs of the mice are thoroughly sectioned and both corpus and antrum are evaluated. Surface epithelium and the lamina propria of the gastric mucosa are all examined. Acute gastritis may exist in an earlier or milder nonerosive

Table 2
UV, IR and ^{1}H NMR spectral data of the compounds

Comp.	$UV_{max}^{ethanol}$ (log ε)	IR (KBr) ν (/cm)	^{1}H NMR (DMSO-d_6) δ ppm (J in Hz)
1a	228.0 (4.78); 259.8 (4.16)	3336, 3197 (N-H), 1713 (C=O), 1216 (C=S), 1057 (Ar–O–CH_2)	2.85 (3H; d; -CH_3), 4.65 (2H; s; Ar–O–CH_2–), 7.10–8.00 (7H; m; aromatic prot.), 8.15 (1H; d; CS–NH–CH_3), 9.30 (1H; s; CO–NH–NH–CS), 10.15 (1H; s; CO–NH–NH–CS)
1b	228.0 (4.58), 260.6 (4.12)	3506, 3364 (N–H), 1677 (C=O), 1217 (C=S), 1066 (Ar–O–CH_2)	1.10 (3H; t; CH_2–CH_3), 3.40–3.60 (2H; q; CH_2–CH_3), 4.70 (2H; s; Ar–O–CH_2–), 7.05–7.95 (7H; m; aromatic prot.), 8.05 (1H; d; CS–NH–CH_3), 9.15 (1H; s; CO–NH–NH–CS), 10.00 (1H; s; CO–NH–NH–CS)
1c	227.2 (4.74), 259.8 (4.17)	3523, 3186 (N–H), 1678 (C=O), 1215 (C=S), 1064(Ar–O–CH_2)	4.10 (2H; m; –CH_2–CH = CH_2), 4.75 (2H; s; Ar–O–CH_2–), 5.05 (1H; dd; –CH_2–CH = CH_2), 5.20 (1H; dd; –CH_2–CH = CH_2), 5.60–6.10 (1H; m; –CH_2–CH = CH_2), 7.15–7.95 (7H; m; aromatic prot.), 8.15 (1H; s; CS–NH–CH_2), 9.35 (1H; s; CO–NH–NHCS), 10.15 (1H; s; CONHNH–CS)
1d	227.4 (4.82), 268.2 (4.13)	3370, 3328 (N–H), 1703 (C=O), 1257 (C=S), 1051 (Ar–O–CH_2)	4.75 (2H; s; Ar–O–C$\underline{H}_2$–), 7.05–7.95 (12H; m; aromatic prot.), 8.20 (1H; d; CS–NH–CH_3), 9.70 (1H; s; CO–NH–NH–CS), 10.35 (1H; s; CO–NH–NH–CS)
2a	220.4(5.15), 264.0(4.13)	3236 (N–H), 1666 (C=N), 1025 (C–O–C ring)	2.85 (3H; d; –CH_3), 5.15 (2H; s; Ar–O–CH_2–), 7.10–7.95 (7H; m; aromatic prot.), 7.55 (1H; s; NH–CH_3)
2b	219.8 (5.09), 265.0 (4.19)	3236 (N–H), 1651, 1629 (C=N) 1029 (C–O–C ring)	1.10 (3H; t; –CH_2–CH_3), 3.15(2H; m; –CH_2–CH_3), 5.30 (2H; s; Ar–O–CH_2–), 7.10–7.95 (7H; m; aromatic prot.), 7.70 (1H; s; NH–C_2H_5)
2c	219.8 (5.14), 264.0 (4.12)	3362 (N–H), 1628, 1600 (C=N), 1039 (C–O–C ring)	3.65–3.95 (2H; m; –C$\underline{H}_2$–CH = CH_2), 5.05 (1H; dd; –CH_2–CH = CH_2 H_A), 5.15 (1H; dd; –CH_2–CH = CH_2; H_B), 5.25 (2H; s; Ar–O–CH_2–), 5.6–5.95 (1H; m; –CH_2–CH = CH_2), 7.10–7.95 (7H; m; aromatic prot.), 7.90 (1H; s; NH–CH_2)
2d	220.4 (5.14), 263.8 (4.31)	3253 (N–H), 1628,1600 (C=N), 1039 (C–O–C ring)	5.35 (2H; s; Ar–O–CH_2–), 6.80–8.10 (12H; m; aromatic prot.) 10.55 (1H; s; NH–C_6H_5)
3a	228.0 (5.12), 266.5 (4.11)	3467 (N–H), 1601, 1629 (C=N)	2.83 (3H; d; –CH_3), 4.75 (2H; s; Ar–O–CH_2–), 7.05–7.85 (7H; m; aromatic prot.), 7.90 (1H; s; NH–CH_3)
3b	227.8 (4.90), 267.5 (4.24)	3182 (N–H), 1626, 1599 (C=N), 1057 (Ar–O–CH_2)	1.15 (3H; t; –CH_2–CH_3), 3.35 (2H; m; –CH_2–CH_3), 5.45 (2H; s; Ar–O–CH_2–), 7.05–7.95 (7H; m; aromatic prot.), 8.05 (1H; s; NH–C_2H_5)
3c	226.8 (4.74), 266.0 (4.12)	3319, 3241 (N–H), 1645 (C=N)	4.15 (2H; t; –C$\underline{H}_2$–CH = CH_2), 4.95 (1H; dd; –CH_2–CH = CH_2; H_A), 5.15 (1H; dd; –CH_2–CH = CH_2; H_B), 4.75 (2H; s; Ar–O–CH_2–), 5.55–6.00 (1H; m; –CH_2–CH = CH_2), 6.95–7.95 (7H; m; aromatic prot.), 8.15 (1H; t; NH–CH_2)
3d	227.6 (4.77), 272.6 (4.18)	3436 (N–H), 1614, 1598 (C=N)	5.50 (2H; s; Ar–O–CH_2–), 6.95–8.15 (12H; m; aromatic prot.), 10.45 (1H; s; NH–C_6H_5)
4a	227.0 (4.79), 254.5 (4.28)	3106 (N–H), 1626 (C=N), 254 (C=S)	3.75 (3H; d; N–CH_3), 5.80 (2H; s; Ar–O–CH_2–), 7.80–8.75 (7H; m; aromatic prot.), 15.30 (1H; bs; NH–CH_3)
4b	218.0 (4.53), 259.5 (4.27)	3054 (N–H), 1633 (C=N), 1258 (C=S), 1048 (Ar–O–CH_2)	1.30 (3H; t; N–CH_2–CH_3), 4.15 (2H; q; –CH_2–CH_3), 5.40 (2H; s; Ar–O–CH_2–), 7.05–8.25 (7H; m; aromatic prot.), 13.93 (1H; s; NH–CH_2)
4c	227.0 (4.82) 260.0 (4.34)	3055 (N–H), 1632, 1601 (C=N), 1249 (C=S)	4.75 (2H; d; N–CH_2–CH = CH_2), 4.95 (1H; dd; –CH_2–CH = CH_2), 5.10 (1H; dd; –CH_2–CH = CH_2), 5.25 (2H; s; Ar–O–C$\underline{H}_2$–), 5.70–6.25 (1H; m; –CH_2–CH = CH_2), 7.10–7.95 (7H; m; aromatic prot.) 13.95 (1H; bs; NH–CH_3)
4d	227.8 (4.74), 261.0 (4.41)	3030 (N–H), 1630, 1600 (C=N), 1258 (C=S)	4.95 (2H; s; Ar–O–CH_2–), 6.95–7.90 (12H; m; aromatic prot.), 13.90 (1H; bs; NH–C_6H_5)

s, Singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quarted; m, multiplet.

Table 3
Anti-inflammatory activities of the compounds

Comp. (100 mg/kg)	CPE (% inhibition ± SE) [a]	PMNL ($\times 10^5$/cm^3) ($n = 6$, after 4 h) [b]
Control		122.50 ± 4.10
1a	n.a.	n.t.
1b	n.a.	n.t.
1c	15.45 ± 1.67	104.76 ± 0.80
1d	n.a.	n.t.
2a	67.32 ± 9.26 [c]	30.75 ± 0.75
2b	28.69 ± 5.06 [c]	91.00 ± 1.00
2c	40.42 ± 5.42 [c]	60.75 ± 0.75
2d	40.42 ± 5.42 [c]	63.30 ± 0.33
3a	20.20 ± 4.14	98.75 ± 2.88
3b	35.51 ± 1.21 [d]	69.42 ± 0.96
3c	22.44 ± 3.08 [d]	95.25 ± 1.56
3d	n.a.	n.t.
4a	60.62 ± 8.55 [c]	32.43 ± 3.15
4b	51.41 ± 3.98 [c]	56.25 ± 0.38
4c	45.18 ± 9.89 [c]	50.00 ± 1.32
4d	42.50 ± 3.82 [c]	46.30 ± 0.68
Naproxen (30 mg/kg)	67.50 ± 1.70 [c]	50.20 ± 1.30
Phenylbutazone (100 mg/kg)	53.27 ± 1.83 [c]	53.27 ± 1.71
Indomethacin (10 mg/kg)	32.10 ± 1.38 [c]	68.80 ± 1.80

PMNL, polymorphonuclear leukocytes; n.a.: no activity; n.t.: not tested.
[a] Results are expressed as their mean values ($n = 6$).
[b] Mean ± SEM ($n = 6$).
[c] $P < 0.01$.
[d] $P < 0.05$.

form with merely mucosal congestion and edema, and histologic evidence of inflammation. These earlier changes are known to be transient and completely reversible within few days, but the development of erosions and hemorrhages is more serious and related to an increased risk of major upper gastrointestinal bleeding. In our study, the animals were sacrified 4 h later than the ingestion of the drugs. None of the sections displayed the morphology of the ulceration but non-erosive form of acute gastritis was observed in various degrees of severity with compounds **1a**–**d** and **4b** (Table 4). On microscopic examination of the kidney sections, the morphologic findings such as pronounced edema and interstitial mononuclear cell infiltration, were characteristic for tubulointerstitial nephritis. The presence of some scattered eosinophil leucocytes also provided evidence of the drug effect. Focal areas displaying variable but generally mild degree of tubular regeneration were also present. Acute pyelonephritis should be considered in the differential diagnosis of the tubulointerstitial nephritis, but none of our cases showed interstitial suppurative (inflammation with polymorphonuclear leucocytes) inflammation with microabscesses. Therefore, the presented renal morphologic findings were accepted as the reflection of the effects of the drugs. Liver tissues were also examined thoroughly and the integrity of the basic structure, degree of lobular and portal inflammation, and the presence or absence of necrosis, fatty change, or cholestasis were evaluated. Many NSAIDs, like

Table 4
Histopathological examination results of the compounds

Comp. (100 mg/kg)	Kidney		Stomach	Liver		
	Edema	Infected cell (MNL)	Gastritis	Fatty change	Acute hepatitis/spotty necrosis	Cholestasis
1a	−	−	+	−	+	−
1b	−	++	++	−	−	−
1c	−	−	++	+	+	+
1d	−	++	++	++	+	−
2a	−	−	−	++	++	++
2b	−	+	−	++	+	−
2c	−		−	−	+	−
2d	−	−	−	−	+	−
3a	−	−	−	−	−	−
3b	−	−	−	−	−	−
3c	−	−	−	−	++	−
3d	−	−	−	+	+	+
4a	++	+	−	++	+	+
4b	−	−	++	+	−	−
4c	++	−	−	−	−	−
4d	−	−	−	−	−	−
Indomethacin (10 mg/kg)	++	+	+++	++	++	−
Naproxen (30 mg/kg)	+	+	+	+	+++	−

n.t., not tested; −, no; +, mild; ++, moderate; +++, severe side effects.

phenylbutazone and naproxen, are known to cause acute or chronic hepatitis, confluent or spotty necrosis, cholestatic hepatitis and/or fatty change. This shows the importance of examining the liver tissues to better assess the safety of NSAIDs. In some cases like **1c**, **1d**, **2a**, **2b**, and **4a** (moderate, + +) macro and microvesicular fatty change and several scattered mild (+) focal spotty necrosis were observed. Multiple foci of spotty necrosis (moderate, + +) was seen with compounds **2a** and **3c**. Cholestatic hepatitis was observed with the compounds **1c**, **2a**, **3d**, and **4a** (Table 4).

In conclusion, a new series of compounds showing anti-inflammatory properties was synthesized. Among them in particular compounds 2-(2-naphthyloxymethyl)-5-methylamino-1,3,4-oxadiazole **2a** and 5-(2-naphthyloxymethyl)-4-methyl-1,2,4-triazole-3-thione **4a** were found to have a superior anti-inflammatory profile with low gastric ulceration incidence with similar toxic profiles of reference NSAIDs in the liver.

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