mer), 4.27 (t, 1.83 H, OCH_2, *Z* isomer), 4.35 (t, 0.17 H, OCH_2, *E* isomer), 6.76 (s, 1 H, 3-H), 7.28–7.41 (m, 4 H, 8, 3′, 4′, 5′-H), 7.84 (dd, 2 H, $J_o = 8.07$ Hz, $J_m = 1.60$ Hz, 2′,6′-H), 7.99 (dd, 1 H, $J_{7,8} = 8.71$ Hz, $J_{7,5} = 1.71$ Hz, 7-H), 8.14 (s, 1 H, −CH=N), 8.20 (d, 1 H, $J_{5,7} = 1.64$ Hz, 5-H). MS (EI): m/z (%) 337 (1.04) $[M + 1]^{+\cdot}$, 101.95 (5.00), 70.84 (6.83), 58.15 (100).

2.4. Flavone-6-carboxaldehyde-O-[2-(1-pyrolidino)ethyl]oxime (***FO4***)

IR (KBr) cm^{-1}: 1650 (γ-pyron CO). ^1H NMR ($CDCl_3$): δ = 1.82 (m, 4 H, b), 2.64 (m, 4 H, a), 2.87 (t, 1.85 H, CH_2N, *Z* isomer), 2.94 (t, 0.15 H, CH_2N, *E* isomer), 4.36 (t, 1.85 H, OCH_2, *Z* isomer), 4.42 (t, 0.15 H, OCH_2, *E* isomer), 6.84 (s, 1 H, 3-H), 7.53–7.58 (m, 4 H, 8,3′,4′,5′-H), 7.93 (dd, 2 H, $J_o = 7.28$ Hz, $J_m = 1.62$ Hz, 2′, 6′-H), 8.02 (dd, 1 H, $J_{7,8} = 8.77$ Hz, $J_{7,5} = 2.11$ Hz, 7-H), 8.21 (s, 1 H, −CH=N), 8.28 (d, 1 H, $J_{5,7} = 2.07$ Hz, 5-H), MS (EI): m/z (%) = 363 (0.63) $[M + 1]^{+\cdot}$, 221.2 (1.59), 101.97 (9.55), 84 (100).

2.5. Flavone-6-carboxaldehyde-O-[2-(1-piperidino)ethyl]oxime (***FO5***)

IR (KBr) cm^{-1}: 1649 (γ-pyron CO). ^1H NMR ($CDCl_3$): δ = 1.46 (m, 2 H, c), 1.61–1.64 (m, 4 H, b), 2.45–2.53 (m, 4 H, a), 2.74 (t, 1.8 H, CH_2N, *Z* isomer), 2.79 (t, 0.2 H, CH_2N, *E* isomer), 4.36 (t, 1.8 H, OCH_2, *Z* isomer), 4.40 (t, 0.2 H, OCH_2, *E* isomer); 6.84 (s, 1 H, 3-H), 7.53–7.58 (m, 4 H, 8,3′,4′,5′-H), 7.92 (dd, 2 H, $J_o = 7.68$ Hz, $J_m = 1.97$ Hz, 2′,6′-H), 8.07 (dd, 1 H, $J_{7,8} = 8.75$ Hz, $J_{7,5} = 1.89$ Hz, 7-H), 8.19 (s, 1 H, −CH=N), 8.28 (d, 1 H, $J_{5,7} = 1.77$ Hz, 5-H). MS (EI): m/z (%) = 376 (0.23) $[M]^{+\cdot}$, 249.1 (1.84), 221.2 (2.66), 127.2 (1.72), 98.05 (100), 64.2 (1.41).

2.6. Flavone-6-carboxaldehyde-O-[2-(4-morpholino)ethyl]oxime (***FO6***)

IR (KBr) cm^{-1}: 1645 (γ-pyron CO). ^1H NMR ($CDCl_3$): δ = 2.92 (t, 4 H, a), 3.05 (t, 2 H, CH_2N), 3.92 (t, 4 H, b), 4.52 (t, 2 H, OCH_2), 6.82 (s, 1 H, 3-H), 7.54–7.61 (m, 4 H, 8,3′,4′,5′-H), 7.93 (dd, 2 H, $J_o = 7.48$ Hz, $J_m = 1.57$ Hz, 2′,6′-H), 8.04 (dd, 1 H, $J_{7,8} = 8.77$ Hz, $J_{7,5} = 2.11$ Hz, 7-H), 8.20 (s, 1 H, −CH=N), 8.32 (d, 1 H, $J_{5,7} = 2.06$ Hz, 5-H). MS (EI): m/z (%) = 379.2 (8.28) $[M + 1]^{+\cdot}$, 221 (6.37), 100.08 (100), 69.99 (13.01).

3. Antimicrobial activity

A paper disc (8 mm in diameter) was soaked in a 3000 μg/ml solution of the test compound in propylene glycol (propylene glycol as a blank has not any inhibition zone), and placed on an agar plate containing fungi or bacteria cells, which was incubated at 37 °C for 24 h. The diameter of the growth inhibition zone around the paper disc was measured [13]. Antimicrobial activity results are shown in Table 2.

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Prof. Dr. Rahmiye Ertan
University of Ankara
Faculty of Pharmacy
06100-Tandoğan
Ankara
Turkey

Department of Pharmaceutical Chemistry[1], and Department of Pharmacology[2], Faculty of Pharmacy, Hacettepe University, Ankara, Turkey

Some azolylthioacetamides and their analgesic and antiinflammatory activities

R. Şimşek[1], P. Kelicen[2], C. Şafak[1], H. Akgün[1], and R. Demirdamar[2]

As part of our continuing effort to prepare nonsteroidal anti-inflammatory drugs (NSAIDs), the aim of the present study was to synthesize compounds having 1*R,2S-N*-(2-phenyl-2-hydroxyl-1-methyl)ethyl-2-(2-benzoxazolyl and/or benzothiazolyl)thioacetamide structure and to determine their analgesic and anti-inflammatory activities.

Four benzoxazolyl- and benzothiazolylthioacetamide derivatives **1b**–**4b** were synthesized by the reaction of the acid derivatives **1a**–**4a** with 1*R,2S*-2-methylamino-1-phenylpropanol (Scheme).

Since the m.p.'s of the compounds are very low, they could not been determined. All spectral data are in accordance with the assumed structures. In the IR spectra, OH and NH and amid-I and amid-II bands were seen at expected values. The ^1H NMR spectra of all compounds showed a doublet at 4.90 ppm, a multiplet at 4.35–4.60 ppm, a singlet at 3.90–4.15 pp, attributable to $C\underline{H}-OH$, $C\underline{H}-CH_3$ and $C\underline{H}_2$ protons respectively. Due to deutorium exchange $O-\underline{H}$ and $N-\underline{H}$ protons were not seen. Aromatic and methyl protons were seen at the expexted chemical shift and integral values. ^{13}C NMR spectra of all compounds supported their structures. In MS molecular ion peaks did not appear for all compounds.

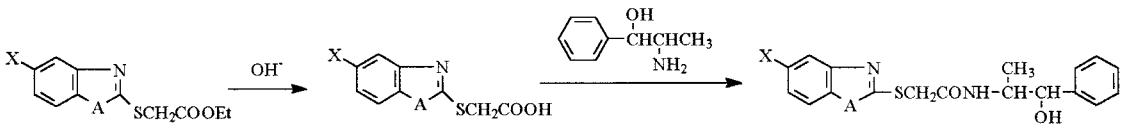
The analgesic activity of the compounds was screened by a "modified Koster's test" using aspirin as a reference analgesic. As seen in the Table, the compounds synthesized did not show analgesic activity.

The anti-inflammatory activity of these compounds was measured in the carrageenan paw edema test. The mechanism of the antiinflammatory action was further investigated by the mice air pouch test. For this purpose, air was injected to the back of mice for three days, thus forming bilateral invagination. The exudate formed within this invagination after carrageenan injection enabled detailed investigation. NSAIDs reduced vasodilatation, edema and hyperalgesia by inhibiting cyclooxygenase activity [1–5]. An ideal NSAID should reduce exudate volume and PMNL accumulation. In our work, compounds **1b**, **2b** and **4b** inhibited carrageenan-induced paw edema and reduced the number of PMNL in mice air-pouch. While compound **3b** was ineffective on carrageenan-induced paw edema, compounds **1b**, **2b** and **4b** inhibit the exudation of plasma proteins, the formation of edema within the tissues, and the emigration of leucocytes from the blood into the tis-

Table: Results of pharmacological studies of the compounds 1b–4b

Compd. (100 mg/kg)	% Analgesic activity (n = 6)	%Anti-inflammatory activity (n = 6)	Number of PMNLs* (PMN × 10^5)
1b	no activity	40	117.33 ± 7.84
2b	no activity	40	84.67 ± 2.81
3b	no activity	6	–
4b	no activity	25	87.33 ± 7.26
Aspirin	56.7	45	102.37 ± 6.30
Control	–	–	157.00 ± 4.88

Scheme



1a-4a 1b-4b

	X	A
1b	H	O
2b	Cl	O
3b	H	S
4b	Cl	S

sue. Introduction of a chlorine subtituent to the phenyl part of the benzoxazole and/or the benzothiazole ring increased the antiinflammatory activity. In addition, asymmetric centers in the side chain can influence the degree of anti-inflammatory activity.

Experimental

1. Chemistry

All chemicals used in this study were supplied from Merck (Darmstadt, Germany) or Aldrich (Steinheim, Germany). ^{1}H NMR Spectra were recorded on a Bruker AC 80 MHz and 250 MHz FT NMR spetrometer (Karlsruhe, Germany). The chemical shifts were reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS). $CDCl_3$ and/or DMSO-d_6 were used as solvents. ^{13}C NMR Spectra were recorded on a Brucker AMX 300 (75.5 MHz) Spectrometer. IR Spectra were obtained with a Perkin Elmer FT-IR Spectrometer 1720X (KBr disc) (Beaconsfield, UK). Mass spectra were recorded on a Hewlett-Packard (70 eV) (Electron Impact) (USA). Analytical TLC was carried out on precoated plates (Silica gel 60 GF_{254}) and spots were visualized with UV light. Elemental analysis were carried out by Hewlett-Packard 185 (USA) and gave satisfactory values.

1.1. 2-(Benzoxazolyl and/or benzothiazolyl)thioacetic acids (**1a**–**4a**)

The sodium salt (0.01 mol) of the appropriate azole derivative benzoxazole and/or benzothiazole was heated with 0.01 mol ethyl bromoacetate in DMF for 3 h. Ester derivatives were converted to acid anologs by alkaline hydrolysis.

1.2 1R,2S-N-(-2-phenyl-2-hydroxy-1-methyl)ethyl-2-(2-benzoxazolyl and/or benzothiazolyl)thioacetamides (**1b**–**4b**)

The appropriate acid (0.01 mol) in 10 ml of THF and 0.01 mol 1*R,2S*-2-methylamino-1-phenyl-1-propanol in 10 ml of CH_2Cl_2 were cooled in a flask with a drying tube. 0.01 mol DCC in 10 ml of CH_2Cl_2 was added. The mixture was stirred for 15 min in an ice bath and for 8 h at room temperature. After cooling, *N,N'*-dicyclohexylurea (DCU) was filtered. The solvent was removed and the crude product was seperated by preparative TLC using Silicagel GF_{254} (E. Merck); ethyl acetaten-hexane (1 : 1) as solvent.

1.3. 1R,2S-N-(-2-phenyl-2-hydroxy-1-methyl)ethyl-2-(2-benzoxazolyl)thioacetamide (**1b**)

Yield: 52% IR: 3291 (OH, NH), 2928 (CH), 1677 (Amid-I), 1644 (Amid-II), 760, 740, 697 cm^{-1} (mono and disübstituted benzene). ^{1}H NMR (DMSO-d_6): δ 0.90 (d; 3 H; C<u>H</u>$_3$), 4.00 (s; 2 H; C<u>H</u>$_2$), 4.50 (m; 1 H; C<u>H</u>−CH$_3$), 4.90 (d; 1 H; C<u>H</u>−OH), 7.20–7.80 ppm (m; 9 H; Ar-H).
$C_{18}H_{17}N_2O_3S$ (341.4)

1.4. 1R,2S-N-(-2-phenyl-2-hydroxy-1-methyl)ethyl-2-(5-chloro-2-benzoxazolyl)thioacetamide (**2b**)

Yield 44%. IR: 3292 (OH, NH), 2963 (CH), 1677 (Amid-I), 1643 (Amid-II), 754, 697 cm^{-1}(mono and trisübstituted benzene).^{1}H NMR ($CDCl_3$-d_1): δ 0.90 (d; 3 H; C<u>H</u>$_3$), 3.90 (s; 2 H; CH_2), 4.30 (m; 1 H; C<u>H</u>−CH$_3$), 4.89 (d; 1 H; C<u>H</u>−OH), 7.00–7.80 ppm (m; 8 H; Ar-H).
$C_{18}H_{16}ClN_2O_3S$ (375.8)

1.5. 1R,2S-N-(-2-phenyl-2-hydroxy-1-methyl)ethyl-2-(2-benzothiazolyl)thioacetamide (**3b**)

Yield 67%. IR: 3310 (OH, NH), 2927 (CH), 1642 (Amid-I), 1540 (Amid-II), 756, 725, 703 cm^{-1} (mono and disübstituted benzene). ^{1}H NMR ($CDCl_3$-d_1): δ 1.10 (d; 3 H; C<u>H</u>$_3$), 4.10 (s; 2 H; C<u>H</u>$_2$), 4.55 (m; 1 H; C<u>H</u>−CH$_3$), 4.90 (d; 1 H; C<u>H</u>−OH), 7.70–7.90 ppm (m; 9 H; Ar-H).
$C_{18}H_{17}N_2O_2S_2$ (357.5)

1.6. 1R,2S-N-(-2-phenyl-2-hydroxy-1-methyl)ethyl-2-(5-chloro-2-benzothiazolyl)thioacetamide (**4b**)

Yield 50%. IR: 3328 (OH, NH), 2927 (CH), 1628 (Amid-I), 1455 (Amid-II), 800, 762, 703 cm^{-1} (mono and disübstituted benzene). ^{1}H NMR ($CDCl_3$-d_1): δ 1.05 (d; 3 H; C<u>H</u>$_3$), 4.15 (s; 2 H; C<u>H</u>$_2$), 4.60 (m; 1 H; C<u>H</u>−CH$_3$), 4.90 (d; 1 H; C<u>H</u>−OH), 7.10–7.90 ppm (m; 8 H; Ar−H).
$C_{18}H_{16}ClN_2O_2S_2$ (391.9)

2. Pharmacology

Female albino mice, weighting 22 ± 2 g, were used (local breed). The animals were housed in groups of six, with food and water ad libidum and were allowed to get accustomed to their environment for at least 2 d before the experiments. Peacock dial Thickness Gauge., Coulter Counter (Model S-Plus VI. Coulter Electronic, Inc. Hialeah, Florida).

2.1. Analgesic activity

A modified Koster test [6] was used. Compounds dissolved in 0.05% carboxymethylcellulose were given orally to mice at a dose level of 100 mg/kg. One h later stretching was induced by i.p. injection of a 3% solution of acetic acid at 300 mg/kg. The control group received 0.05% carboxymethylcellulose 1 h prior to injection of acetic acid. Animals were placed in glass cages 5 min after acetic acid injection and the number of stretches per animal was recorded during a 10 min period. The analgesic activity was calculated using eq. (1).

$$\% \text{ Analgesic activity} = (N - N')/N \times 100 \quad (1)$$

N and N′ indicate the average number of stretches of control and test group, respectively. Aspirin was used as a reference analgesic and administrated according to the test protocol.

2.2. Anti-inflammatory activity

Carrageenan induced mouse paw edema (CPE) was measured using a Peacock Dial Thickness Gauge [7]. Six mice per group were used. After oral administration of the compound (100 mg/kg), 0.01 ml 2% carrageenan was injected subcutaneously into the plantar surface of the right hind paw. After 2 h, the inhibition of edema amount was measured by Peacock Dial Thickness Gauge. Aspirin was used as reference at a dose level of 100 mg/kg. The anti-inflammatory activity was calculated according to eq. (2).

$$\% \text{ Anti-inflammatory activity} = (N - N')/N \times 100 \quad (2)$$

N and N′ indicate edema difference of control and test group respectively. All results were given as average percentage.

2.3. Determination of cell numbers

Male and female albino mice (20–25 g) were used for all experiments. Air-pouches were formed by subcutaneous injection of 1 ml of air for 3 d. On the third day after initial injection of air, 10 mg carrageenan in 1 ml of sterile NaCl solution was injected into the air pouched formed. The carrageenan solution was sterilized and homogenised by storing in an oven at 90 °C for about 1 h. One ml was maintained at 37 °C and injected into the 3-d-old air pouch to induce inflammation [8].
Aspirin (100 mg/kg, oral) was administered as standard by an animal feeding needle suspended in simple syrup. For control, simple syrup was orally administered. Compounds **1b**, **2b** and **4b** (100 mg/kg, oral) were also given. Mice were killed by ether exposure and pouches washed thoroughly with 3 ml of phosphate buffer saline (PBS) containing 50 μg/ml heparin. Lavage fluids were centrifuged at 2000 rpm for 15 min at 4 °C and the

pellet was resuspended in 1 ml of PBS-heparin. The total number of leukocytes was measured using a Coulter Counter. The number of PMNLs recovered from each pouch was then calculated.
Treatment was given 1 h before injection of the carrageenan into the pouch. Pouches were washed and 4 h later, PMNLs infiltration was measured as described above. Mean leukocyte numbers per ml of exsudate for each compound were compared to control values obtained from a similar group of animals receiving vehicle alone. The degree of anti-inflammatory response, produced in the air-pouch cavity was assessed by measuring the total cell number of the exudate. All the compounds tested produced a dose-dependent reduction in carrageenan-induced leucocyte migration *in vivo*. The dose of 10 mg/ml carrageenan caused a time-dependent PMNLs infiltration into the pouch with a maximal rate of influx between 2 and 4 h. Maximum cell numbers were detected in the cavity 4 h after irritation injection. The results are summarized in the Table.
The degree of inflammatory response was also assessed by the hind-paw edema test in mice. No local irritation and gastrointestinal side effects were observed.

2.4. Gastrointestinal ulceration studies

Mice were fasted for 24 h (with water ad libitum). Compounds were suspended in a methyl cellulose vehicle and administered orally by gavage in a volume of 0.5 ml/100 g of body weight. The animals were sacrificed after 4 h, and the stomachs were examined for lesions under a dissecting microscope [9].

2.5. Statistical analysis

Data were statistically evaluated by analysis of variance and Man-Whitney U.p value of less than 0.05 was considered to be significant.

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Rahime Şimşek
Department of Pharmaceutical Chemistry
Faculty of Pharmacy
Hacettepe University
Ankara
Turkey

Medical Faculty, Department of Chemistry and Biochemistry, Stara Zagora, Bulgaria

Syntheses and antitumor activity of 4-{*N′*-[*N*-(2-chloroethyl)-*N*-nitrosocarbamoyl]hydrazono}-2,2,6,6-tetramethylpiperidine-1-oxyl

V. Gadjeva and Z. Raikov

Chloroethylnitrosoureas are an important class of alkylating antitumor agents with a broad range of activity in experimental systems. Some of them, CCNU, BCNU and MeCCNU have been applied for treatment of human cancer, mainly lymphomas, melanomas, gliomas and a few solid tumors [1]. The clinical application of nitrosoureas however is still limited because of their delayed and cumulative toxic effects [2]. In the search for more active and less toxic analogues, nitroxyl radicals have been utilized as carriers of cytotoxic groups. The replacement of the cyclohexyl moiety in CCNU with a nitroxyl radical led to the development of the spin labelled chloroethylnitrosourea 1-(2-chloroethyl)-3-(2,2,6,6-tetramethylpiperidine-1-oxyl)-1-nitrosourea (SLCNU) [3] and other spin labeled analogues of CCNU [4]. It has been established that the nitroxyl radical moiety (spin label) can exhibit a beneficially modifying effect on the toxicity and activity of nitrosourea derivatives [4]. On the other hand halogenoethylhydrazines, halogenoethylhydrazides and halogenoethylnitrososemicarbazides possess marked antitumor effects [5, 6]. In this paper we report syntheses, antitumor activity and toxicity of a new spin labelled chloroethylnitrosourea analogue of CCNU, 4-{*N′*-[*N*-(2-chloroethyl)-*N*-nitrosocarbamoyl]hydrazono}-2,2,6,6-tetramethylpiperidine-1-oxyl (**5**), containing a hydrazone structure. The spin labelled compound **5** was prepared by three different synthetic pathways as shown in the Scheme. The conventional path through condensation of the 4-hydrazono-(2,2,6,6-tetramethylpiperidine-1-oxy) (**1**) with 2-chloroethyl isocyanate and nitrosation of intermediate urea **2** with a mixture of dinitrogen tetraoxide and sodium acetate afforded **5** in only 10% yield. In order to increase the yield and to ascertain the position of the nitroso group in the nitrosourea derivative **5**, a regio-selective method was used to transfer the chloroethyl moiety containing the nitroso group to the hydrazone **1**. Compound **5** was also synthesized by different approaches using either *N′*-hydroxysuccinimide-*N*-(2-chloroethyl)-*N*-nitrosocarbamate (**3**) or 2-chloroethylnitrosocarbamoyl azide (**4**) as regio-selective transfer reagent (see Experimental). The chemical struc-

Scheme

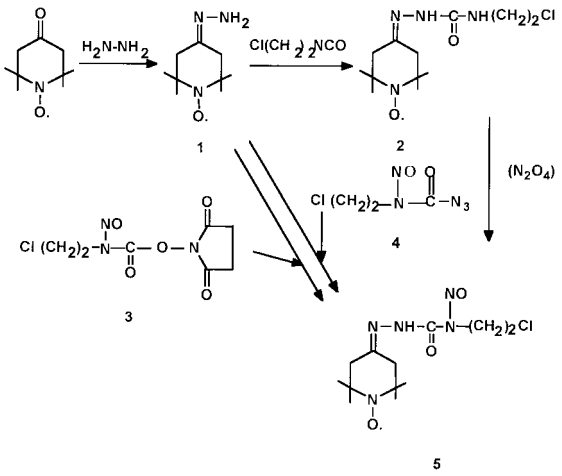

Table: In vivo activity of 5 against lymphoid leukemia L1210 and lymphocytic leukemia P388 in mice CD2F1 in comparison with CCNU and SLCNU

Compd.	L1210		P388		LD_{50} (mg/kg)
	OD (mg/kg)	ILS %	OD (mg/kg)	ILS %	
CCNU	25	646 [10]	16	182 [10]	56 [11]
SLCNU	60	713 [4]	35	542 [4]	123 [4]
5	60	831	25	735	72

OD – optical dose = daily i.p.-administered dose resulting in the maximum increase in life span. Drugs were administrated on day 1 after tumor implantation
ILS – increase in life span = [(T-C)/C] × 100, percentage of animals surviving on day 60 after tumor implantation
T – The mean survival time of the treated animals,
C – The mean survival time of the controls