CYCLIZATION OF 1-{[(4-METHYL-4*H*-1,2,4-TRIAZOL-3-YL)SULFANYL]-ACETYL}THIOSEMICARBAZIDES TO 1,2,4-TRIAZOLE AND 1,3,4-THIADIAZOLE DERIVATIVES AND THEIR PHARMACOLOGICAL PROPERTIES

Alicja MALISZEWSKA-GUZ[a], Monika WUJEC[a1], Monika PITUCHA[a2], Maria DOBOSZ[a,*], Anna CHODKOWSKA[b1], Ewa JAGIEŁŁO-WÓJTOWICZ[b2], Liliana MAZUR[c1] and Anna E. KOZIOŁ[c2]

[a] *Department of Organic Chemistry, Faculty of Pharmacy, Medical University, 6 Staszica Str., 20-081 Lublin, Poland; e-mail: [1] wujec@panaceum.am.lublin.pl, [2] pitucha@panaceum.am.lublin.pl*

[b] *Department of Toxicology, Medical University, 8 Chodzki Str., 20-093 Lublin, Poland; e-mail: [1] ewafarm@asklepios.am.lublin.pl, [2] paulina@asklepios.am.lublin.pl*

[c] *Department of Crystallography, Faculty of Chemistry, Maria Curie-Sklodowska University, 20-031 Lublin, Poland; e-mail: [1] lmazur2@op.pl, [2] akoziol@hermes.umcs.lublin.pl*

Received July 13, 2004
Accepted October 6, 2004

By the reaction of 4-methyl-4*H*-1,2,4-triazole-3-thiol with ethyl bromoacetate, ethyl [(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetate (**1**) was obtained. This compound was converted to [(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**2**). In the reaction of **2** with isothiocyanates, new thiosemicarbazides **3a**–**3g** were obtained. The cyclization of **3a**–**3g** in 2% aqueous solution of sodium hydroxide led to the formation of 4*H*-1,2,4-triazole-3(2*H*)-thione derivatives **4a**–**4g**, whereas the cyclization in acid media led to the formation of 2-amino-1,3,4-thiadiazole derivatives **5a**–**5g**. Molecular structure was confirmed by X-ray structure analysis of **3a**, **4g**, **5a** and **5g**. Compounds **4a**, **4b** and **4g** were investigated pharmacologically to determine their effect on the central nervous system (CNS) in mice.

Keywords: 1,2,4-Triazoles; 1,3,4-Thiadiazoles; Thiosemicarbazides; Cyclizations; X-ray diffraction; Crystal structure determination; Pharmacological screening; Antidepressives.

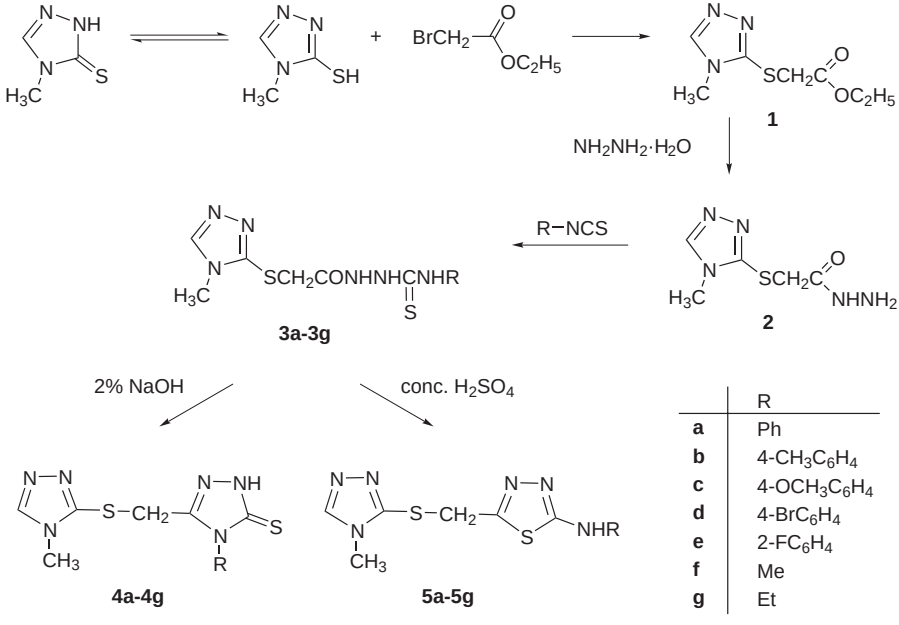
4-Methyl-4*H*-1,2,4-triazole-3-thiol can exist in two tautomeric forms shown in Scheme 1. Nucleophilic substitution reactions, depending on the conditions used, can led either to the *S*- or *N*-derivatives. In this paper the substitution reaction with bromoacetate ester was investigated. The *S*-derivative, ethyl [(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetate (**1**) was obtained. This compound was converted to thiosemicarbazide derivative **3** and, after cyclization in alkaline media, a group of new 5-{[(4-methyl-4*H*-1,2,4-triazol-

3-yl)sulfanyl]methyl}-4*H*-1,2,4-triazole-3(2*H*)-thiones **4** was obtained, while the cyclization in acidic media gave new 2-(alkyl(aryl)amino)-5-{[(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazoles **5**.

Depending of the nature of substituents, the 1,2,4-triazole and 1,3,4-thiadiazole derivatives can show various pharmacological activity. New derivatives of 1,2,4-triazole, which were presented in previous papers, show strictly defined pharmacological activity, e.g. potential action on the central nervous system[1] and anticholinergic[2], antiseptic[3] and analgesic[4] action. The 1,3,4-thiadiazole derivatives show also some pharmacological activity, e.g. anticonvulsion[5–7], antiviral and antibacterial[8,9], chemotherapeutic[10] as well as anthelmintic[11] action.

The 1,2,4-triazole and 1,3,4-thiadiazole derivatives could be prepared via the cyclization of acyl derivatives of thiosemicarbazide. It has been found that cyclization in alkaline media led to the 1,2,4-triazole system[12–34] while in acidic media the 1,3,4-thiadiazole system was obtained[16,17,26,27,29,30].

This paper is a continuation of the systematic investigations on the cyclization of new acyl derivatives of thiosemicarbazide. The reactions are presented in Scheme 1. Some of the obtained compounds (**4a**, **4b**, **4g**) were tested in vivo.



SCHEME 1

RESULTS AND DISCUSSION

Chemistry

4-Methyl-4*H*-1,2,4-triazole-3-thiol is a starting material for the synthesis of new derivatives of 1,2,4-triazole and 1,3,4-thiadiazole. In the reaction with ethyl bromoacetate, ethyl [(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetate (**1**) was obtained. This compound was treated with 100% hydrazine hydrate to give [(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**2**). New thiosemicarbazide derivatives **3** were obtained by reaction of **2** with isothiocyanates. Their structure was confirmed by X-ray analysis of **3a** (Fig. 1a). The reaction was carried out in anhydrous methanol at room temperature. Thiosemicarbazides **3** were cyclized in 2% aqueous solution of NaOH to give derivatives of 1,2,4-triazole-3(2*H*)-thione **4a**–**4g**, whereas the cyclization in acidic media led to the formation of 2-(alkyl(aryl)amino)-1,3,4-thiadiazole derivatives **5a**–**5g**.

The obtained products were characterized by their elemental analysis, IR and ^{1}H NMR spectra. The structures of selected compounds (**3a**, **4g**, **5a**, **5g**) were confirmed by X-ray crystallography.

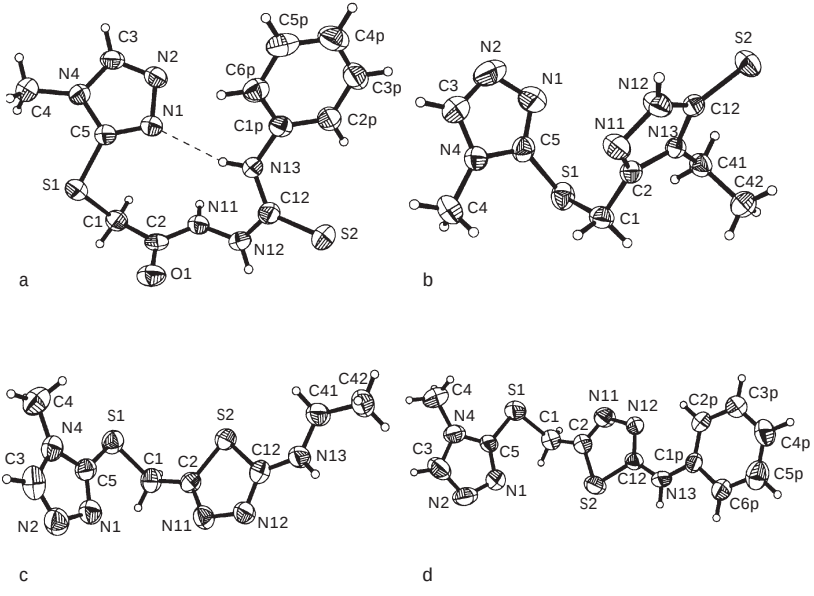


FIG. 1
Molecular structure and atomic numbering scheme for: a **3a**, b **4g**, c **5g**, d **5a**

In the IR spectra of thiosemicarbazide derivatives **3**, the following characteristic absorption bands were observed: 1670–1681 cm^{-1} corresponding to C=O group and 3120–3137 cm^{-1} corresponding to NH group. In the compounds **4** containing the 1,2,4-triazole system, the absorption bands of the C–N group appeared at about 1520 cm^{-1} and those for the C=N group at 1610 cm^{-1}. For 1,3,4-thiadiazole **5**, the absorption bands of the NH group were observed at 3220–3246 cm^{-1} and of the C–S–C group of thiadiazole at 707–719 cm^{-1}.

^{1}H NMR spectra of the thiosemicarbazide derivatives **3** show three-proton signals typical of the NH group in the δ 8.40–10.46 ppm range. In ^{1}H NMR spectra of 1,2,4-triazole derivatives **4**, proton signals of the NH group were observed in the δ 13.58–13.96 ppm ranges. The above observations suggest that the compounds exist mainly in the thione form, both in the solid state and in solution. X-ray analysis confirmed that compound **4g** exists in the thione form (Fig. 1b). The ^{1}H NMR spectra of 1,3,4-thiadiazole derivatives **5** show the proton signals typical of the NH group in the δ range 7.62–10.30 ppm. X-ray analysis of compounds **5a** and **5g** confirmed that the synthesized compounds contain the 1,3,4-thiadiazole system. The numbering scheme and general view of the molecules are shown in Figs 1c and 1d.

Pharmacology

Preliminary pharmacological studies indicated that compounds **4a**, **4b** and **4g** exhibit a weak antidepressive activity in mice. All the derivatives of 1,2,4-triazole-3(2*H*)-thione given in a dose 100 mg/kg i.p. significantly prolonged the sleep time induced by thiopental (Table I), as well as analgesic activity (Table II). Only one compound, **4a**, given in a dose 50 mg/kg significantly decreased the pain reactivity of mice (ca. 38% with respect to control group). In the remaining tests, the new compounds did not produce any significant activity on the CNS of mice.

EXPERIMENTAL

Chemistry

Melting points were determined in a Fisher–Johns block and are not corrected. IR spectra (ν, cm^{-1}) were recorded in KBr using a Specord IR-75 spectrophotometer. ^{1}H NMR spectra were recorded on a Tesla BS-567 A spectrometer (100 MHz) in DMSO-d_6 with TMS as an internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Chemicals were purchased from Merck Co. or Lancaster and used without further purification.

TABLE I
The influence of investigated compounds on the thiopental-induced sleep (N = 10)

Compound	Dose mg/kg. i.p.	Sleeping time	
		min ± SEM	%
Control	–	19.4 ± 5.5	100.0 ± 28.4
4a	50	10.4 ± 4.9	53.6 ± 25.3
	100	44.7 ± 17.9[a]	230.4 ± 92.3[a]
4b	50	13.0 ± 1.1	67.0 ± 5.7
	100	71.4 ± 14.2[a]	368 ± 73.2[a]
4g	50	30.4 ± 14.6	156.7 ± 74.4
	100	55.9 ± 15.8[a]	288.1 ± 81.4[a]

[a] $p < 0.001$ vs the control group.

TABLE II
Analgesic activity of compounds of "writhing syndrome" test in mice (N = 10)

Compound	Dose mg/kg i.p.	Mean writhing number	Inhibition[a] %
Control	–	15.6 ± 2.5	0
4a	25	16.0 ± 1.7	0.7
	50	10.3 ± 2.1[b]	38[b]
	100	8.3 ± 1.9[b]	47[b]
4b	50	13.7 ± 2.7	12
	100	4.9 ± 1.5[b]	68[b]
4g	50	13.8 ± 2.1	12.5
	100	8.4 ± 1.2[b]	46[b]

[a] % of inhibition obtained by comparison with control groups. [b] $p < 0.001$ vs the control group.

Ethyl [(4-Methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetate (**1**)

A mixture of 4-methyl-4*H*-1,2,4-triazole-3-thiol (1.15 g, 10 mmol), anhydrous potassium carbonate (2 g) and ethyl bromoacetate (1.84 g, 11 mmol) in anhydrous acetone (20 ml) was refluxed for 15 h. After cooling, the solid was filtered off, and the solvent was evaporated under reduced pressure. The residue was extracted with diethyl ether and the residue after evaporation was purified by crystallization from ethanol. Yield 1.7 g (85%), m.p. 48–49 °C. For $C_7H_{11}N_3O_2S$ (201.2) calculated: 41.78% C, 5.51% H, 20.88% N; found: 41.67% C, 5.46% H, 20.78% N. IR (KBr): 3060 (CH_{ar}); 2972, 1445 (CH_{al}); 1710 (C=O); 1550 (C=N). ^{1}H NMR (DMSO-d_6): 1.16 t, 3 H, J = 7 (CH_3); 3.61 s, 3 H (CH_3); 3.98 s, 2 H (CH_2); 4.11 q, 2 H, J = 7 (CH_2); 8.55 s, 1 H (CH).

[(4-Methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetohydrazide (**2**)

Hydrazine hydrate 100% (1 ml) was added to a solution of 1 (2.01 g, 10 mmol) in anhydrous ethanol (5 ml) and the mixture was kept at room temperature for 24 h. Precipitated hydrazide was then filtered off and dried. Yield 1.6 g (85%), m.p. 169–170 °C. For $C_5H_9N_5OS$ (187.2) calculated: 32.07% C, 4.84% H, 37.41% N; found: 31.92% C, 4.64% H, 37.62% N. IR (KBr): 3300 (NH); 3064 (CH_{ar}); 2970, 1445 (CH_{al}); 1710 (C=O); 1556 (C=N). ^{1}H NMR (DMSO-d_6): 3.64 s, 3 H (CH_3); 3.80 s, 2 H (CH_2); 4.34 s, 2 H (NH_2); 8.60 s, 1 H (CH); 9.33 s, 1 H (NH).

1-{[(4-Methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetyl}thiosemicarbazides **3a**–**3g**. General Procedure

A mixture of hydrazide **2** (1.87 g, 10 mmol) and the corresponding isothiocyanate (11 mmol) in anhydrous methanol (25 ml) was kept at room temperature for 4 days. The formed product was then filtered off, washed with diethyl ether and crystallized from methanol.

1-{[(4-Methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}-4-phenylthiosemicarbazide (**3a**). Yield 2.96 g (92%), m.p. 166–167 °C. For $C_{12}H_{14}N_6OS_2$ (322.4) calculated: 44.70% C, 4.37% H, 26.07% N; found: 44.50% C, 4.35% H, 26.00% N. IR (KBr): 3130 (NH); 3050 (CH_{ar}); 2930, 1450 (CH_{al}); 1680 (C=O); 1530, 1360 (C=S). ^{1}H NMR (DMSO-d_6): 3.67 s, 3 H (CH_3); 3.90 s, 2 H (CH_2); 7.19–7.58 m, 5 H (5 × CH_{ar}); 8.63 s, 1 H (CH_{ar}); 9.78, 9.87, 10.39 3 s, 3 H (3 × NH).

1-{[(4-Methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}-4-(p-tolyl)thiosemicarbazide (**3b**). Yield 3.16 g (94%), m.p. 162–163 °C. For $C_{13}H_{16}N_6OS_2$ (336.4) calculated: 46.41% C, 4.79% H, 24.98% N; found: 46.34% C, 4.68% H, 24.79% N. IR (KBr): 3120 (NH); 3048 (CH_{ar}); 2934, 1452 (CH_{al}); 1679 (C=O); 1528, 1364 (C=S). ^{1}H NMR (DMSO-d_6): 2.34 s, 3 H (CH_3); 3.66 s, 3 H (CH_3); 3.90 s, 2 H (CH_2); 7.16–7.43 m, 4 H (4 × $CH_{ar.}$); 8.62 s, 1 H (CH_{ar}); 9.71, 9.79, 10.37 3 s, 3 H (3 × NH).

4-(4-Methoxyphenyl)-1-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}thiosemicarbazide (**3c**). Yield 3.27 g (93%), m.p. 163–164 °C. For $C_{13}H_{16}N_6O_2S_2$ (352.4) calculated: 44.31% C, 4.58% H, 23.85% N; found: 44.11% C, 4.47% H, 23.65% N. IR (KBr): 3133 (NH); 3045 (CH_{ar}); 2931, 1452 (CH_{al}); 1678 (C=O); 1536, 1361 (C=S). ^{1}H NMR (DMSO-d_6): 3.66 s, 3 H (CH_3); 3.80 s, 3 H (CH_3); 3.90 s, 2 H (CH_2); 6.92–6.95 d, 2 H, J = 9 (2 × CH_{ar}); 7.38–7.40 d, 2 H, J = 9 (2 × CH_{ar}); 8.62 s, 1 H (CH_{ar}); 9.67, 9.76, 10.35 3 s, 3 H (3 × NH). ^{13}C NMR: 30.90 (CH_3); 35.20 (CH_2); 55.30 (CH_3); 113.20, 114.60, 127.30, 129.60 (4 × CH_{ar}); 131.90, 156.80 (2 × C_{ar}); 147.00 (CH); 149.10 (C-S); 167.20 (C=S); 181.10 (C=O).

4-(4-Bromophenyl)-1-{[(4-Methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}thiosemicarbazide (**3d**). Yield 3.69 g (92%), m.p. 176–177 °C. For $C_{12}H_{13}BrN_6OS_2$ (401.3) calculated: 35.91% C, 3.26% H, 20.94% N; found: 35.76% C, 3.31% H, 20.97% N. IR (KBr): 3121 (NH); 3041 (CH_{ar}); 2927, 1449 (CH_{al}); 1680 (C=O); 1547, 1355 (C=S). ^{1}H NMR (DMSO-d_6): 3.61 s, 3 H (CH_3); 3.83 s, 2 H (CH_2); 7.50–7.52 m, 4 H (4 × CH_{ar}); 8.58 s, 1 H (CH_{ar}); 9.86, 9.92, 10.38 3 s, 3 H (3 × NH).

4-(2-Fluorophenyl)-1-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}thiosemicarbazide (**3e**). Yield 3.20 g (94%), m.p. 182–183 °C. For $C_{12}H_{13}FN_6OS_2$ (340.4) calculated: 42.34% C, 3.85% H, 24.69% N; found: 42.46% C, 3.72% H, 24.52% N. IR (KBr): 3133 (NH); 3061 (CH_{ar}); 2934, 1447 (CH_{al}); 1679 (C=O); 1540, 1362 (C=S); 1 247 (C–O–C). ^{1}H NMR (DMSO-d_6): 3.59 s, 3 H (CH_3); 3.88 s, 2 H (CH_2); 7.13–7.30 m, 4 H (4 × CH_{ar}); 8.51 s, 1 H (CH_{ar}); 9.72, 9.89, 10.46 3 s, 3 H (3 × NH). ^{13}C NMR: 30.80 (CH_3); 35.00 (CH_2); 115.60, 115.90, 124.00, 127.10 (4 × CH_{ar}); 130.70, 160.80 (2 × C_{ar}); 146.20 (CH); 149.10 (C-S); 167.10 (C=S); 182.30 (C=O).

4-Methyl-1-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}thiosemicarbazide (**3f**). Yield 2.49 g (96%), m.p. 160–161 °C. For $C_7H_{12}N_6OS_2$ (260.3) calculated: 32.30% C, 4.65% H, 32.28% N; found: 32.15% C, 4.48% H, 32.34% N. IR (KBr): 3127 (NH); 3070 (CH_{ar}); 2931, 1445 (CH_{al}); 1681 (C=O); 1539, 1366 (C=S). ^{1}H NMR (DMSO-d_6): 2.93 s, 3 H (CH_3); 3.59 s, 3 H (CH_3); 3.83 s, 2 H (CH_2); 8.59 s, 1 H (CH_{ar}); 8.40, 9.37, 10.21 3 s, 3 H (3 × NH). ^{13}C NMR: 30.90 (CH_3); 31.00 (CH_3); 34.20 (CH_2); 146.30 (CH); 149.40 (C-S); 167.00 (C=S); 182.20 (C=O).

4-Ethyl-1-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}thiosemicarbazide (**3g**). Yield 2.60 g (95%), m.p. 168–169 °C. For $C_8H_{14}N_6OS_2$ (274.4) calculated: 35.02% C, 5.14% H, 30.63% N; found: 35.17% C, 5.31% H, 30.74% N. IR (KBr): 3137 (NH); 3055 (CH_{ar}); 2937, 1452 (CH_{al}); 1670 (C=O); 1533, 1359 (C=S). ^{1}H NMR (DMSO-d_6): 1.13 t, 3 H, J = 7 (CH_3); 3.58 q, 2 H, J = 7 (CH_2); 3.65 s, 3 H (CH_3); 3.86 s, 2 H (CH_2); 8.62 s, 1 H (CH_{ar}); 8.43, 9.33, 10.23 3 s, 3 H (3 × NH).

5-{[(4-Methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-4*H*-1,2,4-triazole-3(2*H*)-thione Derivatives **4a**–**4g**. General Procedure

A mixture of thiosemicarbazide **3a**–**3g** (10 mmol) and 2% aqueous solution of sodium hydroxide (15 ml) was refluxed for 2 h (**3a**–**3e**) or kept at room temperature for 5 days (**3f**, **3g**). Then, the solution was neutralized with acetic acid and the formed precipitate was filtered off and crystallized from methanol.

5-{[(4-Methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4-phenyl-4H-1,2,4-triazole-3(2H)-thione (**4a**). Yield 2.62 g (86%), m.p. 193–194 °C. For $C_{12}H_{12}N_6S_2$ (304.4) calculated: 47.34% C, 3.97% H, 27.61% N; found: 47.26% C, 3.76% H, 27.47% N. IR (KBr): 3101 (CH_{ar}); 2922, 1462 (CH_{al}); 1610 (C=N); 1520 (C–N). ^{1}H NMR (DMSO-d_6): 3.54 s, 3 H (CH_3); 4.21 s, 2 H (CH_2); 7.50–7.64 m, 5 H (5 × CH_{ar}); 8.60 s, 1 H (CH_{ar}); 13.89 s, 1 H (NH).

5-{[(4-Methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4-(p-tolyl)-4H-1,2,4-triazole-3(2H)-thione (**4b**). Yield 2.80 g (88%), m.p. 213–214 °C. For $C_{13}H_{14}N_6S_2$ (318.4) calculated: 49.03% C, 4.43% H, 26.39% N; found: 49.22% C, 4.64% H, 26.52% N. IR (KBr): 3098 (CH_{ar}); 2919, 1455 (CH_{al}); 1608 (C=N); 1524 (C–N). ^{1}H NMR (DMSO-d_6): 2.40 s, 3 H (CH_3); 3.49 s, 3 H (CH_3); 4.15 s, 2 H (CH_2); 7.30–7.38 m, 4 H (4 × CH_{ar}); 8.52 s, 1 H (CH_{ar}); 13.81 s, 1 H (NH).

4-(4-Methoxyphenyl)-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (**4c**). Yield 2.97 g (89%), m.p. 205–206 °C. For $C_{13}H_{14}N_6OS_2$ (334.4) calculated: 46.69% C, 4.22% H, 25.13% N; found: 46.48% C, 4.13% H, 25.34% N. IR (KBr): 3099 (CH_{ar});

2931, 1459 (CH_{al}); 1608 (C=N); 1526 (C–N); 1280 (C–O–C). ^{1}H NMR (DMSO-d_6): 3.50 s, 3 H (CH_3); 3.84 s, 3 H (CH_3); 4.14 s, 2 H (CH_2); 7.08–7.37 m, 4 H (4 × CH_{ar}); 8.56 s, 1 H (CH_{ar}); 13.78 s, 1 H (NH). ^{13}C NMR: 27.70 (CH_2); 30.90 (CH_3); 55.50 (CH_3); 114.60, 129.60 (4 × CH_{ar}); 125.70 (C-N); 146.60, 159.80 (2 × C_{ar}); 146.90 (CH); 148.60 (C-S); 168.50 (C=S).

4-(4-Bromophenyl)-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (**4d**). Yield 3.25 g (85%), m.p. 223–224 °C. For $C_{12}H_{11}BrN_6S_2$ (383.2) calculated: 37.61% C, 2.89% H, 21.93% N; found: 37.50% C, 2.78% H, 21.73% N. IR (KBr): 3091 (CH_{ar}); 2925, 1459 (CH_{al}); 1 620 (C=N); 1 530 (C–N). ^{1}H NMR (DMSO-d_6): 3.51 s, 3 H (CH_3); 4.19 s, 2 H (CH_2); 7.45 d, 2 H, J = 9 (2 × CH_{ar}); 7.78 d, 2 H, J = 9 (2 × CH_{ar}); 8.56 s, 1 H (CH_{ar}); 13.86 s, 1 H (NH).

4-(2-Fluorophenyl)-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (**4e**). Yield 2.64 g (82%), m.p. 199–200 °C. For $C_{12}H_{11}FN_6S_2$ (322.4) calculated: 44.71% C, 3.44% H, 26.07% N; found: 44.58% C, 3.19% H, 26.23% N. IR (KBr): 3075 (CH_{ar}); 2933, 1457 (CH_{al}); 1619 (C=N); 1522 (C–N). ^{1}H NMR (DMSO-d_6): 3.49 s, 3 H (CH_3); 4.18 d, 2 H, J = 5 (CH_2); 7.38–7.65 m, 4 H (4 × CH_{ar}); 8.55 s, 1 H (CH_{ar}); 13.96 s, 1 H (NH). ^{13}C NMR: 27.40 (CH_2); 30.80 (CH_3); 116.70, 120.70, 130.70, 132.60 (4 × CH_{ar}); 125.40 (C-N); 146.60, 158.80 (2 × C_{ar}); 146.80 (CH); 148.40 (C-S); 168.70 (C=S).

4-Methyl-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (**4f**). Yield 2.18 g (90%), m.p. 174–175 °C. For $C_7H_{10}N_6S_2$ (242.3) calculated: 34.69% C, 4.16% H, 34.68% N; found: 34.61% C, 4.21% H, 34.49% N. IR (KBr): 3078 (CH_{ar}); 2930, 1466 (CH_{al}); 1603 (C=N); 1519 (C–N). ^{1}H NMR (DMSO-d_6): 3.57 s, 3 H (CH_3); 3.77 s, 3 H (CH_3); 4.37 s, 2 H (CH_2); 8.59 s, 1 H (CH_{ar}); 13.58 s, 1 H (NH). ^{13}C NMR: 28.50 (CH_2); 31.00 (CH_3); 31.80 (CH_3); 125.50 (C-N); 147.70 (CH); 149.50 (C-S); 168.20 (C=S).

4-Ethyl-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-4H-1,2,4-triazole-3(2H)-thione (**4g**). Yield 2.38 g (93%), m.p. 175–176 °C. For $C_8H_{12}N_6S_2$ (256.3) calculated: 37.48% C, 4.72% H, 32.78% N; found: 37.37% C, 4.63% H, 32.53% N. IR (KBr): 3098 (CH_{ar}); 2 927, 1462 (CH_{al}); 1616 (C=N); 1523 (C–N). ^{1}H NMR (DMSO-d_6): 1.28 t, 3 H, J = 7 (CH_3); 3.57 s, 3 H (CH_3); 4.04 q, 2 H, J = 7 (CH_2); 4.41 s, 2 H (CH_2); 8.60 s, 1 H (CH_{ar}); 13.59 s, 1 H (NH).

2-(Alkyl(aryl)amino)-5{[(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole Derivatives **5a**–**5g**. General Procedure

Starting compound **3** (10 mmol) was dissolved in concentrated sulfuric acid (5 ml) and the solution was kept at room temperature for 10 days. The corresponding product **5** was obtained after neutralization of the solution with dilute ammonium hydroxide. The isolated product was filtered off, dried and crystallized from methanol.

2-Anilino-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (**5a**). Yield 2.46 g (81%), m.p. 189–190 °C. For $C_{12}H_{12}N_6S_2$ (304.4) calculated: 47.34% C, 3.97% H, 27.61% N; found: 47.16% C, 3.68% H, 27.48% N. IR (KBr): 3220 (NH); 3051 (CH_{ar}); 2938, 1449 (CH_{al}); 710 (C–S–C). ^{1}H NMR (DMSO-d_6): 3.55 s, 3 H (CH_3); 4.64 s, 2 H (CH_2); 6.96–7.59 m, 5 H (5 × CH_{ar}); 8.61 s, 1 H (CH_{ar}); 10.30 s, 1 H (NH).

5-{[(4-Methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-2-(p-tolylamino)-1,3,4-thiadiazole (**5b**). Yield 2.64 g (83%), m.p. 191–192 °C. For $C_{13}H_{14}N_6S_2$ (318.4) calculated: 49.03% C, 4.43% H, 26.39% N; found: 48.90% C, 4.18% H, 26.18% N. IR (KBr): 3240 (NH); 3056 (CH_{ar}); 2938, 1455 (CH_{al}); 710 (C–S–C). ^{1}H NMR (DMSO-d_6): 2.35 s, 3 H (CH_3); 3.56 s, 3 H (CH_3); 4.64 s, 2 H (CH_2); 6.92–7.43 m, 4 H (4 × CH_{ar}); 8.61 s, 1 H (CH_{ar}); 10.28 s, 1 H (NH).

2-(4-Methoxyanilino)-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (**5c**). Yield 2.81 g (84%), m.p. 167–168 °C. For $C_{13}H_{14}N_6OS_2$ (334.4) calculated: 46.69% C, 4.22% H, 25.13% N; found: 46.50% C, 4.12% H, 25.16% N. IR (KBr): 3242 (NH); 3043 (CH_{ar}); 2931, 1449 (CH_{al}); 1260 (C–O–C); 709 (C–S–C). ^{1}H NMR (DMSO-d_6): 3.56 s, 3 H (CH_3); 3.73 s, 3 H (CH_3); 4.60 s, 2 H (CH_2); 6.93–7.48 m, 4 H (4 × CH_{ar}); 8.60 s, 1 H (CH_{ar}); 10.09 s, 1 H (NH). ^{13}C NMR: 30.90 (CH_3); 31.50 (CH_2); 55.20 (CH_3); 114.30, 119.20 (4 × CH_{ar}); 146.60 (CH); 147.90, 153.70, 165.90 (3 × C-S); 154.60, 155.20 (2 × C_{ar}).

2-(4-Bromoanilino)-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (**5d**). Yield 3.06 g (80%), m.p. 197–198 °C. For $C_{12}H_{11}BrN_6S_2$ (383.2) calculated: 37.61% C, 2.89% H, 21.93% N; found: 37.48% C, 2.65% H, 21.69% N. IR (KBr): 3241 (NH); 3048 (CH_{ar}); 2931, 1449 (CH_{al}); 716 (C–S–C). ^{1}H NMR (DMSO-d_6): 3.55 s, 3 H (CH_3); 4.65 s, 2 H (CH_2); 7.48–7.59 m, 4 H (4 × CH_{ar}); 8.61 s, 1 H (CH_{ar}); 9.83 s, 1 H (NH).

2-(2-Fluoroanilino)-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (**5e**). Yield 2.77 g (86%), m.p. 158–159 °C. For $C_{12}H_{11}FN_6S_2$ (322.4) calculated: 44.71% C, 3.44% H, 26.07% N; found: 44.69% C, 3.50% H, 26.02% N. IR (KBr): 3246 (NH); 3055 (CH_{ar}); 2934, 1446 (CH_{al}); 719 (C–S–C). ^{1}H NMR (DMSO-d_6): 3.56 s, 3 H (CH_3); 4.64 s, 2 H (CH_2); 7.03–8.35 m, 4 H (4 × CH_{ar}); 8.61 s, 1 H (CH_{ar}); 10.13 s, 1 H (NH). ^{13}C NMR: 30.90 (CH_3); 31.40 (CH_2); 115.30, 120.50, 123.00, 124.70 (4 × CH_{ar}); 146.70 (CH); 147.80, 153.50, 165.40 (3 × C-S); 150.20, 157.30 (2 × C_{ar}).

2-(Methylamino)-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (**5f**). Yield 1.94 g (80%), m.p. 135–136 °C. For $C_7H_{10}N_6S_2$ (242.3) calculated: 34.69% C, 4.16% H, 34.68% N; found: 34.47% C, 4.34% H, 34.75% N. IR (KBr): 3244 (NH); 3050 (CH_{ar}); 2931, 1446 (CH_{al}); 707 (C–S–C). ^{1}H NMR (DMSO-d_6): 2.83 d, 3 H, J = 5 (CH_3); 3.54 s, 3 H (CH_3); 4.54 s, 2 H (CH_2); 7.62 d, 1 H, J = 5 (NH); 8.59 s, 1 H (CH_{ar}). ^{13}C NMR: 30.80 (CH_3); 31.10 (CH_3); 31.70 (CH_2); 146.60 (CH); 147.80, 153.80, 170.40 (3 × C-S).

2-(Ethylamino)-5-{[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]methyl}-1,3,4-thiadiazole (**5g**). Yield 2.18 g (85%), m.p. 160–161 °C. For $C_8H_{12}N_6S_2$ (256.3) calculated: 37.48% C, 4.72% H, 32.78% N; found: 37.19% C, 4.57% H, 32.59% N. IR (KBr): 3237 (NH); 2927, 1450 (CH_{al}); 713 (C–S–C). ^{1}H NMR (DMSO-d_6): 1.14 t, 3 H, J = 7 (CH_3); 3.19–3.28 m, 2 H (CH_2); 3.53 s, 3 H (CH_3); 4.53 s, 2 H (CH_2); 7.66 t, 1 H, J = 5 (NH); 8.59 s, 1 H (CH_{ar}).

Crystallography

Single-crystal X-ray diffraction data for compounds **3a**, **4g**, **5a** and **5g** were measured at room temperature in the ω/2θ scan mode on a KM4 four-circle diffractometer using CuKα radiation. Details of the crystal data, X-ray data collection and refinement are given in Table III. Crystal structures were solved by direct methods using the SHELXS97 [35] program and refined by the full-matrix least-squares method on F^2 using the SHELXL97 [36] program. The non-hydrogen atoms were refined with anisotropic displacement parameters. H-atom positions were located from the geometry and isotropic factors $1.2U_{eq}$ of the bonded C-atoms were given; for the C–H bond, “riding model” was used in the refinement.

CCDC 229220, 229221, 229222 and 229223 (for compounds **3a**, **4g**, **5a** and **5g**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

Pharmacology

The experiments were carried out on male Albino Swiss mice (20–25 g). The animals were housed in colony cages with free access to tap water and food (standard laboratory pellets, Bacutil, Motycz, Poland) and maintained in the 12/12 h light-dark cycle (light on from 7 a.m. to 7 p.m.). Experimental and control groups consisting of 10 animals. The experiments were performed between 8 a.m. and 3 p.m. The investigated compounds **4a**, **4b** and **4g** were administered intraperitoneally (i.p.) in doses of 25, 50 and 100 mg/kg (equivalent to 0.025, 0.05 and 0.1 of their LD_{50}) as suspensions in a 1% Tween 80 in the constant volume 10 ml/kg. Control animals received the equivalent volume of solvent. Effects of new compounds on the CNS of mice were studied in eight tests:

1. Chimney test. The effect of compounds **4a**, **4b** and **4g** in a dose of 100 mg/kg i.p. on motoric impairment was quantified with the chimney test[37]. Briefly, mice had to climb up backwards in a plastic tube (3 cm inner diameter, 25 cm long). Mice unable to perform the task within 60 s were considered to display motor impairment. Motoric impairment was quantified as the percentage of animals that failed to complete the test.

2. Body temperature. The rectal body temperature in mice (measured with an Ellab thermometer) was recorded 15, 30, 45, 60, 90 and 120 min after the administration of investigated compounds in a dose 100 mg/kg i.p.

TABLE III
Crystallographic data and details of the structure refinement

Parameter	**3a**	**4g**	**5g**	**5a**
Crystal system	monoclinic	monoclinic	monoclinic	orthorhombic
Space group	$P2_1/c$	$P2_1/n$	$P2_1/c$	*Pbca*
a, Å	8.184(1)	8.699(1)	15.235(3)	13.988(3)
b, Å	20.954(4)	12.328(2)	7.267(1)	8.665(1)
c, Å	8.551(1)	11.013(2)	10.441(2)	22.360(4)
α, °	90	90	90	90
β, °	100.15(1)	97.41(1)	101.44(3)	90
γ, °	90	90	90	90
Volume, $Å^3$	1443.4(4)	1171.2(3)	1133.0(3)	2710.2(8)
θ range, °	4.22–80.23	5.41–76.15	2.96–76.16	5.06–76.17
No. of unique reflections	3135	2383	2222	2780
No. of parameters	192	146	147	182
Final R_1 indices	0.0576	0.0517	0.0572	0.0514
wR_2, $[I > 2\sigma(I)]$	0.0985	0.1340	0.1527	0.1031
$\Delta\rho_{max}$; $\Delta\rho_{min}$, e $Å^{-3}$	0.31; –0.42	0.44; –0.45	0.67; –0.49	0.31; –0.47

3. Four-plate test[38]. Anxiolytic activity was assessed 30 min after injection of the compound in a dose of 100 mg/kg i.p. The number of punished crossings was counted for 1 min.

4. Forced swimming test[39]. The investigated compounds in a dose of 100 mg/kg were administered 30 min before the test. Mice were individually placed and forced to swim in a glass cylinder (27 × 16 cm) containing 15 cm of water (25 °C). A mouse was considered immobile when it floated in the water, in upright position, and showed only small movements to keep its head above water. The total immobility time of mice was measured during the last 4 min of the 6-min test.

5. Thiopental-induced sleep. Thiopental (60 mg/kg i.p.) was given 30 min after administration of the tested derivatives in doses of 50 and 100 mg/kg i.p. The sleeping time (from loss to recovery of righting reflex) was measured.

6. "Writhing syndrome" test[40]. Compounds were administered in doses of 25, 50 or 100 mg/kg i.p., and after 30 min the animals were injected 0.6% acetic acid i.p. The number of writhing episodes was counted for 30 min.

7. Pentetrazole-induced convulsions. Pentetrazole (100 mg/kg sc) was given 30 min after administration of compounds **4a**, **4b** and **4g** in a dose of 100 mg/kg i.p. The observation (during 30 min) of individual animals, to record occurrence of tonic convulsions as well as mortality of mice, started immediately after injection of pentetrazole.

8. Head twitches. The investigated compounds were given in a dose of 100 mg/kg i.p. 30 min before L-5-hydroxytryptophan (L-5-HTP, 180 mg/kg i.p.). The number of head twitch episodes of mice was counted during 60 min after the injection of L-5-HTP.

The obtained results were evaluated statistically with the Student's *t*-test.

REFERENCES

1. Dobosz M.: *Acta Pol. Pharm.* **1984**, *41*, 43.
2. Dobosz M.: *Acta Pol. Pharm.* **1987**, *44*, 420.
3. Dobosz M., Rekas J., Pachuta A.: *Acta Pol. Pharm.* **1989**, *46*, 40.
4. Dobosz M., Pachuta A., Rekas J.: *Acta Pol. Pharm.* **1993**, *50*, 225.
5. Bashir Y., Kann M., Stradling J. R.: *Pulm. Pharmacol.* **1990**, *3*, 151.
6. Feldman M., Goldschmiedt M.: *Am. J. Physiol.* **1991**, *261*, 320.
7. Fremont P., Riverin H., Frenette J., Rogers P. A., Cote C.: *Am. J. Physiol.* **1991**, *260*, 615.
8. Hiremath S. P., Biradar J. S., Kudari S. M.: *J. Indian Chem. Soc.* **1984**, *61*, 74.
9. Schuster G., Just H., Schwarz J.: *Z. Pflanzenkrankh. Pflanzenschutz* **1984**, *91*, 569.
10. Steahly G. W.: U.S. 2,497,825, 1950; *Chem. Abstr.* **1950**, *44*, 5919.
11. Asato G., Berkelhammer G., Gastrock W. H.: U.S. 3,940,411, 1976; *Chem. Abstr.* **1976**, *85*, 5644.
12. Ainsworth C., Jones R. G.: *J. Am. Chem. Soc.* **1954**, *76*, 5651.
13. Jones R. G., Ainsworth C.: *J. Am. Chem. Soc.* **1955**, *77*, 1538.
14. Hoggarth E.: *J. Chem. Soc.* **1949**, 1163.
15. Fry D. J., Lambie A. J.: Brit. 741,228, 1955; *Chem. Abstr.* **1956**, *50*, 9913.
16. Godfrey L. E. A., Kurzer F.: *J. Chem. Soc.* **1961**, 5137.
17. Kurzer F., Canelle J.: *Tetrahedron* **1963**, *19*, 1603.
18. Dobosz M., Sikorska M.: *Acta Pol. Pharm.* **1994**, *51*, 369.
19. Dobosz M., Sikorska M.: *Acta Pol. Pharm.* **1994**, *51*, 377.
20. Purna Ch.: *Weed Res.* **1964**, *4*, 54.

21. Kumar A., Asthana B. P.: *J. Indian Chem. Soc.* **1983**, *60*, 682.
22. Cesur Z., Ergenc N., Iihan E.: *Acta Pharm. Turc.* **1989**, *31*, 103.
23. Eberle M. K., Manning R. E.: U.S. 3,992,396, 1976; *Chem. Abstr.* **1977**, *86*, 89833.
24. Eberle M. K., Manning R. E.: U.S. 3,919,428, 1975; *Chem. Abstr.* **1976**, *84*, 59487.
25. Dobosz M., Rękas-Szylar J.: *Acta Pol. Pharm.* **1994**, *51*, 155.
26. Dobosz M., Pachuta-Stec A.: *Acta Pol. Pharm.* **1994**, *51*, 457.
27. Dobosz M., Pachuta-Stec A.: *Acta Pol. Pharm.* **1995**, *52*, 103.
28. Dobosz M., Maliszewska-Guz A.: *Ann. Univ. Maria Curie-Sklodowska, Lublin, Chem.* **1991/1992**, *46/47*, 50.
29. Dobosz M., Pitucha M., Wujec M.: *Acta Pol. Pharm.* **1996**, *53*, 31.
30. Dobosz M., Pachuta-Stec A.: *Acta Pol. Pharm.* **1996**, *53*, 123.
31. Dobosz M., Wujec M., Pitucha M.: *Ann. Univ. Maria Curie-Sklodowska, Lublin, Chem.* **1995/1996**, *50/51*, 67.
32. Dobosz M., Wujec M.: *Ann. Univ. Maria Curie-Sklodowska, Lublin, Chem.* **1997/1998**, *52/53*, 99.
33. Dobosz M., Struga M., Chodkowska A., Jagiełło-Wójtowicz E., Stępniak K., Kozioł A. E.: *Acta Pol. Pharm.* **2002**, *59*, 281.
34. Dobosz M., Pitucha M., Dybala I., Kozioł A. E.: *Collect. Czech. Commun.* **2003**, *68*, 792.
35. Sheldrick G. M.: *SHELXS97. Program for Crystal Structure Solution*. University of Göttingen, Göttingen 1997.
36. Sheldrick G. M.: *SHELXL97. Program for the Refinement of Crystal Structures*. University of Göttingen, Göttingen 1997.
37. Boissier J. R., Tardy J., Diverres J. C.: *Med. Exp. (Basel)* **1960**, *3*, 81.
38. Aron C., Simon P., Larousse C., Boissier J. R.: *Neuropharmacology* **1971**, *10*, 459.
39. Porsolt R. D., Bertin A., Jalfre M.: *Arch. Int. Pharmacodyn. Ther.* **1977**, *229*, 327.
40. Witkin L., Heubner C., Galdi F., O'Keefe E., Spitaletta P., Plummer A.: *J. Pharmacol. Exp. Ther.* **1961**, *133*, 400.