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Synthesis of 3-(3,4-dimethoxyphenyl)-1*H*-1,2,4-triazole-5-thiol and 2-amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole derivatives exhibiting anti-inflammatory activity

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New 1-acylderivatives of 5-alkylthio-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazole (**5c–f**, **6d–f**) were synthesized by the acylation of 5-alkylthio-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazoles (**3**, **4**) with acyl chlorides. The compounds **3**, **4** were obtained by the alkylation of 3-(3,4-dimethoxyphenyl)-1*H*-1,2,4-triazole-5-thiol (**2**) sodium salt with alkyl iodides. Compound **2** and 2-amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole (**8**) were prepared by the treatment of 3,4-dimethoxybenzoylthiosemicarbazide (**1**) with sodium hydroxide or acetyl chloride (and then sodium hydroxide), respectively. Related 2-acylamino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazoles (**7c**, **e**, **f**) were synthesized by the acylation of compound **8** with acyl chlorides. 3-(3,4-Dimethoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thione (**9**) was *N*-acylated with acyl chlorides or *S*-methylated with iodomethane to give 1-acyl-3-(3,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones (**10a**, **b**) or 3-(3,4-dimethoxyphenyl)-5-methylthio-4-phenyl-4*H*-1,2,4-triazole (**11**) respectively. The synthesized compounds **6d**, **7a**, **c**, **10a**, **b**, **11** exhibit anti-inflammatory activity.

1. Introduction

Following reports of anti-inflammatory activity of 6-arylmethylidene-3-(2-pyrimidinyl)-5,6-dihydro[1,3]thiazolo-[2,3-*c*][1,2,4]triazol-5-ones [1] and 6,7-dialkoxy-2-arylmethylidene-2,3-dihydrobenzo[4,5]imidazo[2,1-*b*][1,3]thiazol-3-ones [2] a series of compounds having similar structure – 1-acyl-5-alkylthio-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazoles (**5a**, **c–f**, **6d–f**), 2-acylamino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazoles (**7c**, **e**, **f**), 1-acyl-3-(3,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones (**10a**, **b**) and 3-(3,4-dimethoxyphenyl)-5-methylthio-4-phenyl-4*H*-1,2,4-triazole (**11**) were synthesized and investigated for anti-inflammatory activity.

2. Investigations, results and discussion

1-Acyl-5-alkylhylthio-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazoles (**5a**, **c–f**, **6d–f**) were synthesized treating 5-alkylthio-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazoles (**3**, **4**) with acyl chlorides in trichloromethane solution in the presence of triethylamine. The compounds **3**, **4** were obtained by alkylation of 3-(3,4-dimethoxyphenyl)-1*H*-1,2,4-triazole-5-thiol [3] sodium salt (**2**) with alkyl iodides. 2-Amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol (**8**) was prepared by treatment of 3,4-dimethoxybenzoylthiosemicarbazide [4] (**1**) with acetyl chloride. The obtained by product – 2-acetylamino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole (**7a**) – was hydrolysed in aqueous sodium hydroxide solution to give **8**. 2-Acylamino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazoles (**7c**, **e**, **f**) were synthesized by the acylation of compound **8** with acyl chlorides in trichloromethane solution in the presence of triethylamine. 3-(3,4-Dimethoxyphenyl)-5-methylthio-4-phenyl-4*H*-1,2,4-triazole (**11**) was obtained by the methylation of sodium salt of 3-(3,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thione (**9**) [5] with iodomethane in aqueous ethanol. 1-Acyl-3-(3,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiones (**10a**, **b**) were synthesized treating compound **9** with acyl chlorides (Scheme).

Some routes of the synthesis of compounds **7c**, **e**, **f** were investigated. They can be obtained by the cyclisation of **1** with corresponding aromatic chloranhydrides (36–51%) [6] or by the acylation of **8** (47–88%). Compound **8** can be synthesized by treatment of **1** with polyphosphoric acid (35%) [6], or by the cyclisation of **1** with acetyl chloride and hydrolysis of the by-product **7a** (79%)

The postulated structures of the newly synthesized compounds **3**, **4**, **5a**, **c**, **d**, **e**, **f**, **6d**, **e**, **f**, **7a**, **c**, **e**, **f**, **8**, **10a**, **b** and **11** are in agreement with their IR, ^{1}H NMR spectral and elemental analysis data.

As expected, the means of $\nu_{C=O}$ in the IR spectra of the methylthioderivatives **5d**, **e**, **f** and the butylthioderivatives

Scheme

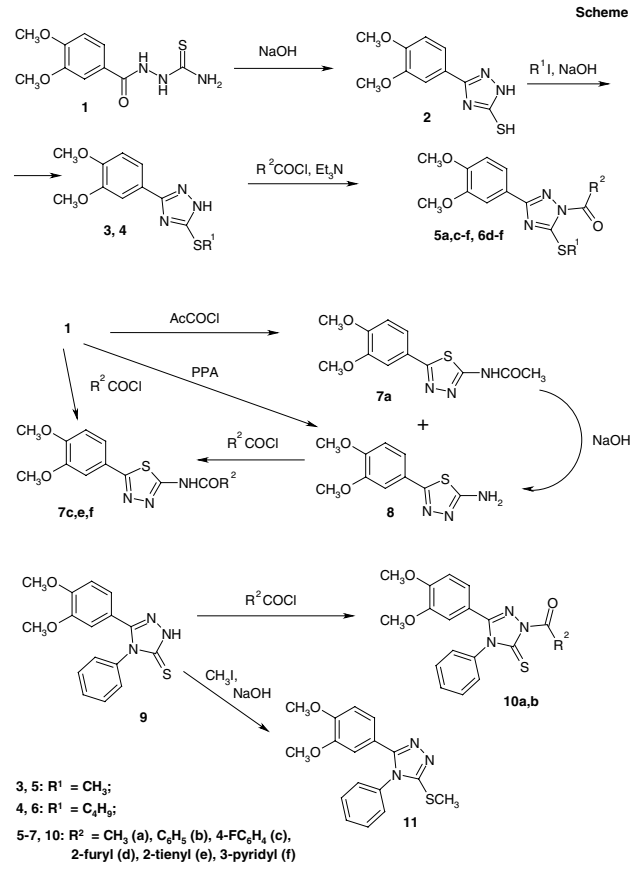

Table 1: Experimental, physico-chemical and spectral data for compounds 3, 4, 5a, c–f, 6d–f, 7a, c, e, f, 8, 10a, b, 11

Compd.	Formula	Yield (%)	M.p. (°C)	IR (cm^{-1})	^{1}H NMR			
					CH_3O	S-alkyl	ArH	Other
3	$C_{11}H_{13}N_3O_2S$	62	119–122	3500 (N–H)	a[a] 3.77 and 3.86	s 2.60 SCH_3	d[b] 6.78 5′H, d 7.45 2′H, dd 7.55 6′H	
4	$C_{14}H_{19}N_3O_2S$	79	105–107	3550 (N–H)	s 3.85	t[c] 0.92 CH_3, m[d] 1.22–1.85 $(CH_2)_2$, t 3.15 CH_2S	d 7.09 5′H, d 7.53 2′H, dd 7.61 6′H	
5c	$C_{18}H_{17}F_3O_3S$	67	157–158	1680 (C=O)	s 3.86	s 2.80 SCH_3	d 6.97 5′H, m 7.08–8.50	
5d	$C_{16}H_{15}N_3O_4S$	97	163–165	1685 (C=O)	s 3.93 and 3.94	s 2.78 SCH_3	d 6.95 5′H, m 7.12–7.31 and 7.67–7.90, d 8.46	
5e	$C_{16}N_{15}N_3O_3S_2$	89	176–178	1680 (C=O)	s 3.95 and 4.00	s 2.77 SCH_3	d 6.95 5′H, m 7.21–8.48	
5f	$C_{17}H_{16}N_4O_3S$	56	149–152	1680 (C=O)	s 3.90 and 3.95	s 2.77 SCH_3	d 7.04 5′H, m 7.45–7.75 and 8.47–9.40	
6d	$C_{19}H_{21}N_3O_4S$	91	132–135	1685 (C=O)	s 3.87 and 4.02	t 0.99 CH_3, m 1.08–2.07 $(CH_2)_2$, t 3.35 CH_2S	d 6.95 5′H, m 7.21–8.48	
6e	$C_{19}H_{21}N_3O_3S_2$	71	131–132	1680 (C=O)	s 3.98 and 4.14	t 0.98 CH_3, m 1.08–2.07 $(CH_2)_2$, t 3.33 CH_2S	m 6.70–8.48	
6f	$C_{20}H_{22}N_4O_3S$	48	116–117	1675 (C=O)	s 3.94 and 4.05	t 0.99 CH_3, m 1.20–1.95 $(CH_2)_2$, t 3.37 CH_2S	d 7.10 5′H, dd 7.56 6′H, d 7.65 2′H, m 8.17–8.70	
7a	$C_{12}H_{13}N_3O_3S$	65	280–282	1687 (C=O)	s 4.05		d 7.09 3H, dd 7.50 2H, d 7.68 6H	2.61 $COCH_3$, 13.35 NH
7c	$C_{17}H_{14}FN_3O_3S$	47	279–280	1665 (C=O)	s 3.88 and 3.90		d 7.13 5′H, m 7.35–8.51	
7e	$C_{15}H_{13}N_3O_3S_2$	88	274–276	1655 (C=O)	s 3.86 and 3.90		m 7.04–7.96	
7f	$C_{16}H_{14}N_4O_3S$	86	259–261	1675 (C=O)	s 3.86 and 3.90		d 7.12 5′H, dd 7.52 6′H, d 7.55 2′H, m 8.38–9.34	
8	$C_{10}H_{11}N_3O_2S$	79	197–199	3396, 3277 (NH_2)	s 3.81 and 3.83		7.00 5′H, dd 7.22 6′H, d 7.37 2′H	7.33 NH_2
10a	$C_{18}H_{17}N_3O_3S$	68	138–138	1744 (C=O)	s 3.50 and 3.75		6.82 and 7.00, m 7.26–7.62	2.77 $COCH_3$
10b	$C_{22}H_{19}N_3O_3S$	75	201–202	1726 (C=O)	s 3.57 and 3.85		6.56–7.62, dd 8.05	
11	$C_{16}H_{18}N_3O_2S$	61	145–148		s 3.54 and 3.75	s 2.62 SCH_3	6.92, m 7.33–7.61	

6d, **e**, **f** respectively are similar and lies in the range of 1675–1685 cm^{-1}. The means of $\nu_{C=O}$ of compounds **7e**, **f** are less than those of **5e**, **f** and **6e**, **f** (1655–1675 cm^{-1} and 1675–1680 cm^{-1}, respectively). The expansion of the conjugated system in compounds **10a**, **b** causes the increased means of $\nu_{C=O}$ (1726–1744 cm^{-1}). The means of the chemical shift in the ^{1}H NMR spectra of CH_3O groups of compounds **10a**, **b**, **11** are less than those of all other synthesized compounds (3.50–3.85 and 3.77–4.14 ppm, respectively). The difference between the means of chemical shift of 3- and 4-CH_3O groups of compounds **10a**, **b**, **11** is also unexpectedly high (0.2–0.28 ppm instead of 0–0.14 ppm for all other compounds) (Table 1).
Compounds **6e** and **9** possess weak anti-inflammatory activity. Compounds **7a**, **c** and **10a** possess anti-inflammatory activity comparable with that of acetylsalicylic acid and the compound **6d** shows activity similar to that of ibuprofen. Compounds **10b** and **11** are significantly more active than ibuprofen. In some cases the anti-inflammatory activity of butylthioderivatives (**6d**, **e**) was higher than that of the corresponding methylthioderivatives (**5d**, **e**). The *N*-acylation or *S*-methylation of compound **9** significantly enhanced activity (**10a**, **b**, **11**). The acute toxicity (LD_{50}) of the most active compounds **6d**, **7a**, **10a**, **b**, **11** was less than that of acetylsalicylic acid and significantly less than that of ibuprofen (Table 2).

3. Experimental

3.1. Chemistry

M.p.'s were determined in open capillaries and are uncorrected. The UV spectra were recorded on a Lambda 20 (Perkin-Elmer, Sweden), IR spectra – on a FT-IR Spectrum BX (Perkin-Elmer, Sweden) in nujol and ^{1}H NMR spectra – on a BS-587A (80 MHz, Tesla, Czechoslovakia) in $(CD_3)_2SO$ with TMS as an internal standart. Chemical shifts (δ) are reported in ppm, coupling constants (J) are given in Hz. All new compounds were analyzed for C, H and N and the results were in an acceptable range.
3-(3,4-Dimethoxyphenyl)-1*H*-1,2,4-triazole-5-thiol (**2**) [3] and 3-(3,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thione (**9**) [5] were synthesized by the known methods.

3.1.1. 5-Alkylthio-3-(3,4-dimethoxyphenyl)-4H-1,2,4-triazoles (**3**, **4**) *and 3-(3,4-dimethoxyphenyl)-5-methylthio-4-phenyl-4H-1,2,4-triazole* (**11**)

A mixture of 0.01 mol 3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazole-5-thiol (**2**) or 3-(3,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thione (**9**) and 0.0105 mol sodium hydroxide was solved in 50% aqueous

Table 2: Anti-inflammatory activity (50 mg/kg p.o.) and acute toxicity (LD_{50}) data for compounds 5a, c–f, 6d–f, 7a, c, e, f, 9, 10a, b, 11

Compd.	0.1 ml of 1% Carrageenin solution		0.1ml of 5% Bentonite suspension		LD_{50} (mg/kg)
	Cross-section of rat paw (relative units)	Inhibition of rat paw oedema (%) over control	Cross-section of rat paw (relative units)	Inhibition of rat paw oedema (%) over control	
Control	96.27	0	95.30	0	
4	90.90	5.5	95.35	0	
5c	96.31	0	85.11	10.7	
5d	86.83	9.8	95.27	0	
5e	88.18	8.4	89.77	5.8	
5f	96.22	0	74.90	21.4	
6d	64.52	33.0	74.64	21.7	>1500
6e	78.08	18.9	81.77	14.2	
6f	90.92	5.5	95.25	0	
7a	71.34	25.9	85.98	19.8	>1600
7c	73.35	23.8	82.05	13.9	
7e	89.77	6.5	92.44	3.0	
7f	91.26	5.2	89.96	5.6	
9	86.35	10.3	80.33	15.7	
10a	73.93	23.2	69.95	26.6	
10b	48.04	50.1	49.81	47.7	>1500
11	51.21	46.8	51.74	45.7	>1500
Acetylsalicylic acid	77.21	19.8	74.71	21.6	1216
Ibuprofen	59.69	38.0	75.57	20.7	500

2-propanol, 0.011 mol corresponding iodoalkane was added. The reaction mixture was kept at room temperature for 5 d or refluxed for 4 h, 2-propanol was evaporated. The product was extracted with $CHCl_2$ and recrystallyzed from EtOH.

3.1.2. 2-Acetylamino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol **(7a)** *and 2-Amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol* **(8)**

Acetyl chloride 8 ml was added dropwise to 10 g (0.04 mol) of 1-(3,4-dimethoxybenzoyl)thiosemicarbazide (**1**) and the mixture was refluxed for 15 min. The reaction mixture was poured into 200 ml H_2O and 5 N NaOH was added until pH 12. 2-Amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol (**8**) was filtered off and recrystallized from methanol. The filtrate was neutralized with acetic acid, the obtained precipitate of 2-acetylamino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazol (**7a**) was filtered off and recrystallized from dimethylsulfoxide.

3.1.3. 1-Acylderivatives of 5-alkylthio-3-(3,4-dimethoxyphenyl)-4H-1,2,4-triazole **(5c–f, 6d–f)***, 2-acylamino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazoles* **(7c, e, f)***, 1-acyl-3-(3,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thiones* **(10a, b)**

A mixture of 0.01 mol 5-alkylthio-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazoles (**3**, **4**), 2-amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole (**8**) or 3-(3,4-dimethoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thione (**9**), 0.01 mol corresponding acyl chloride, 0.02 mol triethylamine and 30 ml anh. $CHCl_3$ were refluxed for 4 h. Then the reaction mixture was cooled, washed with H_2O, dried over Na_2SO_4 and evaporated in vacuum. The acylderivatives (**5c–f**, **6d–f**), (**7c**, **e**, **f**) and (**10a**, **b**) were obtained as solids after crystallyzation from ethanol.

3.2. Pharmacology

Adult male Wistar strain rats weighing 180–220 g and male BALB/C strain mices weighing 18–22 g were used. The animals were alowed food and water *ad libitum*. They were haused in rooms at 18–20 °C with a 12-h light/dark cycle and a relative humidity of 55–60%. The animals were randomly allocated into groups at the beginning of all the experiments. All test compounds and the reference drugs were administered orally suspended in 0.5% carboxymethylcellulose solution. Carrageenin-induced hind-paw oedema in rats was produced by the method of Winter et al. [7]. Carrageenin solution (1.0% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was injected subcutaneously into the subplantar region of the right hind paw 1 h after administration of the test compound. Control animals received only 0.5% carboxymethylcellulose solution. Right hind paw volumes were measured with an electronic onkograph immediately before and 1, 2, 3, and 5 h after the carrageenin injection. The results were matched with those of control rats. Each experiment was made with 5 groups of rats, 10 animals each (the 1-st one was control).

Analogously was studied the bentonite-induced hind paw oedema [8]. Bentonite suspension (5% in sterile 0.9% NaCl solution) in a volume of 0.1 ml was used. The data were evaluated statistically using Student's t-test. A level of $p < 0.05$ was adopted for the test of significance.

The tests of acute toxicity of the compounds were done on mice fasted for 24 h, water *ad libitum.* Groups of 6 mice were treated perorally with the test compound at various dose levels. The animals were wached for mortality and symptoms until day 8 [9].

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