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Short communication

Synthesis and biological evaluation of 2-[substituted acetyl]amino-5-alkyl-1,3,4-thiadiazoles

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Summary — Some new N-substituted acetyl derivatives of 2-amino-5-alkyl-1,3,4-thiadiazoles have been prepared and investigated for antihistaminic and spasmolytic activity on guinea pig ileum. A few of the compounds showed encouraging effects. In addition, some of the compounds also showed anti-inflammatory activity.

1,3,4-thiadiazoles / spasmolytic activity / anti-inflammatory activity / antihistaminic activity

Introduction

Among pharmacologically active compounds the arrangement of atoms >N-C-C-N<, -O-C-C-N< and >N-C-C-O-CO- are of importance, as far as inhibitors of histamine, serotonin (5-HT) and acetyl choline are concerned [1]. Our interest in 2-substituted amino-5-alkyl-1,3,4-thiadiazoles as biological active compounds [2–4] prompted us to prepare a few title compounds having -NH-CO-CH_2-N< arrangement and to undertake their biological evaluation for antihistaminic, spasmolytic activity together with anti-inflammatory activity.

Chemistry

The reaction of 2-amino-5-alkyl-1,3,4-thiadiazoles **1**, **2** with chloroacetyl chloride gave the 2-chloro acetyl amino-5-alkyl-1,3,4-thiadiazoles **3**, **4**. Condensation of **3**, **4** with an excess of secondary amines gave the desired products (**5–16**) (fig 1).

The structures of derivatives were confirmed by elemental analysis and spectral data. The IR and ^1H NMR spectra were in agreement with the proposed structure.

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Pharmacological results and discussion

Pharmacological results of the synthesized compounds are reported in table I. The ALD_{50} of the synthesized compounds ranged between 681–1000 mg/kg (ip) in mice except for compound **14** which was found to be 215 mg/kg (ip).

In the studies of the effect of the compounds on isolated organs all the compounds showed antihistaminic activity as expected, and all showed competitive antagonism towards histamine except for compound **12**, which showed non-competitive antagonism. (Figure 2 represents log dose *versus* response curve for compound **8**.) The 50% inhibitory concentration (IC_{50}) of the compounds ranged

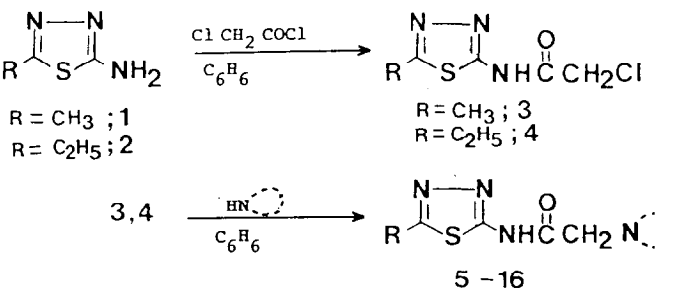


Fig 1.

Table I. Pharmacological data of synthesized compounds.

Product	*ALD_{50} mg/kg ip*	*Anti-inflammatory activity[a] % inhibition*	*IC_{50} (x 10^{-7}, mol/l) against spasmogen*				*pA_2 value Drug-hist*
			Hist	*Ach*	*Nicotine*	*5-HT*	
5	681	27.6	2.47	1.25	2.89	2.68	6.0
6	1000	13.6	1.56	2.34	2.40	2.34	6.2
7	681	41.6	1.20	–	–	–	7.5
8	681	–	0.568	–	–	8.71	8.0
9	681	–	1.89	–	–	–	7.1
10	681	–	1.26	–	–	–	7.4
11	> 1000	–	1.96	–	–	–	6.1
12	1000	20.2	2.74	1.96	1.92	8.50	–
13	> 1000	–	4.15	–	–	–	6.0
14	215	22.1	2.06	1.89	–	–	6.2
15	681	24.0	2.15	–	–	–	6.3
16	681	–	2.60	–	–	–	6.2
PB	–	46.5	–	–	–	–	–
MP	–	–	0.08	1.86	–	–	9.3

[a]Dose 1/10th of ALD_{50} *po*, PB: phenylbutazone, MP: mepyramine.

between 1.20 x 10^{-7} to 4.15 x 10^{-7} mol/l except for **8** which was found to be very potent (IC_{50} 5.68 x 10^{-8} mol/l). The pA_2 values of compounds ranged between 6.0–8.0. However, compounds **5**, **6** and **12** were found to show spasmolytic activity as well.

The introduction of 1,3,4-thiadiazoles nucleus in structure -NH-CO-CH_2-N< showed anti-inflammatory activity in addition to antihistaminic and spasmolytic activity in a few compounds *ie* **5**, **6**, **7**, **12**, **14** and **15** determined by carrageenan-induced paw oedema method. In particular the effect showed by **7** was comparable to that of the test drug *ie*, phenylbutazone ($P < 0.01$).

The compounds were also screened on the cardiovascular system. None of the compounds antagonised the effects of adrenaline, acetylcholine, isoprenaline and histamine *in vivo*. Compound **12** produced a concentration dependent inhibition of the blood pressure. The maximum fall in BP (↓80 mm for 50 min) was observed at a dose of 5 mg/kg bw (iv) (fig 3).

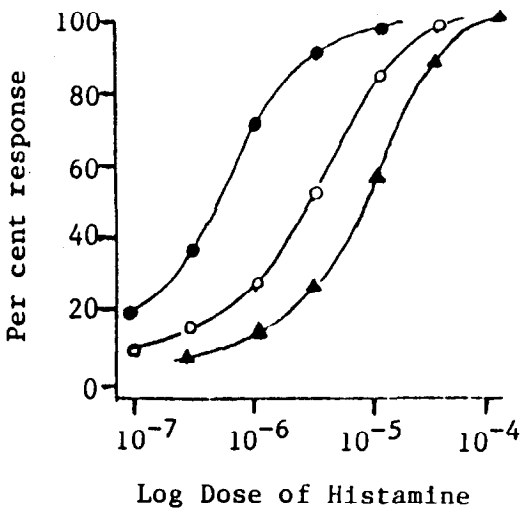


Fig 2. Log dose–response curves of histamine in the absence and in the presence of compound **8**. Histamine alone ●—●; in the presence of compound **8**, ○—○ 3.5 x 10^{-8} mol/l; ▲—▲ 7.0 x 10^{-8} mol/l.

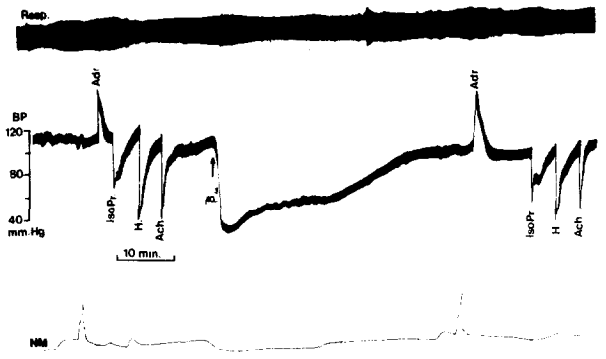


Fig 3. Effect of intravenously administered compound **12**, 5 mg/kg on blood pressure, respiration and nictitating membrane of anaesthetized cat.

Experimental protocols

Chemistry

Melting points were taken on a Toshniwal melting point determination apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian EM-360 (90 MHz) spectrometer using tetramethyl silane (TMS) as the internal standard. IR spectra were recorded on a Perkin-Elmer 157 spectrophotometer. Microanalysis was performed using Carlo-Erba 1106 instruments.

*2-Amino-5-alkyl-1,3,4-thiadiazoles **1**, **2***
These were prepared by treating thiosemicarbazide, aliphatic carboxylic acid and concentrated sulphuric acid according to the reported method [5]. 5-Methyl: yield 92%, mp 221–223°C (reported 223°C). 5-Ethyl: 86%, 192–194°C (194°C).

*2-Chloroacetyl amino-5-alkyl-1,3,4-thiadiazoles **3**, **4***
Compound **1**, **2** (0.10 mol) and chloroacetyl chloride (0.11 mol) were refluxed in benzene (250 ml) for 3 h on a water bath. Benzene was then distilled off and the crude product obtained was recrystallized from ethanol (95% v/v). 5-Methyl: yield 90%, mp 235–36°C. 5-Ethyl: 92%, 224–225°C.

*2-[Substituted acetyl]amino-5-alkyl-1,3,4-thiadiazoles **5–16***
To a stirred suspension of 0.05 mol of compound **3**, **4** in 100 ml of benzene, appropriate amine (0.125 mol) was added dropwise. The reaction mixture was stirred and refluxed for 2–3 h. After separation of the hydrochloride of amine the organic layer was extracted several times with 1 N HCl. On neutralization with 1 N NH_4OH, the crude product separated out. Recrystallization was done using appropriate solvents. Yields, melting points, recrystallization solvents and elemental analysis are given in table II; IR and ^{1}H NMR spectral data are reported in table III.

Pharmacology

For the pharmacological studies, adult cats (2–4 kg), guinea pigs (300–400 g), albino rats (100–120 g) and albino mice (20–25 g) of either sex were used. Hydrochloride salts of the compounds were utilised. Depending on the requirement compounds were dissolved in either distilled water or normal saline. The control group received vehicle only.

Approximate lethal dose (ALD_{50})
The albino mice were divided into different groups of 4 animals each. These were then administered with the graded doses of the compounds intraperitoneally (ip). Observation for mortality was then made. The ALD_{50} was taken from Horn's table [6].

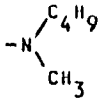
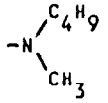
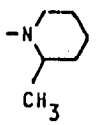
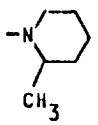
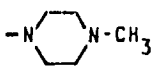
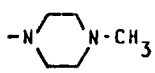
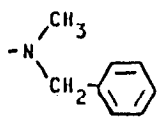
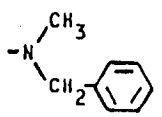
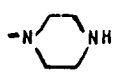
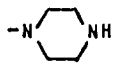
Smooth muscle relaxant activity in vitro
A 2–3-cm long piece of ileum from a freshly killed guinea pig was suspended in an organ bath containing aerated Tyrode solution (pH 7.4) at 34°C [7]. Contractions were recorded on a kymograph through a frontal writing lever. Spasmolytic activity of the compounds was assessed by its ability to prevent the contraction induced by a submaximal concentration (g/ml) of acetyl choline (2.5×10^{-8}) histamine (3.0×10^{-8}), serotonin (5-HT, 5.0×10^{-7}) or nicotine (2.5×10^{-6}). In all the isolated preparations, graded doses of the spasmolytic compounds were tested against the submaximal concentration of the spasmogen and the IC_{50} was calculated graphically in each experiment.

The organ bath was then connected to 2 bottles so that it could be filled either with the one containing Tyrode solution or that containing the antagonist (compounds **5–16**). The submaximal contractions were first obtained with histamine (3×10^{-7}-8×10^{-4}) in absence and in presence of antagonist. The dose ratio (DR) was determined by plotting log dose *versus* response curve. The pA_2 value was then determined by plotting log (DR-1) *versus* log molar concentration graph.

Anti-inflammatory activity
Carrageenan-induced paw oedema method [8] was adopted. The albino rats were divided into groups of 5 animals each. The paw oedema was determined plethysmographically. One percent suspension of carrageenan in normal saline was injected (0.1 ml) into the subplantar surface of the right hind paw through a 26-gauge needle. The compounds were administered 1 h prior to carrageenan injection (dose 1/10th of ALD_{50} *po*). After 3 h the paw volume was again determined. The results were compared with that of standard drug phenylbutazone (dose 30 mg/kg *po*).

Effect on the cardiovascular system
Cats of either sex were anaesthetised by injecting pentobarbitone sodium (40 mg/kg ip) [9]. The femoral vein was exposed, cannulated and connected with rubber tubing to a burette filled with normal saline. A midline incision was made on the neck to expose the trachea and one end of the tracheal cannula was inserted into it. The other end of the cannula was connected to Mayer's tambour by rubber tubing for recording respiratory changes. The common carotid artery was then exposed and clipped at the lower end. An arterial cannula was inserted and tied. The other end of the cannula was connected to a manometer with rubber tubing. A 10% w/v sodium citrate solution ip was filled in the manometer and the rubber tubing. The clip on the carotid artery was released, the blood pressure was then recorded on a slowly moving kymograph with a pointer floating on the mercury column. During the experiment the sympathetic nerve was also stimulated electrically. The drugs were then administered through the cannulated femoral vein and the effects recorded.

Table II. Physico-chemical data of 2-[substituted acetyl]amino-5-alkyl-1,3,4-thiadiazoles.

Compd	*R*	*X*	*Formula*	*Elemental analysis found (calculated)* *C*	*H*	*N*	*Yield*	*mp °C*[a]	*Recryst solvent*
5	$-CH_3$		$C_{10}H_{18}N_4OS$	49.05 (49.58)	7.20 (7.44)	23.32 (23.14)	72	97	EtOH/AcOEt
6	$-C_2H_5$		$C_{11}H_{20}N_4OS$	51.32 (51.56)	7.65 (7.81)	21.74 (21.87)	70	75	EtOH
7	$-CH_3$		$C_{10}H_{16}N_4OS$	49.89 (50.00)	6.52 (6.67)	23.11 (23.34)	52	161	EtOH/AcOEt
8	$-C_2H_5$		$C_{11}H_{18}N_4OS$	51.67 (51.96)	7.01 (7.08)	21.89 (22.04)	56	148	MeOH/AcOEt
9	$-CH_3$		$C_{11}H_{18}N_4OS$	51.81 (51.96)	6.94 (7.08)	21.76 (22.04)	65	114	EtOH/H_2O
10	$-C_2H_5$		$C_{12}H_{20}N_4OS$	53.57 (53.73)	7.39 (7.46)	20.77 (20.89)	55	145	EtOH/H_2O
11	$-CH_3$		$C_{10}H_{17}N_5OS$	46.91 (47.05)	6.54 (6.67)	27.39 (27.45)	36	98	EtOH
12	$-C_2H_5$		$C_{11}H_{19}N_5OS$	49.00 (49.07)	6.96 (7.06)	25.85 (26.02)	45	141	EtOH
13	$-CH_3$		$C_{13}H_{16}N_4OS$	56.42 (56.52)	5.65 (5.79)	20.15 (20.28)	51	140	EtOH
14	$-C_2H_5$		$C_{14}H_{18}N_4OS$	57.88 (57.93)	6.03 (6.20)	19.23 (19.31)	52	83	EtOH
15	$-CH_3$		$C_9H_{15}N_5OS$	44.55 (44.81)	6.15 (6.22)	29.00 (29.04)	53	120	EtOH
16	$-C_2H_5$		$C_{10}H_{17}N_5OS$	46.89 (47.05)	6.59 (6.67)	27.37 (27.45)	50	109	EtOH

[a]Melting points were determined in open capillary and are uncorrected.

Table III. Spectral data of compounds **5–16**.

Compd	*IR(KBr) ν (cm^{-1})*	*^{1}H NMR CDCl$_3$/TMS*
5	3200, 1645, 1400	0.90 (t, 3H, CH_3); 1.35 (m, 6H, -$(CH_2)_3$ CH_3); 2.05 (s, 3H, CH_3 td); 2.70 (s, 3H, CH_3-N); 3.90 (s, 2H, -CO-CH_2); 5.75 (br, 1H, NH, D_2O exchangeable)
6	3145, 1620, 1415	0.80 (t, 3H, CH_3); 1.00 (t, 3H, CH_3); 1.60 (m, 6H, $(CH_2)_3CH_3$); 2.60 (br, 3H, CH_3-N); 4.20 (s, 2H, -CO-CH_2); 7.50 (br, 1H, NH)
7	3150, 1655, 1425	1.75 (br, 6H, CH_2, pipd); 2.25 (s, 3H, CH_3, td); 2.80 (m, 4H, CH_2-N-CH_2, pipd); 3.90 (s, 2H, -CO-CH_2); 6.50 (br, 1H, NH)
8	3175, 1640, 1420	1.15 (t, 3H, CH_3-CH_2-); 1.60 (br, 6H, CH_2 pipd); 2.65 (m, 4H, -CH_2-N-CH_2-pipd); 3.20 (q, 2H, CH_3-CH_2-); 3.75 (s, 2H, -CO -CH_2); 5.80 (br, 1H, NH)
9	3210, 1650, 1445	1.10 (d, 3H, -CH_3, pipd); 1.95 (s, 3H, CH_3 td); 2.4 (m, 9H pipd); 3.75 (s, 2H,-CO-CH_2); 7.50 (br, 1H, NH)
10	1700, 1440	0.95 (d, 3H, -CH_3 pipd); 1.25 (t, 3H, -CH_2-CH_3); 2.25 (br, 9H, pipd); 2.90 (q, 2H, -CH_2-CH_3); 4.15 (s, 2H, -CO-CH_2); 8.50 (br, 1H, NH)
11	3300–3100, 1650, 1440	2.05 (s, 3H, CH_3 td); 2.60 (s, 3H, CH_3); 3.75 (s, 8H, pipz); 4.25 (s, 2H, -CO-CH_2-); 5.50 (br, 1H, NH)
12	3285–3100, 1645	1.25 (t, 3H, CH_3-CH_2); 2.75 (s, 3H, CH_3); 3.45 (s, 8H, pipz); 3.95 (q, 2H, CH_3-CH_2-) 4.15 (s, 2H, -CO-CH_2-); 7.25 (br, 1H, NH)
13	3150, 1680, 1440, 1350	2.20 (s, 3H, CH_3 td); 2.55 (s, 3H, CH_3-N); 3.15 (s, 2H, -CO-CH_2); 3.65 (s, 2H, CH_2); 6.85 (br, 1H, NH); 7.15 (t, 5H, Ar)
14	3150, 1660, 1410, 1320	0.95 (t, 3H, CH_3-CH_2); 2.70 (s, 3H, CH_3-N); 3.65 (q, 2H, CH_3-CH_2-); 4.05 (s, 2H, -CO-CH_2); 4.20 (s, 2H, CH_2); 7.30 (t, 5H, Ar); 8.15 (br, 1H, NH)
15	3180, 1650, 1420, 1350	1.95 (s, 3H, CH_3 td); 3.45 (br, 8H, pipz); 3.90 (s, 2H, -CO-CH_2); 8.05 (br, 2H, NH)
16	3100, 1625, 1435, 1325	1.10 (t, 3H, CH_3-CH_2); 3.65 (br, 8H, pipz); 4.10 (q, 2H, CH_3-CH_2); 4.20 (s, 2H, -CO-CH_2-); 5.85 (br, 2H, NH)

pipd = piperidine; pipz = piperazine; td = thiadiazole.

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