Arch. Pharm. Res. Vol.21, No.6, pp. 753-758, 1998

Synthesis and Biological Evaluation of Novel 2-[Substituted acetly]-amino-5-alkyl]-amino-5-alkyl-1,3,4-thiadiazoles

Ashok K. Shakya[1,*], Pradeep Mishra[2], G. K. Patnaik, Rakesh Shukla and R. C. Srimal[3]

[1]*Clinical Pharmacology & Pharmacokinetics Ranbaxy Research Laboratories, PLOT 20, Sector 18, Udyog Vihar, Gurgoan (Haryana), India,* [2]*Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya [Formerly University of Saugor] Sagar 470003 (MP), India and Division of Pharmacology Central Drug Research Institute, Lucknow 226001 (UP), India*

(Received September 5, 1997)

Sixteen novel 2-substituted acetyl amino-5-alkyl-1,3,4-thiadiazole were synthesized and screened for their pharmacological activities. A few of the compounds namely 11, 12 and 16 showed anti-inflammatory activities comparable to phenylbutazone. Compound 12 also showed significant non-specific spasmolytic activity. Diuretic activity of compound 15 at a dose level of 90 mg/kg p.o. was two fold higher compared to 50 mg/kg p.o. of furosemide. Comparable diuresis was also produced by compounds 9, 10 and 16.

Key words : 2-[Substituted acetyl]-amino-5-alkyl-1,3,4-thiadiazoles, 2-[Substituted-ethanamido]-5-alkyl-1,3,4-thiadiazoles, 1,3,4-Thiadiazole, Diuretic, Anti-inflammatory agent

INTRODUCTION

Biological activities like CNS stimulant (Pandey *et al.*, 1982), anticholinergic (Muhi-Eldeen *et al.*, 1982), hypoglycemic (Hussain *et al.*, 1986) and anticonvulsant activity (Bhatnager *et al.*, 1986; Chapleo *et al.*, 1986) associated with 1,3,4-thiadiazoles are well documented. CNS depressant (Mishra *et al.*, 1989) activity of a few derivatives has been reported from this laboratory. Among the 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 and acetylcholine is concerned. While screening for pharmacological activities in 2-substituted-acetyl-amino-5-alkyl-1,3,4-thiadiazole we observed not only spasmolytic activity but also CNS depressant and anti-inflammatory activities (Mishra *et al.*, 1990 a,b; Shakya *et al.*, 1992) Therefore, it was of interest to prepare few title compounds of this series having >N-CO-CH_2-N< arrangement and screen them biologically.

EXPERIMENTAL SECTION

Chemistry

The reaction of thiosemicarbazide with aliphatic carboxylic acid in presence of concentrated sulfuric acid gave the 2-amino-5-alkyl-1,3,4-thiadiazole (I). Compound I was then acetylated with 2-chloroacetyl chloride. The chloroacetyl derivative (II) on reaction with secondary amines gave 2-substituted acetyl amino-5-alkyl-1,3,4-thiadiazoles (III, 1-16).

The melting points were measured in open capillary tubes with Toshniwal (India) melting point determination apparatus and are uncorrected. The progress of reaction and purity of the compounds were checked on TLC, that was performed on precoated silica gel (60G 254, Merck, chloroform for solvent, 30% H_2SO_4 vanillin solution).

PMR spectra were recorded in $CDCl_3$ on a Varian EM-390 (90 MHz) spectrometer, and chemical shifts are

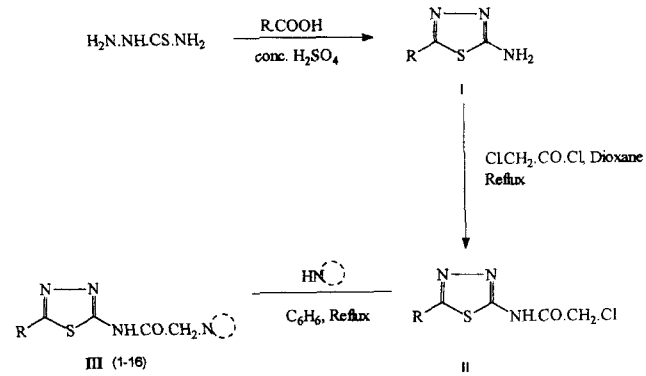


Fig. 1.

Correspondence to: Ashok K. Shakya, Faculty of Pharmacy and Medical Sciences, Post Box 282, Amman University, Amman 19328, Jordan

given in δ ppm with tetramethyl silane (TMS). IR spectra were taken from Shimadzu IR-470 Spectrophotometer in KBr pellet. Microanalysis were performed using Garlo-Erba 1106 instrument.

Synthesis of 2-amino-5-alkyl-1,3,4-thiadiazoles (1)

These were synthesized in good yield following the method of Funatsukuri and Ueda (1967). The results are 5-methyl:yield 92%, m.p. 221~223°C (literature 223°C), 5-ethyl:86%, 192~194 (194°C).

Synthesis of 2-chloroacetyl amino 5-alkyl-1,3,4-thiadiazole (II)

Chloroacetyl chloride (0.20 mole) was added dropwise to the compound I (0.20 mole) dissolved in dioxane (250 ml). The reaction mixture was refluxed for 3 hours, then cooled by the pouring on crushed ice. The precipitated product was filtered and washed repeatedly with aqueous potassium carbonate (1% w/v) and then washed with ice cold water. Recrystallised was done using absolute alcohol. 5-methyl:yield 90%, mp 235~36°C:5-ethyl:92%, 224~25°C.

General procedure for synthesis of of 2-[substituted acetyl]-amino-5-alkyl-1,3,4-thiadiazole (III, 1-16)

To a stirred suspension of 0.05 mole of II in 100 ml of benzene, appropriate amine (0.10 mol) was added dropwise. It was then refluxed for 5~6 hours. After cooling, the benzene layer was washed several times with water. The organic phase was made free from water using exsicated sodium sulfate. Removal of the organic phase, under vaccum gave the product. Recrystallisation was done from suitable solvents.

Yield, melting points, recrystallisation solvents and elemental analysis are given in Table I, PMR and IR spectral date are reported in Table II.

Pharmacology

For the pharmacological studies, adult cats (2.0~3.5 kg), guinea pigs (300~400 gm), albino rats (150~200 g) and albino mice (20~25 g) of either sex were used, wherever mentioned. hydrochloride salts of the compounds were used. Compounds were dissolved in either distilled water or normal saline depending upon the requirement. The control group received vehicle only.

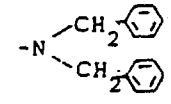
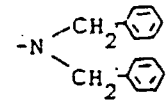
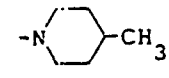
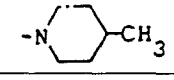
Lethal dose (LD_{50})

The albino mice were divided into different groups of four animals each. These were then administered 215, 464, 1000 and 2150 mg/kg (i.p.) of the drugs intra-peritoneally and observations made for mortality upto 24 hours. In cases where cent per cent mortality was observed even at a dose of 215 mg/kg., further studies were undertaken with 10% of the above doses of the drugs to find out the acute toxicity. The lethal dose was then taken from the Horn's Table (Horn, 1956).

Anti-inflammatory activity

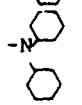
Anti-inflammatory activity of the compounds was determined following carrageenan induced paw-edema method (Winter *et al.*, 1962). Adults rats of either sex were divided into the groups of 5 animals each. The edema was induced in one of the hind paws by in-

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

Comp. No.	R	X	Mole. formula	Elemental Analysis (%) Found (Calculated) C	H	N	Yield (%)	Melt.* point (°C)	Recryst. solvent
1	CH_3		$C_9H_{14}N_4OS$	47.56 (47.79)	6.02 (6.19)	24.59 (24.78)	40	135	Methanol
2	C_2H_5		$C_{10}H_{16}N_4OS$	49.89 (50.00)	6.56 (6.67)	23.26 (23.33)	80	140-1	Methanol
3	CH_3		$C_{19}H_{20}N_4OS$	64.59 (64.77)	5.52 (5.68)	15.78 (15.90)	50	115	Ethanol
4	C_2H_5		$C_{20}H_{22}N_4OS$	65.43 (65.57)	5.93 (6.01)	15.17 (15.30)	45	105	Ethanol
5	CH_3		$C_{11}H_{18}N_4OS$	51.81 (51.97)	7.00 (7.09)	21.89 (22.05)	46	130	Ethanol
6	C_2H_5		$C_{12}H_{20}N_4OS$	53.69 (53.73)	7.40 (7.46)	20.80 (20.90)	58	80	Ethanol

*Melting points were determined in open capillaries and are uncorrected.

Table I. Continued

Comp. No.	R	X	Mole. formula	Elemental Analysis (%) Found (Calculated) C	H	N	Yield (%)	Melt.* point (°C)	Recryst. solvent
7	CH_3		$C_9H_{12}N_4O_2S$	45.16 (45.00)	4.97 (5.00)	23.40 (23.33)	43	129-30	Ethanol
8	C_2H_5		$C_{10}H_{14}N_4O_2S$	47.30 (47.24)	5.47 (5.51)	22.00 (22.05)	45	182	Ethanol
9	CH_3		$C_{12}H_{20}N_4OS$	53.61 (53.73)	7.41 (7.46)	20.83 (20.90)	50	107	Ethyl acetate
10	C_2H_5		$C_{13}H_{22}N_4OS$	55.12 (55.32)	7.85 (7.80)	19.78 (19.86)	40	105	Ethyl acetate
11	CH_3		$C_{17}H_{28}N_4OS$	60.59 (60.71)	8.20 (8.33)	16.59 (16.67)	40	101	Ethanol
12	C_2H_5		$C_{18}H_{30}N_4OS$	62.53 (61.73)	8.49 (8.57)	15.89 (16.00)	46	81	Ethanol
13	CH_3		$C_{12}H_{20}N_4OS$	53.70 (53.73)	7.42 (7.46)	20.84 (20.90)	40	89	Ethanol
14	C_2H_5		$C_{13}H_{22}N_4OS$	55.21 (55.32)	7.75 (7.80)	19.79 (19.86)	46	101	Ethanol
15	CH_3		$C_{10}H_{18}N_4OS$	49.05 (49.58)	7.20 (7.44)	23.32 (23.14)	72	97	Ethanol
16	C_2H_5		$C_{11}H_{20}N_4OS$	51.32 (51.56)	7.65 (7.81)	21.74 (21.87)	70	75	Ethanol

jection of 0.1 ml of 1% carrageenan aqueous suspension into planter aponeurosis. Volume of the paw was measured plethysmographically immediately and after 3 hours of the injection of the irritants. The difference in the volume gives the amount of edema developed. Per cent inhibition of the edema between the control group and the compound treated group was calculated and comparaed with the group receiving standard drug phenylbutazone (30 mg/kg p.o.).

Diuretic activity

Diuretic activity was determined in male adult rats weighing 175~200 g of Sprague Dawley strain of Central Drug Research Institute, Lucknow, India according to the method of Kau *et al.* (1984). Rats were fasted overnight with free access to water and were given 5 ml/100 g body weight normal saline orally by specially designed cannula. Immediately after saline loading, each animal was placed in an individual hanging metabolic cage and urine was collected into a measuring cylinder hourly interval over a period of 5 hours. Osmolality (micro-osmometre), Na^+ & K^+ (flame photometer) in urine was measured. Animals whose osmolality, urine volume or electrolyte output were not within normal range, during initial screening, were discarded. Suitable rats were paired to obtain similar cumulative urine volume, osmolality and electrolyte levels. They were weighed, color coded and administered orally with test agents or standard drug suspended in 0.5% gum acacia in a volume of 1 ml/100 g. The urinary bladder was emptied by gentle compression of the pelvic area and by gentle pull of the tail and left in metabolic cages. After 5 hours the bladder were again emptied as before and urine volume determined. Furosemide (50 mg/kg) was taken as the standard. The results are expressed in terms of per cent urine volume excretion taking furosemide activity as 100%. One group of rats served as control. All animals were used twice a week.

Effects on cardio-vascular system

The effects on cardio-vascular system was evaluated by procedure described by Ghosh (1984). Cats of either sex were anesthetized by injecting pentobarbitione

Table II. Spectral data of synthesized compounds 1-16

Compound No.	IR (cm^{-1})	^{1}HNMR (ppm)
1	3250, 2850, 1690, 1625, 1525	1.60~2.20 (m, 4H, -CH_2CH_2-, pyrro); 2.50 (s, 3H, -CH_3td); 2.60~3.00 (m, 4H, -CH_2-N-CH_2- pyrro); 3.50 (s, 2H, -$COCH_2$-); 5.00-6.20 (br, 1H, NH, D_2O) exchangeable)
2	3185, 2905, 2895, 1700, 1637, 1505	1.40 (t, 3H, 3=7.5 Hz, -CH_2CH_3); 1.70~1.95 (m, 4H, -CH_2CH_2-, Pyrro.); 2.55~2.88 (m, 4H, -CH_2N-CH_2- pyrro.); 3.05 (q, 2H, J=7.0 Hz, -CH_2CH_3); 3.40 (s, 2H, -$COCH_2$-); 6.50~7.50 (br, 1H, NH)
3	3400, 3085, 3000, 1700, 1605, 1562, 775, 725	2.65 (s, 3H, CH_3); 3.45 (s, 2H, -$COCH_2$-); 3.85 (s, 4H, -$CH_2C_6H_5$); 7.30 (s, 10H, -$CH_2C_6H_5$), 8.00~9.00 (br, 1H, NH).
4	3285, 3090, 3015, 1690, 1600, 1580, 760, 720	1.32 (t, 3H, J=9.0 Hz, -CH_2CH_3); 2.95 (q, 2H, J=7.0 Hz, -CH_2CH_3); 3.30 (s, 2H, -$COCH_2$-); 3.67 (s, 4H, -$CH_2C_6H_5$); 7.23 (s, 10H, -$CH_2C_6H_5$); 7.90~8.10 (br, 1H, NH)
5	3250, 2860, 1705, 1633, 1560	0.90 (d, 3H, J=3.5 Hz, -CH_3 pipd.); 1.80~2.55 (br, 5H, -CH_2-CH-CH_2- pipd.); 2.65 (s, 3H, -CH_3 td.); 2.80~3.10 (br, 4H, -CH_2-N-CH_2-pipd.); 3.40 (s, 2H, -$COCH_2$-); 5.50-6.90 (br, 1H, NH).
6	3320, 2915, 2850, 1705, 1620, 1545	0.90 (d, 3H, J=3.0 Hz, -CH_3 pipd.); 1.25 (t, 3H, J=7.0 hz, -CH_2CH_3); 2.00~2.45 (br, 5H, -CH_2CHCH_2- pipd.); 2.60-3.05 (br, 4H, -CH_2-N-CH_2-pipd.); 3.10 (q, 2H, -CH_2CH_3); 3.35 (s, 2H, -$COCH_2$-); 6.50~7.80 (br, 1H, NH)
7	3400, 2985, 2850, 1700, 1670, 1605, 1560	1.30~1.95 (br, 6H, -$CH_2CH_2CH_2$- pyrro.); 2.55 (s, 3H, -CH_3 td.); 3.25 (s, 2H, -$COCH_2$-); 6.70~7.50 (br, 1H, NH)
8	3350, 2956, 2885, 1710, 1685, 1620, 1540	0.90 (t, 3H, J=7.5 Hz, CH_2CH_3); 1.35~2.15 (br, 6H, -$CH_2CH_2CH_2$- pyrro.); 2.85 (q, 2H, J=6.0 Hz, -CH_2CH_3); 3.30 (s, 2H, -$COCH_2$-); 5.40~6.90 (br, 1H, NH)
9	3300, 2910, 2860, 1690, 1610, 1520	0.90 (t, 3H, J=7.0 Hz, CH_2CH_3 pipd.); 1.20~2.00 (br, 8H, -$CH_2CH_2CH_2$- & -$CH_2^*CH_3$ pipd) 2.35 (s, 3H, -CH_3 td.); 2.40~2.90 (br, 3H, -CH_2-N-CH); 3.15, 3.40 (dd, 2H, -$COCH_2$-); 7.00~8.10 (br, 1H, NH).
10	3300~3210, 2950, 2885, 1700, 1615, 1535	0.90~1.15 (br, 6H, -CH_2CH_3); 1.20~1.90 (br, 8H, -$CH_2CH_2CH_2$- pipd. and -$CH_2CH_2CH_3$); 2.20~2.80 (br, 3H, -CH_2-N-CH pipd.); 3.10 (q, 2H, -CH_2CH_3); 3.25, 3.42 (dd, 2H, -$COCH_2$-); 5.60~6.90 (br, 1H, NH)
11	3250, 2910, 1700, 1620, 1555	1.10~1.70 (br, 12H, -$CH_2CH_2CH_2$- Cyclo hexyl group); 1.75~2.60 (br, 10H, CH_2-CH-CH_2- cyclohexyl group); 2.65 (s, 3H, -CH_3 td.); 3.20 (s, 2H, -$COCH_2$-); 8.00-9.05 (br, 1H, NH)
12	3250, 2930, 2910, 1690, 1620, 1535	0.90 (t, 3H, -CH_2CH_3); 1.15~1.70 (br, 12H, -$CH_2CH_2CH_2$- cyclohexyl groups); 1.80~2.55 (br, 10H, -CH_2CHCH_2- cyclo hexyl group); 3.00 (q, 2H, -CH_2CH_3); 3.25 (s, 2H, -$COCH_2$-); 6.80~7.60 (br, 1H, NH).
13	3200, 2930, 2880, 1690, 1600, 1540	1.05~1.60 (br, 6H, -$CH_2CH_2CH_2$- cyclo-hexyl group), 1.62~2.30 (br, 5H, -CH_2-CH-CH_2-, Cy. Hexyl group), 2.45 (S, 3H, -N-CH_3), 2.67 (S, 3H, -CH_3 td.), 3.30 (S, 2H, -$COCH_2$-), 5.40~6.80 (br, 1H, NH).
14	3340, 2925, 2900, 1700, 1615, 1545	0.85 (t, 3H, -CH_2CH_3 fused signal), 1.00~1.52 (br, 6H, -$CH_2CH_2CH_2$ - Cy.Hexyl group), 1.60~2.40 (br, 5H, -CH_2CH-CH_2-, Cy.hexyl group); 2.50 (S, 3H, -N-CH_3); 3.05 (q, 2H, J=6.5 Hz, -CH_2CH_3); 3.25 (S, 2H, -$COCH_2$-); 5.05~6.15 (br, 1H, NH)
15	3200, 1720, 1645, 1550	0.90 (t, 3H, CH_3); 1.35 (m, 6H, -$(CH_2)_3CH_3$); 2.05 (S, 3H, CH_3 td.); 2.70 (S, 2H, N-CH_3), 3.90 (S, 2H, -$COCH_2$-); 5.75 (br, 1H, NH, D_2O exchangeable)
16	3145, 1710, 1620, 1545	0.80 (t, 3H, CH_3); 1.00 (t, 3H, CH_3); 1.60 (m, 6H, -$(CH_2)_3CH_3$); 2.60 (br, 3H, N-CH_3); 3.20 (S, 2H, -$COCH_2$-); 7.50 (br, 1H, NH)

sodium (40 mg/kg i.p.). The femoral vein was exposed, cannulated and connected with a rubber tubing to a burette filled with normal saline. A middle incision was made on the neck to expose the trachea and one 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 mercury manometer with rubber tubing. A 10% w/v sodium citrate solution i.p. 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 effect recorded.

In-vitro smooth muscle relaxant activity

The smooth muscle relaxant activity of the compounds were determined by the method described by Burn (1952). A 2~3 cm long piece of ileum from a freshly killed guinea pig was suspended in a organ bath containing aerated Tyrode solution (pH 7.4) at 34°C. Contractions were recorded on a kymograph through a frontal writing lever. Spasmolytic activity of compounds was assessed by its ability to prevent the contraction induced by a sub maximal concentration (g/ml) of acetylcholine (2.5×10^{-8}), histamine (3.0×10^{-7}) or nicotine (2.5×10^{-6}). In all the isolated preparations, graded doses of the spasmolytic compounds were tested against the sub maximal concentrations of the spasmogen and the IC_{50} was calculated graphically in each experiment.

RESULTS AND DISCUSSION

The title compounds 2-(Substituted acetyl)-amino-5-alkyl-1,3,4-thiadiazoles were prepared by refluxing the 2-chloroacetyl amino-5-alkyl-1,3,4-thiadiazoles with appropriate amine in benzene for 5~6 hours. These were characterized by elemental analysis, IR and PMR spectroscopy (Table I).

The IR spectra of the compounds showed characteristic absorption band at 3300~3100 cm^{-1} [N-H], 1720~1680 [C=O], 1540 [C=N], 750~720 [substituted benzene] (Table II).

In the PMR spectra, the protons of -CO-CH_2- were observed between 3.00~3.90 ppm. Aromatic protons (phenyl group) were seen at about 7.15 ppm. The protons of -NH- were observed as a broad band between 4.50~9.00 ppm (Table II).

Lethal dose (LD_{50}) of the compound ranged from 147~1000 mg/kg intraperitoneally. So far as the anti-inflammatory activity is concerned, compound 11, 2-(N,N-dicyclohexyl-amino)-acetylamino-5-methyl-1,3,4-thiadiazole, showed 43% inhibition in rat paw-edema as compared to phenylbutazone (41%), while 5-ethyl subtituent showed only 35% inhibition (compound 12). The compound 13, 2-(N-cyclohexyl-N-methylamino)-acetylamino-5-methyl-1,3,4-thiadiazole, showed only 24% inhibition while 5-ethyl substitutent was inactive (compound 14). The compound 16, 2-(N-n-butyl-N-methylamino)-acetylamino-5-ethyl-1,3,4-thiadiazole, showed approximately equal activity (42% inhibition) as compared to phenylbutazone, but compound 15, with 5-methyl substituent, had no anti-inflammatory activity. Also the compounds with nitrogen atom did not have any anti-inflammatory activity (Table III).

Only compound no. 9, 10, 15 and 16 showed appreciable diuretic activity comparable to furosemide (50 mg/kg p.o.). The diuretic activity of compound 15, 2-(N-n-butyl-N-methylamino)-acetylamino-5-methyl-1,3,4-thiadiazole was 184%, while that of 5-ethyl substituted compound (16) was only 81%. The 2-(2-ethyl-piperidino)-acetylamino-5-methyl/5-ethyl-1,3,4-thiadiazole (compound 9 and 10) produced 85% diuretic acitivity. None of these compounds had any significant effects on

Table III. Pharmacological data of the synthesized compounds (1-16)

Compound No	LD_{50} mg/kg i.p.	Anti-inflammatory activity*	Diuretic activity#	IC_{50} (μg/ml) against spasmogen	
		% inhibition	(%)	Histamine	Acetyl choline
1	>1000	-	20	28.5±1.3	37.2±1.6
2	681	-	18	30.1±1.7	-
3	>1000	-	20	22.6±1.7	32.9±1.8
4	>1000	10	19	34.7±1.1	38.0±1.6
5	>1000	-	11	31.9±1.7	39.0±1.1
6	>1000	-	-	-	-
7	1000	-	-	-	26.9±1.4
8	1000	10	-	31.7±1.5	29.0±1.4
9	1000	-	85	-	-
10	1000	-	85	-	-
11	147	43**	-	22.5±1.4	20.1±1.4
12	681	35	-	4.0±0.3	6.0±0.6
13	681	24	-	31.0±1.5	20.5±1.0
14	681	-	-	24.8±1.2	33.9±1.7
15	464	-	184	12.0±1.1	13.1±1.7
16	681	42**	81	13.0±1.7	18.0±1.9
PB 30 mg/kg p.o.	-	41	-		-
FS 50 mg/kg p.o.	-	-	100		-

*dose 1/10th of LD_{50} p.o., #dose 1/5th of LD_{50} p.o., **p-value$\leq$0.05, PB=Phenylbutazone, FS=Furosemide

osmolality, Na^+ and K^+ concentration in urine.

The compounds under study showed, non-specific spasmolytic activity *in vitro*. The IC_{50} (μg/ml) values of the compounds ranged from 4.0~34.7 μg/ml against histamine and 6.0~39.0 μg/ml against acetylcholine.

No significant change in the blood pressure, heart rate and respiration was observed in the studies on the anaesthetized cat. Also the synthesized compounds failed to antagonize the effect of adrenaline, acetylcholine, isoprenaline and histamine *in-vivo*.

The series of derivatives of 1,3,4-thiadiazoles synthesized for the present study showed varied pharmacological effects such as anti-inflammatory, diuretic and spasmolytic activity. The compounds with substituted aliphatic amino- group showed anti-inflammatory activity but the compounds with cyclic nitrogen had no significant activity except for two compounds 9 and 10 showed weak diuretic activity. The compounds 15 and 16 containing N-*n*-buty-N-methyl amino-group showed significant diuretic activity. However, the compounds with N-cyclohexyl-N-methyl amino- and N,N-dicyclohexyl amino- group showed on diuretic activity. The compound having cyclic nitrogen atom showed weak non-specific spasmolytic activity as compared to disubstituted aliphatic amino compounds.

ACKNOWLEDGMENTS

The authors wish to thank the Head, Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar (M.P.) and Head, Division of Pharmacology, Central Drug Research Institute for providing necessary facilities. Thanks are also due to Head, RSIC, CDRI, Lucknow (U.P.) for providing spectral and microanalysis data. One of us (AKS) is grateful to council of Scientific and Industrial Research, Ministry of Science and Technology, New Delhi, India for financial assistance in the form of senior research fellowship.

REFERENCES CITED

Bhatnagar, S., Kamthan, D., Mehra, S. C. and Tandon, S. K., Synthesis of some new N^3-substituted hydantoin as potential anticonvulsant agent. *Indian J. Pharmac*, 18, 235-238 (1986).

Burn, J. H., *Practical Pharmacology*, Blackwell Scientific Publication, Oxford, pp.25-30, 1952.

Chapleo, C. B., Myers, M., Myers, P. L., Savilla, J. F., Smith, A. C. B., Stillings, M. R., Tulloch, J. F., Walter, D. S. and Welbourn, A. P., Substituted 1,3, 4-thiadiazoles with anticonvulsant activity. 1: Hydrazine. *J. Med, Chem.*, 29, 2273-2280 (1986).

Funatsukuri, G. and Ueda, M., 5-amino-1,3,4-thiadiazoles. *Chem. Abstr.*, 66, 46430f (1967).

Ghosh, M. N., *Textbook of Experimental Pharmacolgy*, J. Sinha and Co., Calcutta, pp.88-92, 1984.

Horn H. J., Simplified LD_{50} (or (ED_{50}) calculations. *Biometrics*, 22, 311-317 (1956).

Hussain, M. I., Kumar, A. and Shrivastava, R. C., Synthesis of N-(2-naphthyl oxyacetyl)-thiosemicarbazide and 2-aryl-amino-5-(2-naphthyloxy methyl)-1,3,4-thiadiazoles oxadiazoles as oral hypoglycemic agent. *Current Sci.* (India) 55, 644-646 (1986).

Kau, S. T., Keddie, J. R. and Andrews, D., *J. Pharmacol. Method*, 11, 67-75 (1984).

Mishra, P., Reddy, U. M. and Agarwal, R. K., Synthesis of Mannich bases of 2-amino-5-alkyl-1,3,4-thiadiazoles and their pharmacologial studies. *J. Inst. Chem.* (India), 61, 31-32 (1989).

Mishra, P., Shakya, A. K., Agarwal. R. K. and Patnaik, G. K., A few 2-(substituted acetyl) amino-5-alkyl-1, 3,4-thiadiazoles as CNS depressants. *J. Indian Chem. Soc.*, 67, 520-521 (1990a).

Mishra, P., Shakya, A. K., Agarwal, R. K. and Patnaik, G. K., Pharmacological screening of some new 2-(substituted acetyl) amino-5-alkyl-1,3,4-thiadiazoles. *Indian J. Pharmac.*, 22, 113-116 (1990b).

Muhi-Eldeen, Z., Al-Jawed, F., Eldin, S., Abdul-Kadir, S. and Ganotus, H., Carabet, M., Synthesis and biological evaluation of 2-(4-*tert*.-amino-2-butynyl)-thio-5-aryl-1,3,4-thiadiazoles. *Eur. J. Med. Chem.*, 17, 479-481 (1982).

Pandey, V. K., Lohani, H. C. and Agarwal, A. K., Studies on 3,6-dibenzoyl-1,4,-di 2'-(5"-alkyl)-1',3', 4'-thiadiazoyl-1,4-dihydro-1,2,4,5-tetrazines. *Indian Pharm. Sci.*, 44, 155-157 (1982).

Shakya, A. K., Patnaik, G. K. and Mishra, P., Synthesis and biological evaluation of 2-(substituted acetyl) amino-5-alkyl-1,3,4-thiadiazoles. *Eur. J. Med. Chem.*, 27, 67-71 (1992).

Winter, C. A., Risley, E. A. and Nuss, G. W., Carrageenan induced oedema in hind paw of rats as assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.*, 111, 544-547 (1962).