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PHARMACOLOGICAL SCREENING OF FEW NEW 2-(SUBSTITUTED ACETYL) AMINO-5-ALKYL-1,3,4-OXADIAZOLES

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Abstract: Nine new 2-(substituted acetyl) amino-5-alkyl-1,3,4-oxadiazoles were synthesised and confirmed on the basis of IR and nitrogen analysis. These were screened for spasmolytic, anti-inflammatory and their effects on blood pressure after determining ALD_{50}. Compounds GK-4 i.e. 2-(diethylaminoacetyl)- amino-5-methyl-1,3,4-oxadiazole and GK-8 i.e. 2-(din-propylamino acetyl)- amino-5-ethyl-1,3,4-oxadiazole were found to be spasmolytic. Compound GK-6 i.e. 2-(diethylaminoacetyl)- amino-5-n-propyl-1,3,4-oxadiazole was found to be a potent hypotensive agent with the effect lasting for more than two hours.

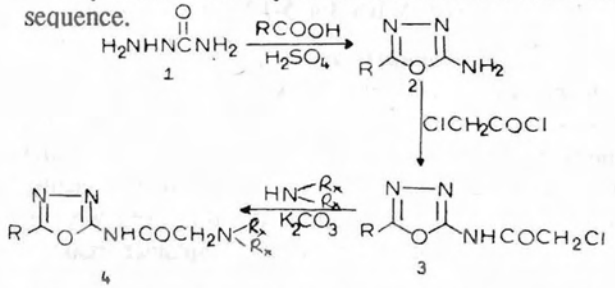
Key words: 1,3,4-oxadiazole spasmolytic hypotensive

INTRODUCTION

Our studies on 2 - (substituted acetyl)-amino-5-alkyl 1,3,4-thiadiazoles showed some of them to be CNS depressant (1) and anti-inflammatory agents (2) in addition to their being antihistaminics. Various biological activities like analgesic, anti-inflammatory (3), oral hypoglycemic (4), fungitoxic (5) and hypotensive (6) reported with 1,3,4,- oxadiazoles promoted us to synthesise a series of new 2-(substituted acetyl) amino-5-alkyl 1,3,4-oxadiazoles. Their pharma-cological screening for antiinflammatory, spasmolytic and effect on cardiovascular system were done to find out whether oxadiazoles too have parallel activities to thiadiazoles.

METHODS

Synthesis : The synthesis involved the following sequence.



Synthesis of 2-amino -5-alkyl - 1,3,4-oxadiazole (2) : Semicarbazide HCl (0.15 mole) was added to 100 ml of the fatty acid and maintained at 70-80°C for 2 hr. Concentrated sulphuric acid Analar (specific gravity 1.84, 10 ml) was added and the contents were heated to 90-100°C for another 3 hr. After cooling the reaction mixture was poured on crushed ice with constant stirring. It was made alkaline with potassium bicarbonate. The crude product thus separated was filtered and recrystallised from 70% aqueous methanol. Yield : 2a (R=methyl) (72%), m.p. 212-15°C, 2b (R=ethyl) (80%), m.p.–230-33°C, 2c (R=n-propyl) (85%), m.p. 200-03°C.

2-chloroacetylamino-5-alkyl-1,3,4-oxadiazole (3) : Chloroacetyl chloride (0.11 mole) was added dropwise to compound (2) (0.10 mole) in dioxane (40 ml). The reaction mixture was refluxed for 3 hours. After cooling the mixture was poured on crushed ice. The crude product thus precipitated was recrystallized from methanol (95% v/v). Yield : 3 a(R=methyl) (80%), m.p. 210-12°C, 3b (R=ethyl) (85%), m.p. 232-35°, 3c (R=n-propyl) (90%), m.p. 240-43°C.

2(substituted acetyl)-amino-5-alkyl-1,3,4-oxadiazoles (4) : A mixture of (3) (0.02 mole), appropriate secondary amine (0.04-0.05 mole), potassium carbonate

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(2 g) and benzene (25 ml) was refluxed for 4 hr at 60-70°C. The amine hydrochloride which crystallized out on cooling was filtered off. The filtrate was extracted several times with hydrochloric acid (1N). The combined acid extract was made alkaline with sodium hydroxide (1N) and extracted with diethylether several times.

The etheral extract was washed with ice cold distilled water. Ether was distilled off and last traces evaporated on hot water bath. The crude product was crystallized from aqueous methanol (90% v/v). All the compounds were analysed for nitrogen. All compounds showed characteristic IR bands at 3300-300 cm^{-1} (N-H), 1700-1680 cm^{-1} (C=O), 1660 cm^{-1}, 1080 cm^{-1} (1,3,4-oxadiazole nucleus), thus confirming their structures.

TABLE I : Physical data of compounds (4).

S. No.	Compound Code	-R	Rx	Molecular formula	m.p. °C	Yield %	Nitrogen % Found	Nitrogen % (calculated)
1	GK-1	Methyl	Methyl	$C_7H_{12}O_2N_4$	212	80	30.33	(30.43)
2	GK-2	Ethyl	Methyl	$C_8H_{14}O_2N_4$	237	83	28.14	(28.28)
3	GK-3	n-propyl	Methyl	$C_9H_{16}O_2N_4$	216-20	89	26.34	(26.42)
4	GK-4	Methyl	Ethyl	$C_9H_{16}O_2N_4$	168	75	26.30	(26.42)
5	GK-5	Ethyl	Ethyl	$C_{10}H_{18}O_2N_4$	205	78	24.67	(24.77)
6	GK-6	n-propyl	Ethyl	$C_{11}H_{20}O_2N_4$	209	65	23.18	(23.34)
7	GK-7	Methyl	n-propyl	$C_{11}H_{20}O_2N_4$	242	82	23.23	(23.34)
8	GK-8	Ethyl	n-propyl	$C_{12}H_{20}O_2N_4$	197	20	21.81	(22.04)
9	GK-9	n-propyl	n-propyl	$C_{13}H_{24}O_2N_4$	182	63	20.67	(20.89)

Pharmacological studies : 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 wherever mentioned. Hydrochloride salts of the compounds were used. 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 four animals each. These were then administered graded doses of the compounds (215, 464, 1000 and 2150 mg/kg i.p.) and observations made for mortality upto 24 hours. The lethal dose was then taken from the Horn's Table (7).

Smooth muscle relaxant activity (Spasmolytic Activity) : A 2-3 cm long piece of ileum from freshly killed guinea pig was suspended in an organ bath (20 ml) containing aerated Tyrode solution (pH 7.4) at 37°C. Contractions were recorded on a kymograph through a frontal writing lever. The effects of the compounds (1.0×10^{-6} M and 5.0×10^{-6} M) *per se* and on spasm induced by submaximal concentration of acetyl choline (6.25×10^{-8} M), histamine (6.25×10^{-8}) and serotonin (1.25×10^{-6} M) were studied.

Effect on cardiovascular system (9): Cats were anaesthetised by injecting pentobarbitone sodium (40 mg/kg, i.p.). The effect of the compounds (1.0 and 5.0 mg/kg, i.v.) on carotid blood pressure and respiration were recorded on a symograph. During the experiment the sympathetc nerve was also stimulated electrically (10 Hz, 1-5m sec, 5-10v for 5-10 sec.).

Anti-inflammatory activity (10) : Carrageenin induced paw oedema method was adopted. The albino rats were divided into group of five animals. Each compound was given (dose 1/10th of ALD_{50} P.O.) and the paw volume was determined plethysmographically. After one hr, the carrageenin (0.1 ml, 1.0% w/v) in sterile saline was injected into the subplanter tissue of

the rat's right hind paw. After three hr the paw volume was again measured.

Rectal temperature : Albino rats were divided into groups of 5 animals each. The rectal temperature of animals before and after drug adminstration were noted using a telethermometer (Aplab) at 1, 2 and 3 hr.

RESULTS

1. *Determination of ALD_{50}* : The ALD_{50} of the compounds ranged between 562-1000 mg/kg when administered intraperitoneally.
2. *Smooth muscle relaxant activity* : Compound GK-4 and GK-8 showed non-specific spasmolytic activity. The activity shown by compound GK-8 was significant (Fig. 1).

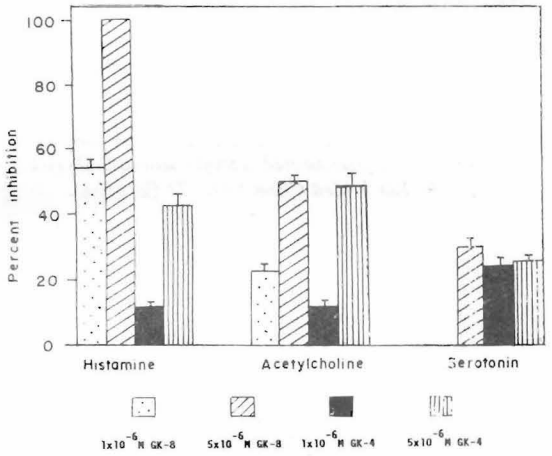

Fig. 1 : Showing the percent inhibition of the responses of guinea-pig ileum to Histamine, Acetylcholine and Serotonin by GK-8 and GK–4.

3. *Effect of Cardiovascular system:* None of the componds modified the effects of 2-4 µg/kg of adrenaline, acetylcholine, isoprenaline and histamine in -vivo. Compound GK-6 produced significant hypotensive activity. The maximum fall of blood pressure was 68 mm and 60 mm for two hr at the doeses level of 1.0 and 5.0 mg/kg respectively (Fig. 2).

   The compound GK-4 also showed lesser hypotensive activity. The blood pressure fall at 1.0 mg/kg was 40 mm (for 16 min) and at 5.0 mg/kg was 50 mm (for 20 min). No significant effect was observed on respiration.
4. *Anti-inflammatory activity* : None of the compounds showed anti-inflammatory activity.
5. *Rectal temperature* : None of the compounds showed any significant change.

DISCUSSION

Though the chain -NH–C(=O)–CH_2–N– is reported to have antihistaminic activity and spasmolytic activity (1,2,11) only compounds GK-4 and GK-8 showed these non-specific spasmolytic activities. However, none of the compounds showed any anti-inflammatory property which we had observed in the cases of compounds having the same chain with 1:3:4 thiadiazoles. Thus it is concluded that with the substitution of 1,3,4- oxadiazole molecules for 1,3,4-thiadiazole, the anti inflammatory property is lost.

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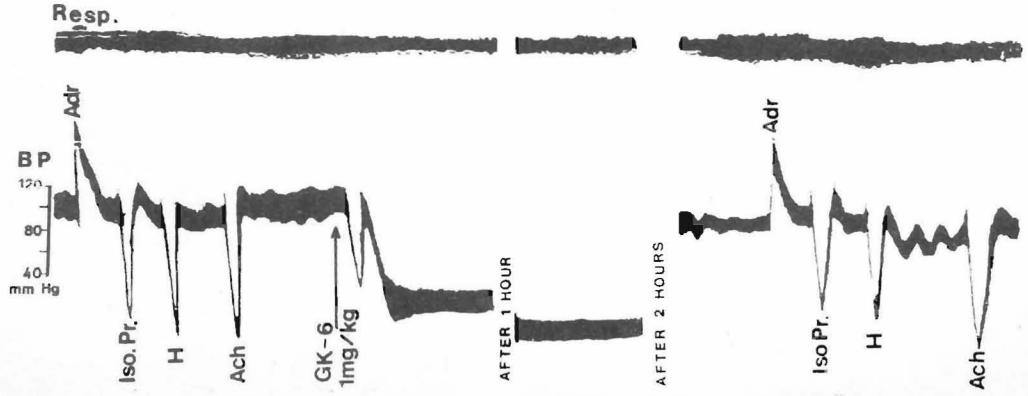

Fig. 2 : Showing the effect of GK-6, on respiration and blood pressure of anesthetised Cat. Note the responses to Adrenaline (Adr), Isoprenaline (Iso Pr), Histamine (H) and Acetylcholine (Ach) were not modified by GK-6.

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