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Indolyl Compounds as Antiinflammatory Agents

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Eight new ω-(indol-3-yl)alkane carboxamides were synthesized and studied for their antiinflammatory activities against carrageenin induced paw oedema in albino rats. Six compounds exhibited various degrees of antiinflammatory activity, two compounds showed no activity. The most potent compound of the present series, 1-(indol-3-yl)-*N*-{4-[1-(4-methylphenyl)piperazinyl]phenyl}acetamide (**7**) as well as phenylbutazone showed dose dependent inhibition of rat paw oedema. The ulcerogenic activities of compound **7** and phenylbutazone were also dose dependent. However, **7** showed a much lower ulcerogenic activity. In view of its potent antiinflammatory activity, minimal ulcerogenic liability and low acute toxicity compound **7** appears promising.

Indolylverbindungen als entzündungshemmende Substanzen

Acht neue Indolyl-3-alkyl-carboxamide wurden synthetisiert und auf ihre entzündungshemmende Aktivität an der carrageenininduzierten Entzündung der Pfoten von Albino-Ratten getestet. Zwei dieser Substanzen hatten keine entzündungshemmende Aktivität, die anderen sechs zeigten in unterschiedlichem Grad eine solche Aktivität. Die stärkste entzündungshemmende Aktivität zeigt Indolyl-3-methyl-1-aminophenyl-4'-(p-tolyl)piperazinocarboxamid (Substanz **7**). **7** besitzt wie auch Phenylbutazon eine ulcerogene Aktivität, jedoch ist diese bedeutend schwächer als die des Phenylbutazons. Substanz **7** scheint vielversprechend wegen ihrer schwächeren ulcerogenen Aktivität und ihrer niedrigen akuten Toxizität.

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Indolic compounds constitute an important group of nonsteroidal antiinflammatory agents. Some of these compounds viz. indole carboxanilides[1] and indometacin[2] have been shown to possess potent antiinflammatory activity. Moreover, indometacin is being used clinically for the treatment of various types of inflammatory processes. It is a misfortune of mankind that antiinflammatory potency imparts a high degree of ulcerogenic liability to most of the agents including indometacin.

In view of the potentialities of the indole nucleus, eight new indolyl-3-alkylcarboxamides were synthesized with a view to obtain new antiinflammatory agents with smaller ulcerogenic liability. These compounds were studied for their antiinflammatory activity against carrageenin induced rat paw oedema. The most active compound of the series was compared with phenylbutazone at three dose levels for the antiinflammatory activity and ulcerogenic potentialities. ALD_{50} of the compound was also determined.

Experimental

All the compounds as a matter of routine were checked by thin layer chromatography (TLC) on silica gel G. IR spectrum was recorded on Perkin Elmer infra cord in KBr pellets. UV spectra were determined in Pye – Unichem UV/visible spectrophotometer model SP 8 – 100 (solutions in ethanol).

Substituted phenylpiperazines[3], 1-(4′-aminophenyl-4-arylpiperazines, 1-(4′-aminophenylmorpholine[4] and 4-aminomorpholine[5,6] were prepared by the reported methods.

Indolyl-3-alkyl carboxamides

Dicyclohexylcarbodiimide (DCC,) 0.01 mole was added to a cooled solution of indole-3-acetic acid or butyric acid and substituted morpholine, piperazine or piperidine (0.01 mole) in 60 ml dry ethyl acetate. The reaction mixture was stirred at room temp. for 8 h. Dicyclohexylurea was filtered off and the filtrate was washed with 1N-HCl and 1N-Na_2CO_3 and finally with a saturated solution of sodium chloride and dried. Removal of the solvent yielded a residue which was dissolved in 20 ml dry benzene and kept for 2 h. The precipitate was filtered off and benzene was distilled off at reduced pressure, the residue obtained was crystallized from a mixture of benzene/petroleum ether. Analytical data of compounds thus synthesized are given in table 1.

Indolyl-3-methyl-(1′-(p-aminophenyl)-morpholino)-carboxamide (**2**) showed characteristic bands of -CO stretching (1685 cm^{-1}), -NH stretching (3350 cm^{-1}) and -CH_2 stretching (2875 cm^{-1}) in IR.

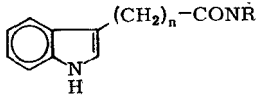
Indolyl-3-methyl-(2′-methyl piperidino)-carboxamide (**1**) showed a absorption maximum at 280 nm due to the carbonyl chromophoric group in the UV spectrum.

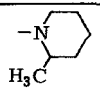
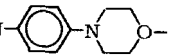
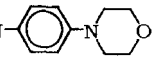
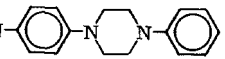
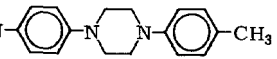
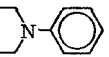
Biological studies

I. *Determination of antiproteolytic activity*

The antiproteolytic activity of the compounds was determined by the spectrophotometric method of *Kishore* et al.[7]. The reaction mixture consisted of 0.5 M tris-buffer (pH 8.2), 0.75 mg of crystalline trypsin (Sigma), $3.5 \cdot 10^{-5}$ M bovine serum albumin (BSA, substrate), glass distilled water and test compound in a total vol. of 1 ml. The compounds were tested at a final concentration of $5 \cdot 10^{-4}$ M, and were dissolved in dimethyl formamide (DMF). Equivalent amount of DMF was added to control tubes. The test compounds were preincubated with trypsin for 10 min at 37 °C prior to the addition of BSA and the reaction mixture was further incubated for 5 min. The reaction was stopped by addition

Table 1: *Indolyl-3-alkyl carboxamides and their antiproteolytic and antiinflammatory activity*



Compd. No.	n	–NR	M.P. °C	Yield %	Molecular formula	Antiproteolytic activity % inhibition $5 \cdot 10^{-4}$ M conc. (a)	Antiinflammatory activity % inhibition 50 mg/kg p.o.
1	1		120	52	$C_{16}H_{20}N_2O$	53.2	9.8
2	1		138	48	$C_{20}H_{21}N_3O_2$	65.3	10.7
3	3		101	46	$C_{22}H_{25}N_3O_2$	62.9	Nil
4	1		57	42	$C_{14}H_{17}N_3O_2$	72.1	5.8
5	3		82	45	$C_{16}H_{21}N_3O_2$	67.7	Nil
6	1		104	47	$C_{26}H_{26}N_4O$	70.2	9.6
7	1		118	44	$C_{27}H_{28}N_4O$	72.6	29.4
8	1		161	40	$C_{20}H_{21}N_3O$	55.1	13.4

[a] Values are the mean of two replicates. [b] All the compounds were analysed for N, analysis were found within limits.

of 0.5 ml of trichloroacetic acid (15 %, w/v). It was centrifuged and the acid soluble products of protein catabolism were determined by the method of *Lowry* et al.[8].

II. *Antiinflammatory activity*

Antiinflammatory activity was evaluated against carrageenin induced paw oedema in albino rats.

Adult albino rats of either sex weighing 80–120 g were divided into groups of six animals each. Freshly prepared suspension of carrageenin, 0.05 ml (1.0 % in 0.9 % saline) was injected under the planter aponeurosis of right paw of the rats by the method of *Winter* et al.[9]. One group was kept as control and the animals of other groups were pretreated with the test drugs suspended in gum acacia given orally 1 h before the carrageenin injection. The vol. of the foot was measured before and 3 h after carrageenin treatment by the micro pipette method as described by *Buttle* et al.[10]. The mean increase in paw vol. in each group was measured and percent antiinflammatory activity was calculated.

III. *Ulcerogenic activity*

Adult albino rats of either sex weighing 80–120 g were divided into groups of five animals each. Pregnancy was excluded and they were fasted for 24 h before the administration of drugs. Water was allowed ad libitum to the animals. The most active compound and phenylbutazone were given intraperitoneally and the animals were sacrificed 8 h following the drug treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of (a) shedding of epithelium (b) petechial and frank haemorrhages and (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

IV. *Acute Toxicity*

The compound with marked antiinflammatory activity against carrageenin induced oedema was investigated for acute toxicity (approximate LD_{50}) in albino mice.

Results and Discussions

Antiproteolytic activity

The compounds were tested for their antiproteolytic activity at a final concentration of $5 \cdot 10^{-4}$ M. The results are shown in Table 1. **7** showed maximum activity (72.6 %). The minimum activity (53.2 %) was found in indolyl-3-methyl-(3'-methyl piperidino)-carboxamide (**1**).

Antiinflammatory Activity

Six compounds of the newly synthesized indolyl-3-alkyl carboxamide series exhibited varying degree of antiinflammatory activity at 50 mg/kg oral dose. Two compounds (**3** and **5**) had no antiinflammatory activity. **7** showed most potent antiinflammatory activity (29.4 % at 50 mg/kg p.o. dose). Therefore **7** was compared with phenylbutazone at three dose levels for their antiinflammatory activity and ulcerogenic liability (table 2). Like phenylbutazone, **7** showed dose dependent inhibition of rat paw oedema (Fig. 1).

Ulcerogenic Activity

Graded doses of **7** and phenylbutazone were injected intraperitoneally in albino rats and incidence of gastric ulceration was determined (table 3). Fig. 2 shows the regression lines of **7** and phenylbutazone. The results show that the ulcerogenic activity of both agents is dose dependent.

Table 2: *Antiinflammatory activity of indolyl 3-methyl-1'-aminophenyl-4'-(p-tolyl)-piperazino-carboxamide* **(7)** *and phenylbutazone*

Compound	Dose mg/kg p.o.	Antiinflammatory activity % inhibition	Antiinflammatory ED_{30} mg/kg p.o.
Phenylbutazone	25	30.9	
	50	38.9	25.1
	100	50.2	
Compound **7**	50	29.4	
	100	31.0	56.2
	150	44.3	

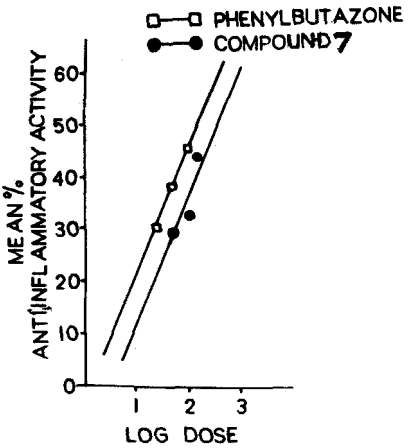


Fig. 1: Regression lines of antiinflammatory activity of phenylbutazone and compound **7**.

Table 3: *Ulcerogenic activity of indolyl-3-methyl-1'-aminophenyl-4'-(p-tolyl)-piperazino-carboxamide* **(7)** *and phenylbutazone*

Compound	Dose mg/kg	No. of rats with ulcer / No. of rats studied	% ulcer	UD_{50} mg/kg i.p.	Ulcer index
Phenylbutazone	50	4/10	40		16
	150	8/10	80	66.8	24
	250	10/10	100		28
Compound **7**	100	2/10	20		7
	200	4/10	40	251.1	16
	300	6/10	60		22

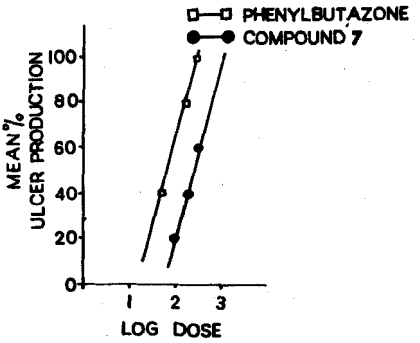


Fig. 2: Regression lines of ulcerogenic activity of phenylbutazone and compound **7**.

Acute Toxicity

The approximate 50 % lethal dose of **7** given orally was found to be more than 2000 mg/kg the maximum dose tested.

The results of the present investigation revealed that six out of eight compounds possess varying degree of antiinflammatory activity. An important feature of the present investigation was the potent dose dependent antiinflammatory activity of compound **7**. However, it was less potent than phenylbutazone (Table 2).

In view of its minimal ulcerogenic liability (UD_{50} – 25.12 mg/kg i.p.) as compared to that of phenylbutazone (UD_{50} – 66.83 mg/kg i.p.) and low acute toxicity (LD_{50} > 2000 mg/kg) the compound seems promising.

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