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STUDIES ON NON-STEROIDAL ANTI-INFLAMMATORY AND RELATED BIOLOGICAL ACTIVITIES OF 5-(INDAN-1'-YL)TETRAZOLES AND THEIR INTERMEDIATES*

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Abstract: Anti-inflammatory, analgesic and anti-pyretic activities of three new 5-(Indan-1'-yl)tetrazoles and anti-inflammatory activity of corresponding carboxamides were compared to those of standard drugs, phenylbutazone and aspirin. The results indicated 5-(Indan-1'-yl)tetrazole as the most promising compound in chronic anti-inflammatory and anti-pyretic tests.

Key words : indanyltetrazoles indanylcarboxamides anti-inflammatory analgesic anti-pyretic

INTRODUCTION

In our earlier studies, we reported on the synthesis and the anti-inflammatory activity of some indanylmethyltetrazoles (1, 2). Among these compounds 5-(6'-Methoxyindan-1'-yl)methyltetrazole and 5-(5', 6'- Dimethoxyindan-1'-yl)methyltetrazole exhibited promising anti-inflammatory and related biological activities. These findings prompted us to undertake further synthesis (3) and biological screening of 5-(Indan-1'-yl)tetrazoles and their intermediate carboxamides. Relatively poor performance of Indan-1-acetonitriles (4) led to exclusion of the corresponding - carbonitriles from screening programme.

METHODS

Male albino Charles Foster rats, 160 ± 10 g and 225 ± 25 g, were used for inflammation and pyrexia models respectively. Male albino Swiss mice (18 ± 2 g) were used for analgesic screening. National Research Council's guideline was followed for the care and use of all laboratory animals. They were maintained on standard laboratory animal feed (Lipton India) supplemented by fresh greens and clean tap water. The animals were used after acclimatization to the laboratory environment for at least 8 days. Only water was allowed *ad lib* during experiments.

Acute Anti-inflammatory Activity : Carrageenan-induced Oedema : The method was essentially that of Winter et al (5) with minor modifications (6). Test compounds and Phenylbutazone B.P. were dissolved in 50% aqueous Propylene Glycol B.P. with warming if necessary and fed orally. The paw volume upto a fixed mark at the level of lateral malleolus was measured before and after 1, 2, 3, and 24 hr of carrageenan (Marine Colloids, USA) administration. The average % increase in paw volume was calculated and compared against that of the control (vehicle-treated) group. Percent inhibition was calculated using the formula : % Inhibition = $(V_C - V_T) \times 100/V_C$, where V_C and V_T represent average paw volume in control group and treated group respectively. The results were analysed using Student's t-test.

Chronic Anti-inflammatory Activity : "established" Adjuvant-induced Arthritis : The method was essentially that of Newbould (7) with minor modifications. Random distributed rats injected with Freund's complete adjuvant (DIFCO, USA)

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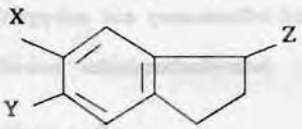
(0.05 ml, sc, plantar surface of right hind paw) were left untreated until day 14. Daily treatment (po) was then continued to 25th day. The effects of treatment were assessed by measurements of both hind paw volume and change in body weight. Severity of secondary lesions was arbitrarily assessed as absent-0, mild-1, moderate-2, moderately severe-3 and severe-4. Joint mobility was assessed by measuring the angle through which the hind paws could be moved easily (8). These records were made on day-0, -14, and then on every second or third day. Each animal served as its own control. The results of day-25 were compared with those of day-14 taking day-0 as base within the group. Percent improvement was calculated using the formula : % improvement = $[\{(V_{14} - V_0) - (V_{25} - V_0)\} \times 100] / (V_{14} - V_0)$, where V_0, V_{14} and V_{25} represent paw volume or joint mobility on day-0, -14 and -25 respectively. Results were analysed using Student's t-test for paired sets for % improvement in right and left paw volumes and joint mobility while Wilcoxon's signed rank test was employed for analysis of secondary lesion scores.

TABLE I : Structure and acute anti-inflammatory activity of Indan derivatives.



Compound No	*Structure substitutents*	*Dose mg/kg*	*Percent increase* in paw volumes ($\bar{X}$ ± SEM)*			
			1 hr	*2 hr*	*3 hr*	*24 hr*
RL-1	x=y=H z=$CONH_2$	100	NS	NS	76.5 ± 7.03[e] (18.64)	NS
		200	20.70 ± 2.95[a] (49.71)	47.55 ± 6.5[a] (39.82)	65.34 ± 2.95[a] (30.51)	39.28 ± 1.98[a] (27.23)
RL-2	x=OCH_3, y=H z=$CONH_2$	100	26.16 ± 3.11[a] (36.44)	60.61 ± 7.08[c] (23.29)	79.12 ± 7.55[f] (15.86)	40.66 ± 3.90[c] (24.68)
		200	24.45 ± 5.47[d] (40.59)	56.15 ± 3.78[a] (28.93)	65.34 ± 6.67[b] (30.51)	32.81 ± 4.14[a] (39.22)
RL-3	x=y=OCH_3 z=$CONH_2$	200	29.78 ± 3.14[c] (27.29)	60.98 ± 5.14[d] (22.82)	66.66 ± 6.18[b] (29.11)	35.84 ± 3.96[b] (33.61)
RL-4	x=y=H z=CN	Not Done				
RL-5	x=OCH_3, y=H z=CN	Not Done				
RL-6	x=y=OCH_3 z=CN	Not Done				
RL-7	x=y=H z=5-tetrazole	100	30.30 ± 0.66[a] (26.85)	53.01 ± 1.18[a] (32.91)	67.67 ± 1.69[a] (28.03)	37.08 ± 0.88[a] (31.31)
RL-8	x=OCH_3, y=H z=5-tetrazole	100	NS	NS	NS	NS
RL-9	x=y=OCH_3 z=5-tetrazole	100	NS	NS	NS	44.24 ± 1.31[a] (18.04)
Phynylbutazone		100	24.11 ± 1.00[a] (41.42)	34.11 ± 0.86[a] (56.83)	49.55 ± 1.88[a] (47.30)	43.59 ± 0.68[a] (19.25)
Control (50% Aq. Propylene Glycol)			41.16 ± 0.79	79.01 ± 1.18	94.03 ± 1.02	53.98 ± 0.67

*After carrageenan administration (in parantheses, % inhibition of oedema). Paw volume is expressed in change of height (in mm) of Hg-bath ($\bar{X}$ ± SEM) (6); n=6 in all groups.

a-f Probablity values (calculated as compared to control using Student's t-test) : a <0.001, b <0.005, c <0.010, d <0.025, e <0.05, f <0.10,
NS - Not Significant

All drugs were administered orally (in 50% propylene glycol) 1 hr before carrageenan administration.

Anti-pyretic Efficiency : Yeast-induced Pyrexia : A test similar to that of Loux *et al* (9) was used. Baker's yeast (Shaw-Wallace, India) was used. Rectal temperatures were recorded at hourly intervals upto 3 hr after dosing (po) with tetrazoles or phenylbutazone or Aspirin I.P. or vehicle. Mean temperatures after treatment were compared with those at 18 hr and expressed as 'Temperature - index' (10).

Analgesic Efficiency : Phenylquinone-induced Writhing in Mice: A test similar to that of Hendershot and Forsaith (11) was used. Phenylquinone (Sigma, USA) solution was prepared following Blumberg et al (12). Compounds were administered (sc) to pre-selected random distributed animals; control group received saline. Phenylbutazone and aspirin were used as reference standards. Data were analysed using Student's t-test for unequal groups.

RESULTS AND DISCUSSION

The results of carrageenan-induced oedema test (Table I) show that the test-compounds exhibit variable anti-inflammatory activity, and a few among them retain significant residual anti-inflammatory activity at 24 hr after a single selected oral dose. A closer look reveals that RL-2 possesses comparatively high initial anti-inflammatory activity; this activity, however, declines rapidly while RL-7 exhibits almost uniform appreciable anti-inflammatory activity over the hours. Both the compounds retain significant residual anti-inflammatory activity at 24 hr. Because of these interesting features RL-2 and -7 were further tested in a chronic inflammatory model and both the compounds exhibited significant activity in the \'established' adjuvant-induced arthritis model in rats. In this biomodel RL-2 was comparable to phenylbutazone in reducing primary inflammation of the right hind paw but unlike phenylbutazone effectively reduced secondary inflammation of the left hind paw and both were ineffective in increasing joint-mobility, while RL-7 was found to be effective in all respect. None of the agents was, however, capable of reducing secondary lesions (Table II-IV).

Relatively poor performance of the carboxamides as anti-inflammatories prompted us to exclude them from other screening programmes.

TABLE II: Curative anti-inflammatory activity of Indan derivatives : Effect on paw volume of "established" adjuvant - induced arthritic rats.

Compound	*Right Paw volume*				*Left paw (non-injected) volume*			
	0 day	*14 day*	*25 day*	*% Improvement on 25 day*	*0 day*	*14 day*	*25 day*	*% Improvement on 25 day*
RL-2	1.75±0.04	3.92±0.20	3.45±0.27	23.18±5.38[d]	1.76±0.03	2.53±0.05	2.33±0.07	25.70±6.55[d]
RL-7	1.85±0.05	3.54±0.08	3.02±0.08	31.41±2.16[a]	1.90±0.04	2.49±0.09	2.22±0.07	44.75±3.25[b]
Phenylbutazone	1.83±0.03	4.44±0.38	3.78±0.27	25.16±2.17[b]	1.83±0.06	2.44±0.16	2.30±0.09	NS
Vehicle (50% Aqueous Propylene Glycol)	1.71±0.05	3.75±0.06	3.99±0.08	-11.44±0.69[a]	1.69±0.06	2.57±0.06	2.73±0.07	-19.20±1.17[a]

All compounds were administered (100 mg/kg) po in 50% aq. propylene glycol.
Paw volume is expressed in change of height (in mm) of Hg-bath (X ± SEM) (6); n = 6, except vehicle treated group (n = 9).
a - d probability values as under Table I but calculated as compared to 14th day within the group.

In phenylquinone-induced writhing test, none of the tetrazoles was comparable to the reference drugs, phenylbutazone or aspirin (Table V). RL-7 was found to be quite active as an anti-pyretic agent in yeast-induced pyrexia model in rats (Table VI) which corroborates our earlier observation that indanylmethyltetrazoles are better anti-pyretics than analgesics (2).

TABLE III: Curative anti-inflammatory activity of Indan derivatives : Effect on joint - mobility of "established" adjuvant - induced arthritic rats.

Compound	*Right paw mobility (°) Loss of Mobility*				*Left paw (non-injected) mobility (°) Loss of mobility*			
	0 day	*14 day*	*25 day*	*% Improvement on 25 day*	*0 day*	*14 day*	*25 day*	*% Improvement on 25 day*
RL-2	118.83 ±1.42	26.17 ±6.70	24.84 ±3.70	NS	118.83 ±1.42	5.50 ±2.23	18.17 ±3.52	-
RL-7	123.00 ±2.94	43.17 ±6.69	22.50 ±3.03	43.96±9.24[c]	119.67 ±1.94	12.33 ±2.09	7.17 ±2.14	44.98±7.90[b]
Phenylbutazone	117.17 ±1.08	30.17 ±11.06	30.50 ±5.12	NS	117.17 ±1.08	8.50 ±2.73	15.30 ±2.60	-
Vehicle (50% aq. propylene glycol)	119.11 ±1.34	24.56 ±3.06	26.00 ±2.98	NS	121.78 ±1.96	11.11 ±2.69	24.22 ±3.20	-

Paw mobility is expressed in degrees
See foot notes to Table II

Experimental results make it clear that RL-7 is the most promising anti-inflammatory test agent in both acute and chronic inflammatory models at the dose level tested. The curative anti-inflammatory activity of the compound as demonstrated in 'established' adjuvant-induced arthritis model may possibly be due to its longer duration of action as observed in the carrageenan-induced oedema test.

The beneficial effects of treatment with RL-2 and -7 were also reflected in relatively higher weight gain compared to that observed in the vehicle-treated group. Both the agents may possibly be less toxic than phenylbutazone as evinced from weight gain (Table IV) and gross observations.

From the pharmacological activity profile of the test agents revealed in this investigation, RL-7 may be considered as the most promising agent among these and is worthy of further investigation.

TABLE IV: Curative anti-inflammatory activity of Indan derivatives: Effect on body weight and secondary lesion score of "established" adjuvant-induced arthritic rats.

	Av. Secondary lesion score			*Body weight ($\bar{X}$, g/100 g change*		
	14 day	*25 day*	*P*	*0 day*	*14 day*	*25 day*
RL-2	1.5±0.22	2.17±0.40	NS	158.3±4.2	+ 5.47	+ 14.84
RL-7	1.17±0.17	1.50±0.43	NS	161.2±4.0	+ 2.07	+ 14.99
Phenylbutazone	2.67±0.49	2.83±0.48	NS	157.5±5.6	+ 5.18	+ 8.25
Vehicle (50% aq. propylene glycol	1.78±0.32	4.00±0.00	< 0.01	166.0±5.34	+ 2.33	+ 6.59

P was calculated using Wilcoxon's signed rank test.
See foot notes to Table II

TABLE V : Analgesic activity of Indan derivatives : Effect on phenyl-p-quinone-induced writhing in mice.

Compound	*n*	*No. of writhing ($\bar{X}$ ± SEM)*	*% Reduction from control*	*P (Difference from control)*
RL-7	44	34.7 ± 3.4	28.2	<0.001
RL-8	39	36.1 ± 3.6	25.3	<0.001
RL-9	30	38.3 ± 4.1	20.7	<0.001
Aspirin	15	19.2 ± 1.6	60.2	<0.001
Phenylbutazone	36	23.1 ± 2.7	52.2	<0.001
Control (Saline)	104	48.3 ± 2.5		

All compounds were administered (100 mg/kg) sc, dissolved in aq. NaOH I.P. (pH 7.5 ± 0.2)

TABLE VI : Anti-pyretic activity of Indan derivatives : Effect on yeast-induced pyrexia in rats.

Compound	*Mean Temperature (°C) after hr of compound administration*				*TI*
	0	1	2	3	
RL-7	39.3	38.2	38.3	38.0	- 3.4
RL-8	39.4	38.7	38.5	38.5	- 2.5
RL-9	39.5	39.2	39.0	39.0	- 1.3
Aspirin	39.3	38.5	38.4	38.5	- 2.5
Phenylbutazone	39.4	38.6	38.2	38.4	- 3.0
Control (pyretic)	39.4	39.5	39.4	39.4	+ 0.1
Control (nonpyretic)	37.5	37.7	38.0	38.0	+ 1.2

Values are mean (n = 6)
All compounds were administered (100 mg/kg) po dissolved in aq. NaOH I.P. (pH 7.5 ± 0.2)

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