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Research Paper :

Synthesis of N-pyrimidino benzamide-2-carboxylic acid and evaluation of its antimicrobial and anti-inflammatory activities

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ABSTRACT

N-pyrimidino benzamide-2-carboxylic acid (NPBCA) has been synthesized. The structure of the synthesized compound has been established by using elemental analysis, molecular weight determination and infrared spectral studies. Antifungal activity of the compound has been screened on common fungi *viz*., *Aspergillus niger, Aspergillus nidulense* and *Candida albicans* at 28^{0}C and antibacterial activity has been determined on gram-positive (*Staphylococcus aureus*) and gram-negative (*Esherichia coli*) bacteria at 37^{0}C. The compound significantly inhibited 21.6 % carrageenan induced rat paw oedema at 100 mg/kg p.o. dose (LD_{50} > 400 mg/kg). It, however, fails to inhibit cotton pellet induced granuloma formation and oedema in adjuvant arthritis test (non established), upto a dose of 200 mg/kg p.o. It elicited ulcerogenic effect at 100 mg/kg dose.

Key words : Benzamide derivative, Anti-inflammatory activity, Ulcerogenic acivity, Biocidal activity

The studies on inflammation and anti-inflammatory drugs have received sufficient attention, yet a satisfactory treatment is not available. Hence, the search for an ideal anti-inflammatory agent still continues. Substituted pyrimidines have been reported to possess anti-inflammatory activity with side effects, specially gastric irritation, equal to anthranilic acid derivatives. Keeping above in view and continuing our studies[1-7], N-pyrimidino benzamide-2-carboxylic acid has been synthesized. We have tried, however, to draw particular attention to physiological and biochemical aspects of the present selection without dwelling on its specific physicochemical approach to the thermodynamic studies which also, of course important facts of these studies.

MATERIALS AND METHODS

All the chemicals used were of analytical reagent grade and purified further either by recrystallization or by distillation.

Synthesis of N-pyrimidino benzamide-2-carboxylic acid :

N-pyrimidino benzamide-2-carboxylic acid was synthesized by the method reported earlier[8]. The observations are, melting point 69-71^{o}C, found %C = 59.08;

H = 3.76; N = 17.23; $C_{12}H_9N_3O_3$ calculated %C = 59.26; H = 3.73; N = 17.28;

Molecular weight found 234; calculated 243.

Physical measurements :

N-pyrimidino benzamide-2-carboxylic acid (NPBCA) was analysed for carbon, hydrogen and nitrogen by standard method[9]. Molecular weight of the synthesized compound was determined by the cryoscopic method in dimethylsulfoxide (DMSO). Infrared spectra were recorded using Perkin Elmer spectrophotometer model-521.

RESULTS AND DISCUSSION

The synthesized compound (NPBCA) was found thermally stable and insoluble in water. It varies in its solubility in most common organic solvents.

Infrared spectral studies :

The study of infrared spectra showed different bands due to the stretching frequencies of active groups. The amide I band appearing at 1730 cm^{-1} indicated the presence of carbonyl oxygen of the amide group[10-11]. A band at 3340 cm^{-1} is observed due to NH stretching vibrations[12]. The carboxylic group stretching frequency vibrations of the compound has been found[13] at 1690 cm^{-1}. Ring breathing modes at 1575 cm^{-1}clearly showed the presence of pyrimidine ring in the synthesized compound[14].

Microbial studies :

The synthesized compound (NPBCA) was screened for its antimicrobial activity on three common fungi *viz*., *Aspergillus niger, Aspergillus nidulense* and *Candida albicans* at 28^{0}C and on both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria at 37^{0}C by serial dilution method[15]. The minimum inhibitory concentration values are given below.

Table : Antimicrobial activity of the synthesized compound (NPBCA)

Fungi/bacteria	MIC*
Aspergillus niger	49.65
Aspergillus nidulense	47.10
Candida albicans	44.55
Staphylococcus aureus	47.75
Escherichia coli	48.95

* Values are minimum inhibitory concentration in µg/ml

Table depicts that the compound is active against the bacteria and fungi used. The proper temperature, pH and the necessary nutrients and growth media free from other microorganisms have been provided for the preparation of the cultures of pathogenic bacteria and fungi, using aseptic techniques[16]. The culture media used for the slant and broth was sterilized by a moist heat sterilization method[17]. The incubating period for fungi was 96 hours (*Aspergillus niger* and *Aspergillus nidulense*) and 48 hours (*Candida albicans*) at 28^{0}C. The incubating period for bacteria was 24 hours at 37^{0}C. It was found that its activity increases with increase in concentration. A comparatively faster diffusion of the compound as a whole through the cells may be one of the important factors. It is evident that the compound is stable and chemically inert having no specific active center. Such compound can exert a powerful inhibitory effect on an intracellular biological process by concentrating at the susceptible site from which it slowly dissolves.

Anti-inflammatory activity :

Overnight fasted male rats weighing between 100-140 g were arranged in three groups of 8 each, one group serving as control.

Carrageenan induced rat paw oedema test :

The test compound NPBCA (100 mg/kg) and standard drug oxyphenbutazone (50 mg/kg) was given intraparitoneally in a 1 % carboxymethyl cellulose suspension. For screening, the method adopted was that of Winter *et al.*[18] with some modifications.

Oedema was induced by injecting 0.1 ml of 1 % carrageenan suspension in normal saline into the plantar aponeurosis of right paw. The NPBCA and oxyphenbutazone was given 1 hour before the carrageenan challenge. The paw volume was measured before and 1hrs, 2 hrs, 3 hrs, 4 hrs and 6 hrs after carrageenan injection by volume differential meter (M 7101, Ugo Basile, Milan, Italy). The percentage inhibition was calculated.

Cotton pellet granuloma test :

Pellets of sterilized (in an air oven for 2 hours) surgical cotton weighing 9.0 ±0 1 mg were implanted in both the axillae and groins under light ether anesthesia according to the method of Meier *et al.*[19]. The drug (N-pyrimidino benzamide-2-carboxylic acid) was given intraperitoneally in the doses of 100/200 mg/kg, oxyphenbutazone at the dose level of 50/80 mg/kg and ibuprofen at the dose level of 34 mg/kg daily for six days. The pellets were dissected out on the 7th day under light anesthesia, kept separately in small glass vials, dried for 2 hours at 150^{0}C and weighed after cooling. Percentage inhibition was calculated.

Adjuvant arthritis (Non-Established) :

Rats were injected 0.1 ml of freund's adjuvant complete (Difco) into the plantar aponeurosis[20]. NPBCA (200 mg/kg) and Ibuprofen (34 mg/kg) were administered orally for 14 days daily as a suspension in carboxy methyl cellulose. The paw volume of the injected paw and uninjected paw and uninjected paw was measured on days 0, 2, 4, 6, 8, 10, 12 and 14 using a water plathysmometer (M 7151, Ugo Basile, Milan, Italy).

The percentage inhibition of NPBCA have been given in the following table :

The above table indicates that NPBCA has exhibited anti-inflammatory activity in Carrageenan oedema test and Cotton pellet granuloma test. Though the activity was much less than oxyhenbutazone.

Compound	Dose mg/kg	Percentage inhibition		
		Carrageenan Oedema	Adjuvant Arthritis (Non Estab.)	Cotton Pellet Granuloma
NPBCA	100	21.6	-	-
	200	-	18.1	23.7
Oxyphenbutazone	50	51.2	-	-
	80	-	-	45.6
Ibuprofen	34	-	34.9*	-

(-) Not tested (*) $p < 0.01$

Ulcerogenic effect in rats :

Male 24 hours fasted rats (140 ± 10 g) were used with free access to water. The test was carried out as per method of Dhawan *et al.*[21]. NPBCA and reference standard (oxyphenbutazone) was administered orally as suspension in carboxymethyl cellulose. Animals were sacrificed 6 hours after giving NPBCA/oxyphenbutazone and their stomachs were removed, opened carefully along the larger curvature, washed and examined under a binocular steroscopic microscope (Meopta). The severity of the lesions appearing in the muscular portion were scored as follows :

0 - Normal
1 - Haemorrhagic effusion
2 - Mucosal ulceration
3 - Deep ulceration
4 - Perforated ulcers

The ulcerogenic index (UI) is calculated as follows:

$$UI = \frac{ADU \times \% RU}{100}$$

where ADU = Average degree of ulceration
% RU = Percentage of rats with ulcer

Compound	Dose Mg/kg p.o.	ADU	% RU	UI
Oxyphenbutazone	50	2.0	100	2.00
	80	4.0	100	4.00
NPBCA	100	1.5	75	1.12
	200	3.0	100	3.00

The above said table indicated that NPBCA is much less ulcerogenic than oxyphenbutazone.

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