ANTI-ARTHRITIC AND ANTI-INFLAMMATORY EFFECT OF SOME NEW ISOXAZOLINE ANALOGS: IT'S MECHANISM OF ACTION

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ABSTRACT

Anti-arthritic and anti-inflammatory action of a novel 4-(5′-substituted-aryl-4′,5′-dihydro-isoxazole-3′-yl-amino) phenols was compared with standard anti-inflammatory drugs in different in vivo and in vitro test models. Treatment with New Isoxazoline Analogs significantly reduced adjuvant-induced non- established and established arthritis in rats and the effect was comparable to Indomethacin. It also provided significant protection against protein denaturation in the in vitro models, Results were comparable to Indomethacin. It is concluded that Synthesized Isoxazoline analogs may prove to be a useful anti-arthritic product in future.

Key words Arthritis, Isoxazoline analogs

1. INTRODUCTION:

Rheumatoid arthritis is a major ailment among rheumatic disorders. It is a chronic condition with multiple causation and affects the people in their most active period of life. The deformities that may develop due to the chronic forms stand as the greatest crippler of mankind (Chaturvedi GN and Singh RH, 1965). A large number of NSAID are *in vogue* for the treatment of various types of arthritis'. A systematic investigation of this class of heterocyclic lead revealed that isoxazole containing pharmacoactive agents play important role in medicinal chemistry. It has been reported that isoxazolines possess analgesic, anti-inflammatory activities(Rani P, 2003; Habeeb G A, 2001; Habeeb G A, 2000; Safak C, 1992). Marketed Valdecoxib as an Isoxazoline derivatives, was reported to be significantly effective in different sets of anti-arthritic and anti-inflammatory experiments in domesticated and laboratory animals. Toxicity study revealed innocuous nature of this product when administered in rats even at ten times higher than recommended dose for a period of 30 days. In view of these above fact, an attempt has been made for Evaluating of novel 4-(5′-substituted-aryl-4′,5′-dihydro-isoxazole-3′-yl-amino) phenols possessing potent biological activities. The synthesized compounds were tested for their possible anti-inflammatory and Protein Denaturation activities.

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3. MATERIALS AND METHODS

The present study is aimed at determining the antiarthritic effect and mechanism of action of novel 4-(5′-substituted-aryl-4′,5′-dihydro-isoxazole-3′-yl-amino) phenols *in* vivo and *in vitro* pharmacological models. *In vivo* anti-arthritic effect of novel 4-(5′-substituted-aryl-4′,5′-dihydro-isoxazole-3′-yl-amino) phenols was assessed using adult male albino rats of Wistar strain (approx.wt: 150 gm). The animals were grouped in plastic cages in a centrally air conditioned room at an ambient temperature of 22± 1°C with 12 hours light and dark cycle.

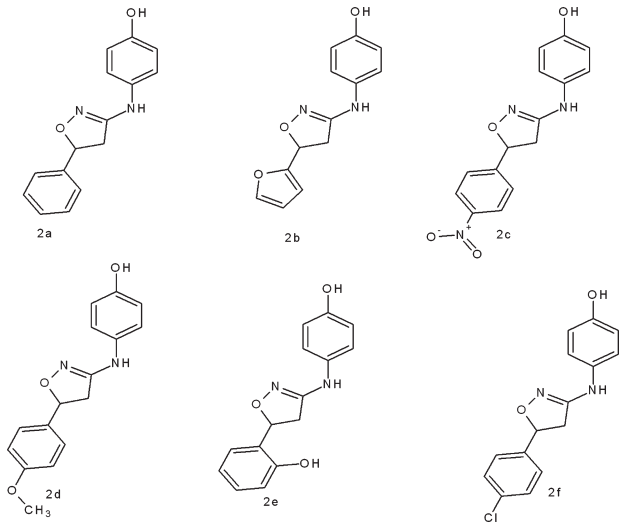
Anti-inflammatory Activity

Anti-inflammatory activity was determined by carrageenan-induced rat paw edema method (Winter CA, 1968) in albino rats (n=6) of either sex (100-140 g). Indomethocin (10 mg/kg) was administered as a standard drug. The test compounds were administered (100 mg/kg) orally 30 min. prior to the administration of carrageenan in the right hind paw of the rats. The paw thickness was measured using vernier callipers at 30, 60, 120 and 180 min. after carrageenan administration. Results are presented in Table 1.

Inhibition of protein denaturation

The reaction mixtures (0.5 mL) consisted of 0.45 ml bovine serum albumin (5 % aqueous solution) and 0.05 mL of synthesized compound (100 ìg/mL of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCI. The samples were incubated at 37 °C for 20 min. and then heated at 57 °C for 3 min. After cooling the samples, 2.5 mL phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm (Chatterjee S and Das SN, 1996). For control tests 0.05 mL distilled water was used instead of synthesized compound while product control tests lacked bovine serum albumin. The results are presented Table 2.

Structure of the New Isoxazoline analogs



3. RESULTS AND DISCUSSION:

Anti-arthritic effect of novel 4-(5′-substituted-aryl-4′,5′-dihydro-isoxazole-3′-yl-amino) phenols against adjuvant induced Inflammation is shown in Table 1. It significantly reduced the diameter of hind paw edema as compared to the untreated control rats, .Effect of synthesized compounds on inhibition of protein denaturation, is shown in Table 2.Isoxazoline analogs at dose levels (100 ìg/mI) provided significant protection against denaturation of proteins.Denaturation of proteins as one of the causes of rheumatoid arthritis is well documented". Production of auto antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins'*.The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Grant NH, 1970). From the results of the present study it can be stated that Synthesized Isoxazoline analogs is capable of controlling the production of auto antigens due to in vivo denaturation of proteins in rheumatic diseases. This finding justifies the usefulness of this product in the management and treatment of inflammation associated diseases like arthritis. From the results obtained in the present studies, it may be concluded that novel 4-(5′-substituted-aryl-4′,5′-dihydro-isoxazole-3′-yl-amino) phenols possesses significant anti-arthritic and anti-inflammatory action, which is comparable to synthetic anti-inflammatory agents. Further clinical studies are needed to establish its safety and usefulness in arthritic patient.

Table 1:- Anti-inflammatory activity carrageenan induced Rat Paw Oedema method of compounds 2a-f.

Compound Code	Dose (mg/kg)	(Percentage of activity) 30 min.	1 hour	2 hour	3 hour
2a	100	27± 0.19 *	34± 0.26 *	40± 0.13 *	29± 0.11 *
2b	100	30± 0.11 *	36± 0.17 *	41± 0.28 *	32± 0.29 *
2c	100	28± 0.33 **	32± 0.61 *	39± 0.68 *	28± 0.41 *
2d	100	25± 0.47 *	31± 0.37 **	34± 0.66 **	26± 0.92 **
2e	100	24± 0.21 *	33± 0.46 *	38± 0.61 *	27± 0.71 *
2f	100	27± 0.11 **	31± 0.28 **	35±0.41 **	26± 0.62 **
Indomethacin	10	52.94±0.023***	55.69±0.023***	50.00±0.005***	44.68±0.009***

Results are expressed in mean ± SEM. (n=6) significance levels * P<0.05, ** P < 0.01 and *** P < 0.001 as compared with the respective control.

Table 2 In vitro anti-inflammatory screening of synthesized compounds by Inhibition of bovine serum albumin denaturation

Compound	Absorbance value	Inhibition of denaturation *Mean (%)
2a	0.094	6.81
2b	0.099	12.5
2c	0.121	37.5
2d	0.117	32.95
2e	0.138	56.81
2f	0.141	60.22
Indomethacin	0.149	63.63
Control	0.088	00

*Average of Three reading

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