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Evaluation of Biological Activity (*In-Vitro*) of Some 2-Phenyl Oxazoline Derivatives

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ABSTRACT

2-phenyl Oxazolones were prepared from hippuric acid, substituted aromatic aldehydes, acetic anhydride and sodium acetate. Further reacting with isoniazid in presence of methanol yields various Schiff's bases of oxazoline derivatives were synthesized. The novel Schiff's bases were also screened for *in-vitro* antibacterial, antifungal activity by agar cup plate method and *in-vitro* anti-inflammatory activity was evaluated by HRBC membrane stabilization method.

INTRODUCTION

The oxazoline ring is an important constituent of bioactive natural products and pharmaceuticals. The oxazoline ring presents an interesting structure on which to build a wide variety of compounds having antioxidant, antimicrobial, anti-inflammatory, antimalarial, antitumor, antiviral, antitubercular, antipyretic and CNS stimulant activities. [1]

The increasing resistance of human pathogens to current antimicrobial agents is a serious medical problem. Many of the drugs currently available have undesirable effects and might be toxic. Considering the fact that the available antimicrobial agent originates from a limited number of sources and that most of them have similar modes of activity, it is very important to explore additional sources for substances with potential antimicrobial activity, which could possibly have different modes of activity or affect different sites in the bacterial and fungal cells. [2]

Inflammation is a basic mechanism by which the body responds to infection, irritation or other injury of the body cells and tissues, and the key feature being redness, warmth, swelling and pain. The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. Nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC membrane is similar to lysosomal membrane, the study was undertaken to check the stability of HRBC membrane by these synthetic organic compounds to predict the anti-inflammatory activity. [3]

MATERIALS AND METHODS

Antibacterial activity [4]

The newly synthesized compounds were screened for their antibacterial activity using agar cup plate method. The antibacterial activity of test compounds was evaluated against gram-positive bacteria, *Staphylococcus aureus* and gram-negative bacteria, *Escherichia coli*. A test tube containing sterile melted top agar (1.5%) previously cooled to room temperature with 0.2mL suspension of the test culture, mixed methodically and poured in the Petri dish containing sterile base agar medium (autoclaved at 121°C for 15min.) then allowed to solidify. With the help of

sterile cup-borer, five and six cups in the agar-plate were marked and were injected with 0.1mL of test solution, 0.1mL of standard solution and 0.1mL of DMSO solvent respectively. Then the plates were allowed to diffuse for 20min. in refrigerator at 4-5°C. The plates were then incubated in upright position at 37°C for 24 hrs. After incubation, the relative susceptibility of the microorganisms to the potential antimicrobial agent is demonstrated by a clear zone of growth inhibition around the cup. The inhibition zone caused by the various compounds on the microorganisms was measured and the activity was rated on the basis of the size of the inhibition zone.

Antifungal activity [5]

The newly synthesized compounds were screened for their antifungal activity using agar cup plate method. The antifungal activity of test compounds was evaluated against *Aspergillus brasiliensis* and *Candida albicans*. The plates were inoculated by dipping a sterile swab into inoculums. The inoculation was dried at room temperature in aseptic condition. The plates were bored and prepared test solutions were added. These plates were placed in an incubator at 22°C within a few minutes of preparation. After 7 days of incubation, the diameter of zone of inhibition was measured in mm.

Anti-inflammatory activity [6]

Preparation of HRBC suspension in isosaline:

The human erythrocytes suspension was used for the *in-vitro* membrane stabilization assay. Blood was collected from healthy volunteers who had not consumed any NSAIDs for two weeks prior to the experiment. Fresh whole human blood (5mL) was collected and transferred to the centrifuged tubes containing EDTA to prevent clotting. The tubes were centrifuged at 3000rpm for 10min and were washed three times with equal volume of isosaline. The volume of blood was measured and reconstituted as 10% v/v suspension with isosaline.

The assay mixture consists of 1.0mL of test sample of different concentrations (100µg/mL, 200µg/mL and 300µg/mL) in normal saline and 0.5mL of 10% HRBC suspension, 1mL of 0.2M phosphate buffer, 1mL hyposaline were incubated at 37^0C for 30min and centrifuged at 3,000

rpm for 20min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. Diclofenac was used as standard.

The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following formula:

$$\%\text{Haemolysis} = (optical\ density\ of\ test / optical\ density\ of\ control) X100$$

$$\%\text{Protection} = 100[(optical\ density\ of\ test / optical\ density\ of\ control) X100]$$

RESULTS AND DISCUSSION

Antimicrobial activity

All the synthesized compounds were found to exhibit moderate to good *in-vitro* antimicrobial activity. The comparative study on the zones of inhibition was carried out by the agar cup plate diffusion method. Compound **S6, S7** were found to be very effective against *Staphylococcus aureus*. Compound **S5, S6** were found to possess more potent activity against *Escherichia coli*. Compound **S1, S3, S7** were found to be very effective against *Aspergillus brasiliensis*. Compound **S6, S7** were found to possess more potent activity against *Candida albicans*. Gentamycin and ketoconazole were used as reference standards for antibacterial and antifungal activities. All the synthesized compounds were shown to exhibit significant anti-microbial activity when compared to INH.

Table 1: Antimicrobial activity of the synthesized compounds

COMPOUND	*Staphylococcus aureus*		*Escherichia coli (E.Coli)*		*Aspergillus brasiliensis*		*Candida albicans*	
	200µg/mL	400µg/mL	200µg/mL	400µg/mL	200µg/mL	400µg/mL	200µg/mL	400µg/mL
S1	9	10	7.5	11	16	20	14	14
S2	11	14.5	9.5	13.5	14.5	19	11	14
S3	10.5	12	11.5	12	15	20	10	14
S4	8.5	11	11	15	12.5	18	R	14
S5	13.5	15.5	13	16.5	13	17.5	13	14
S6	15.5	16.5	13.5	16.5	13	18	12	16
S7	14.5	15.5	9	10.5	15.5	20	14	16
INH	8	10	10	12	10	14	12	16
DMSO	-	-	-	-	-	-	-	-
GENTAMYCIN	18	19.5	15.5	18.5	-	-	-	-
KETOKONAZOLE	-	-	-	-	21	24.5	15	18

R=Resistant

Anti-inflammatory activity

The compounds **S3, S4** exhibited significant membrane stabilizing activity when compared to that of the reference; the other compounds had moderate activity. It was also observed that all the compounds showed dose dependent inhibition of hemolysis. Hence, all the synthesized compounds had potential for anti-inflammatory activity when compared with INH.

Table 2: Anti-inflammatory activity of synthesized compounds

S.NO	COMPOUND	%HAEMOLYSIS			%PROTECTION			IC50
		100µg/mL	200µg/mL	300µg/mL	100µg/mL	200µg/mL	300µg/mL	
1	S1	47.46	41.53	34.53	52.54	58.47	65.47	179.41
2	S2	48.52	38.14	29.87	51.48	61.86	70.13	169.32
3	S3	42.58	33.69	20.55	57.42	66.31	79.45	184.97
4	S4	43.22	33.26	23.31	56.78	66.74	76.69	149.83
5	S5	56.36	44.49	37.5	43.64	55.51	62.5	198.49
6	S6	47.88	40.89	37.71	52.12	59.11	62.29	184.97
7	S7	55.3	42.58	34.11	44.7	57.42	65.89	188.38
8	INH	69.49	62.71	53.81	31.51	38.29	47.19	290.57
9	STD	32.63	25.42	18.22	67.37	74.58	81.78	126.79

STD= Diclofenac

CONCLUSION

In-vitro antibacterial activity of synthesized compounds was carried out against *Staphylococcus aureus, Escherichia coli*. The result of antibacterial activity indicate that compound **S6, S7** showed moderate activity against *Staphylococcus aureus* and compound **S5, S6** showed moderate activity against *Escherichia coli.*

In-vitro antifungal activity of synthesized compounds was carried out against *Aspergillus brasiliensis* and *Candida albicans.* The result of antifungal activity indicates that compound **S1**, **S3, S7** showed moderate activity against *Aspergillus brasiliensis* and compound **S6, S7** showed moderate activity against *Candida albicans.* All the synthesized compounds were shown significant anti-microbial activity when compare to INH.

In-vitro anti-inflammatory activity of all the compounds was evaluated and compared with standard. All the compounds showed significant activity. The compounds **S3, S4** exhibited more potent activity when compared to isoniazid.

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