ANTIMICROBIAL & ANTIFUNGAL ACTIVITY OF SUBSTITUTED 4-ARYLIDENE 2-(HYDROXY PHENYL) OXAZOL-5(4H)-ONES

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Abstract: A series of (4Z)-4-(Arylidene)-2-(2-Hydroxyphenyl)oxazol-5(4H)-ones were synthesized by condensation of substituted hippuric acid with different aromatic aldehydes in the presence of fused sodium acetate, acetic anhydride in catalytic amount, ethanol as solvent under reflux conditions.All the synthesized compounds were confirmed and characterized by using various spectral techniques like IR, H^1NMR, C^{13}NMR, and mass spectral studies. All the synthesized oxazolone derivatives displayed moderate to potent antibacterial activity.

Keywords: **Oxazolones, Azlactones, Antibacterial Activity, Antifungal Activity Studies.**

I. INTRODUCTION

Oxazolones are heterocyclic compounds which are known to be multifunctional as they showed prolific development in synthetic chemistry through C=C,C=N,C=O bonds.

Oxazolone were first reported by Plochl[1] and named it as 'azlactone'.These compounds exhibit important biological activities such as antimicrobial[2], antibacterial[3], analgesic[4], antifungal[5],anticancer[6,7], anti-inflammatory[8],neuroleptic[9], sedative[10], antidiabetic[11] and antiobesity[12]. Azlactones are important intermediates in the preparation of several chemicals including aminoacids[13],peptides[14], some heterocyclic precursors[15]as well as coupling and photosensitive devices for proteins[16].They exhibit promising photophysical and photochemical activities [17,1,8,19] and as P^H sensors[20].Few benzimidazole derivatives were synthesized which showed good antimicrobal activity[21].

Over past few years there is an increase in public concern on environmental pollution and there is a need for new antibiotics either plant based or synthetic or both products.The development of new antibiotics is prime concern which paved way to further develop bacterial resistant drugs.

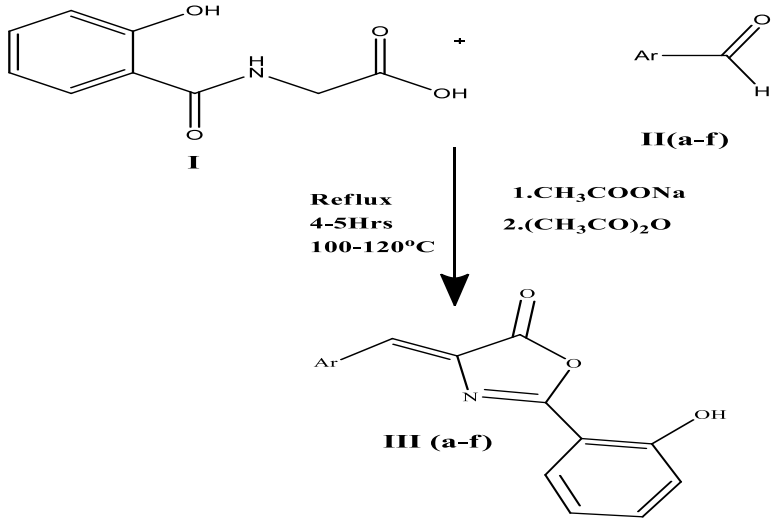
Azlactones posess imidazole nucleus and known to exhibit antifungal, antibacterial and antiinflammatory activities. In this line we synthesized new derivatives of oxazolones[22] and were screened for their anti-bacterial and antifungal activity.These compounds showed moderate to potent activity.

The scheme of synthesis for same compounds in this paper along with their antimicrobial and antifungal activity has been discussed.

II. EXPERIMENTAL

2.1 General method for the preparation of (4Z)-4-(arylidene)-2-(2-hydroxyphenyl)oxazol-5(4H)-ones

A mixture of 1mmol (0.195 gms)of 2-hydroxy hippuric acid,1mmol(0.185gms)of suitable aldehyde,3mmol of acetic anhydride and 1 mmol (0.082gms)of fused anhydrous sodium acetate was heated on an oil bath at 140-150^{0}C for 3-4 Hrs and then cooled.Then 5ml of ethanol is added slowly to the contents of the flask and the mixture is allowed to stand overnight.The compound is filtered under suction,washed with 10ml of ice cold ethanol and then with 10ml of boiling water and air dried and recrystallised from Hexane.



II a)Ar=$OCH_3C_6H_5$ **II d)Ar=4-ClC_6H_5**
II b)Ar=3-$NO_2C_6H_5$ **II e)Ar= 4- BrC_6H_5**
II c)Ar=2-OHC_6H_5 **II f)Ar= $N(CH_3)2C_6H_4$**

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2.2 Antimicrobial testing

The compounds synthesized were screened for their antimicrobial activity by Disc Diffusion Method[23]. In this method the sensitivity of the compounds is measured by determining the zone of inhibition after placing the paper disc dipped in solution of compounds, on LBS agar medium, which was previously inoculated with test organism. These results were compared with the zone of inhibition produced after placing disc dipped in the solution of standard antibiotic. The diameter of zone of inhibition is directly proportional to antimicrobial activity of the compound. The size of zone of inhibition depends on rate of antibiotic diffusion, rate of bacterial growth and incubation condition, concentration of organism.

Materials used 1.Sterilized Petri dishes, 2. Sterilized test tubes and watch glasses, 3.Micropipette and micro-tips, 4. Cotton swabs

2.3 Test organism used in the study

Bacterial cultures used:

1.Pseudomonas aeruginosa– Gram positive bacteria
2. Bacillus subtilism– Gram positive bacteria.
3.Staphylococcus aureus –Gram positive
4.Escherichia Coli-Gram negative

Subculture: One day prior to the testing, the organisms obtained from the laboratory stock were subculture into sterile nutrient broth and incubated at 37 º C for 18-24hrs. The culture growth thus obtained was used as inoculums for the antibacterial testing.

Fungal Cultures used:

1. Candida albicans ,
2. Rizopusmicrosporus var. oligosporus

Subculture:Two days before the testing the culture is prepared by inoculating the fungus from master culture into potato dextrose medium and incubated for 48 hrs at room temperature.

Drugs Control: 1. Penicillin (antibacterial), 2. Griseofulvin (antifungal)

Concentration: all the test compounds were tested at 25 to 100 µg/ml concentrations.

Solvent*:* Methanol.

2.4 Preparation of paper discs

Paper disc of 6mm diameter and 2mm thickness was used for the test. These disks were sterilized by autoclaving at 121º C (15 lbs psi) for 15 minutes.

2.5 Preparation of culture medium

Culture media provides all essential nutrients for the growth of microorganism. Luria Broth (LB) Agar was used to inoculate bacterial strains and PDA (Potassium-dextrose agar) medium for fungal strains.

Nutrient media thus prepared was sterilized by autoclave at 121º C for 20 mins at 15 lbs pressure.

2.6 Procedure

Petri dishes were filled to depth of 3-4mm with a nutrient agar medium. This poured medium was allowed to set and then inoculated with susceptible test organism culture using cotton swab under aseptic conditions under laminar air flow unit. Each plate was divided into six equal positions along the diameter. Each portion was used to place one disc. Five discs of each sample was placed on five portions using sterilized forceps. Two discs were placed one each with ciprofloxacin disc and a disc impregnated with the solvent. The petri dishes were incubated at 37°C for 24 h for bacterial culture and incubated for 28 ºC for 4 days for fungal culture. Diameter of the zone of inhibition was measured and the results are shown in Table.The diameter obtained for the test samples were compared with that produced by standards. Diameter of the zone of inhibition was measured in mm.

Table-1: Activity against Bacillus subtilis

Compd No	Code	Activity against Bacillus subtilis (MTCC No.6544)		
		25µg/ml	50µg/ml	100µg/ml
1	**IIa**	8	14	18
2	**IIb**	9	13	19
3	**IIc**	8	10	19
4	**IId**	10	15	19
5	**IIe**	12	16	20
6	**IIf**	8	14	18
Pencillin	Standard	17	39	48

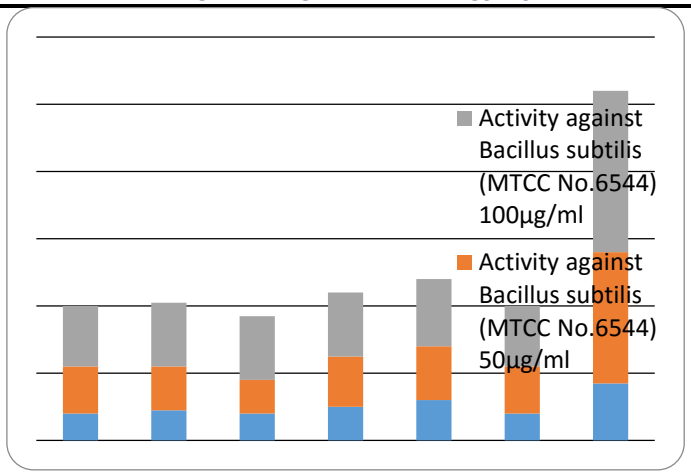


Fig-1: pictorial representation

Table-2: Activity against Staphylococus aureus

Compd No	Code	Activity against Staphylococus aureus (MTCC No.3160)		
		25µg/ml	50µg/ml	100µg/ml
1	**IIa**	5	8	10
2	**IIb**	6	8	10
3	**IIc**	2	8	11
4	**IId**	7	10	13
5	**IIe**	5	5	7
6	**IIf**	6	9	12
Pencillin	Standard	14	36	40

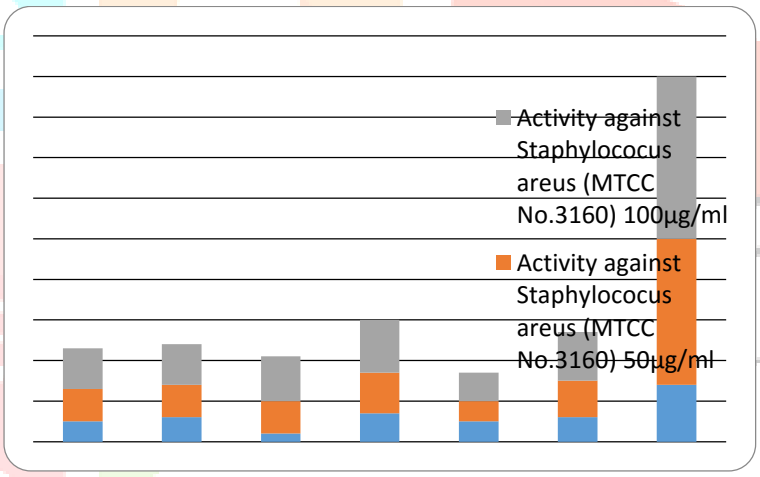


Fig-2: pictorial representation

Table-3: Activity against Pseudomonas aeruginosa

Compd No	Code	Activity against Pseudomonas Aeruginosa (MTCC No.1034)		
		25µg/ml	50µg/ml	100µg/ml
1	**IIa**	4	9	13
2	**IIb**	5	12	15
3	**IIc**	4	12	15
4	**IId**	6	11	13
5	**IIe**	5	8	12
6	**IIf**	6	10	15
Pencillin	Standard	14	36	40

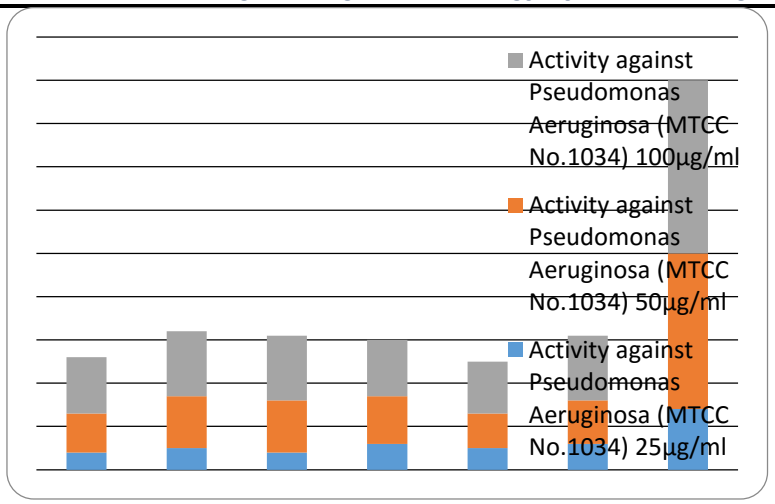


Fig-3: pictorial representation

Table-4: Activity against Escherichia Coli

Compd No	Code	Activity against E.Coli (MTCC No.42)		
		25µg/ml	50µg/ml	100µg/ml
1	**IIa**	5	7	7
2	**IIb**	2	5	9
3	**IIc**	9	11	17
4	**IId**	9	12	17
5	**IIe**	4	10	12
6	**IIf**	8	12	20
Pencillin	Standard	17	39	48

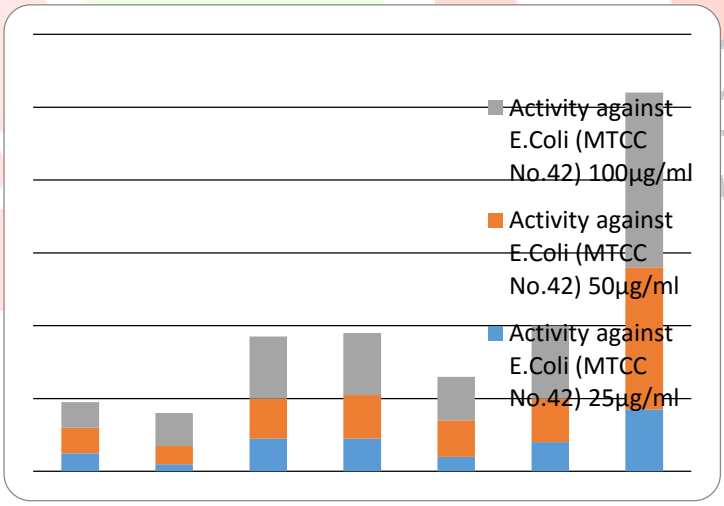


Fig-4: pictorial representation

Table-5: Activity against Candida Parapsolosis

Compd No	Code	Activity against Candida Parapsolosis (MTCC No..3017)		
		25µg/ml	50µg/ml	100µg/ml
1	**IIa**	9	10	12
2	**IIb**	7	8	10
3	**IIc**	-	-	11
4	**IId**	-	-	-
5	**IIe**	-	-	-
6	**IIf**	-	10	12
Griseofulvin	Standard	14	18	23

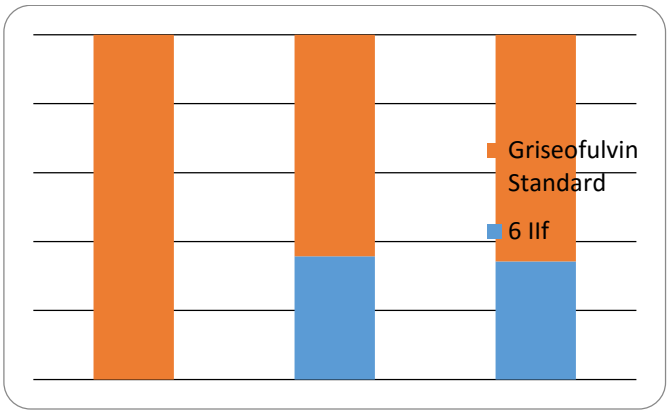


Fig-5: pictorial representation

Table-6: Activity against Rizopus microsporus var.oligosporus

Compd No	Code	Activity against Rizopus microsporus var.oligosporus (MTCC No.2785)		
		25µg/ml	50µg/ml	100µg/ml
1	**IIa**	-	10	14
2	**IIb**	5	10	12
3	**IIc**	2	8	12
4	**IId**	-	4	6
5	**IIe**	4	12	15
6	**IIf**	3	9	12
Griseofulvin	Standard	19	22	33

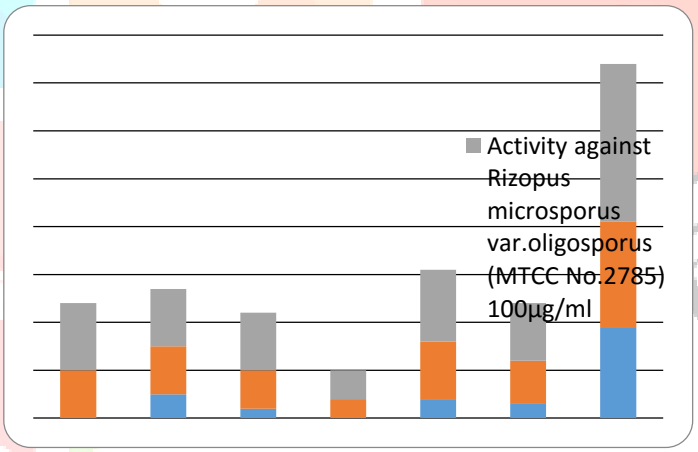
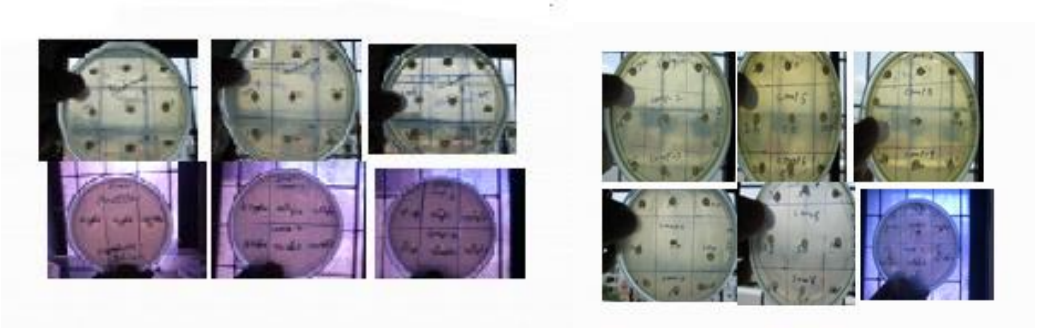
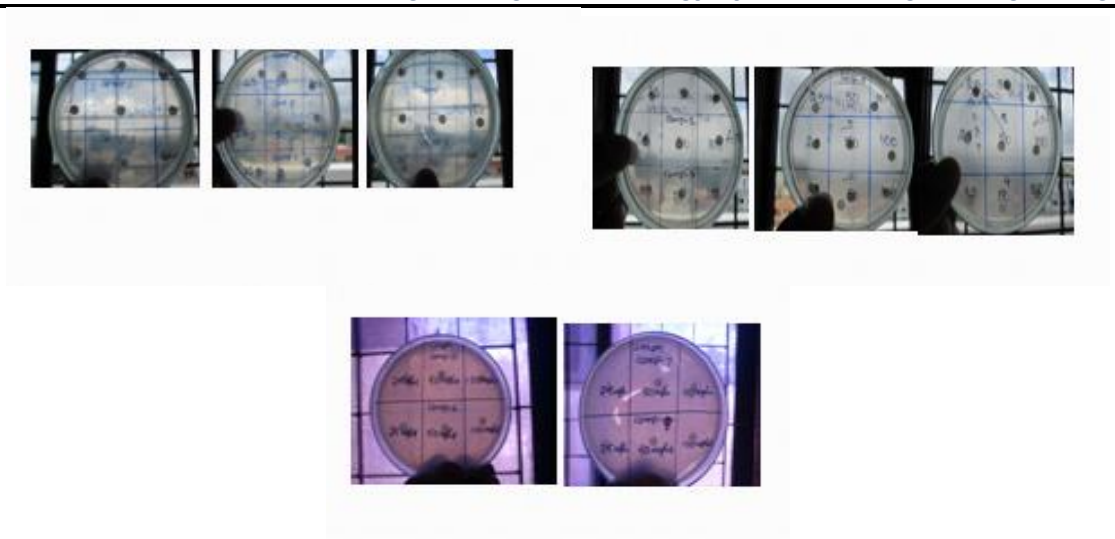


Fig-6: pictorial representation



Images of activity against Bacterial Strains



Images of activity against Fungal Strains

III. CONCLUSION

The activity studies of all the synthesized compounds against gram positive and gram negative bacterial strains revealed that the compounds 1,2,3,4,5 and 6 showed moderate to good activity against bacterial strains compared with standard pencillin.Activity against Fungi Rizopus oligosporus revealed that the compounds 1 and 4 showed activity only above 50µg/ml concentrations and compounds 2,3,5 and 6 showed good to moderate activity compared with standard Griseofulvin.Activity against Candida Parapsolosis revealed that compound 3 showed activity at 100µg/ml only while 4 and 5 showed no activity but 6 showed good activity. Hence these substituted oxazolones displayed moderate to potent activity against microbial and fungal strains.

The antimicrobial screening was carried by disc fusion method. The most eye catching features of these compounds are there utility in pharmaceutical industry[24] and there is further scope for their exploration. The present focus is on DNA binding studies of these synthesized compounds.

IV. ACKNOWLEDGEMENT

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