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Study of antimicrobial and antifungal activities of some synthesized 2-(2-amino-1, 3-Oxazol-4-yl)-4-substitutednaphthalen-1-ol

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ABSTRACT

New oxazole derivative of substitutednaphthol were ecofriendly and efficiently be synthesized like 2-(2-amino-1, 3-oxazol-4-yl)-4-substitutednaphthalen-1-ol by cyclization of 1-[2-(4-Substituted-1-hydroxynaphthalen-2-yl)-2-oxoethyl]thiocarbamide by utilizing elemental sulphur as a chief, easily available, non toxic catalyst. Some of these compounds were screen for their antimicrobial and antifungal activity against some selected microbe like C. albicans, A. niger, S. Typhi, E. coli, S. dysenteriae, K. pneumoniae, S. aureus by agar well diffusion assay method carried out. The compounds screen against selected microbe shows good results.

Keywords: Antimicrobial, antifungal, microbes, agar well diffusion assay method.

I. INTRODUCTION

Human desire for long and healthy living has kept him searching remedies for the suffering. Swiss physician Paracelsus postulated that, life is essentially a chemical process and ills of body can be treating by chemicals. He applied simple chemicals like mercury, sulphur, iron, copper sulphate etc to cure disease. Any chemical substance inhibiting the growth or causing the death of microorganisms is known as 'antimicrobial agent'. In nature, there are various types of chemicals possesses such properties when used in a sufficiently high concentration. There are large number of synthetic compounds are now explored for different antimicrobial activity and the development of non-resistant antimicrobial substance is necessary. Hetrocycles containing nitrogen, oxygen and sulphur in the ring have their own identity and importance since the plant extracts containing these are used in traditional medicine[1] and exhibit various physiological activities[2] such as antihistaminic, anti-inflammatory, estrogenic and anti-implantation etc. Substituted oxazoles have versatile application in the preparation of various biological, medicinal and agriculture compounds as well as in the industrial fields.[3-5] the oxazole ring is present in large number of pharmaceutical products such as antibiotics[6] and antiproliferative[7]. We wish to report a practical and convenient method for the preparation of newly substituted oxazole, using elemental sulphur as cyclising catalyst in PEG 400 a green and ecofreindly solvent medium.

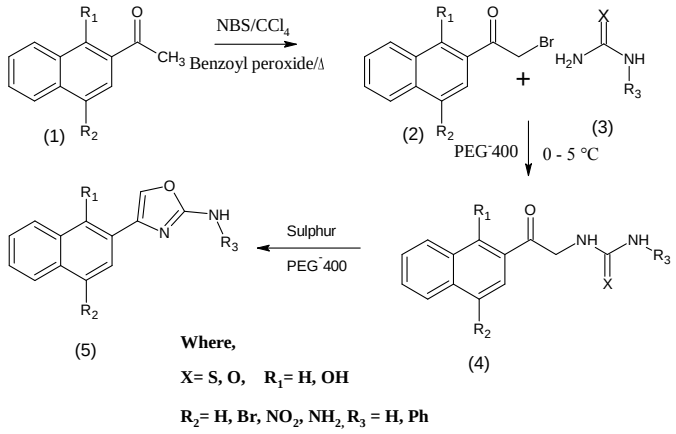

Figure 1. showing the scheme of preparation of substituted oxazole heterocyclic compounds

The wide range of biological activities of oxazoles includes anti-inflammatory, analgesic, antibacterial,

antifungal, hypoglycemic, antiproliferative, antituberculosis, muscle relaxant and HIV inhibitor activity[8-15]. It was reported that oxazoles showed remarkable antimicrobial activity against Staphylococcus aureus; Escherichia coli; Bacillus subtilis; Klebshiella pneumonie.[16-17]

The main aim of the study undertaken was to examine the potency of some Oxazoles moity against pathogenic microorganisms and helps to find better alternative against drug resistant pathogenic microorganism. The study also helps to renovate the medicinal uses of Oxazoles that have been used in the traditional practices. Hence, we have carried out the antimicrobial screening of following some selected synthesized heterocyclic compounds.

1. 2-(2-amino-1,3-oxazol-4-yl)-4-bromonaphthalen-1-ol (5a)
2. 4-amino-2-(2-amino-1,3-oxazol-4-yl)naphthalen-1-ol (5b)
3. 2-(2-amino-1,3-oxazol-4-yl)-4-nitronaphthalen-1-ol (5c)
4. 2-(2-amino-1, 3-oxazol-4-yl) naphthalen-1-ol (5d)

The above synthesized oxazole compounds were screened in vitro for their bactericidal activity against Gram positive bacteria (Staphylococcus aureus) and Gram negative bacteria (Escherichia Coli, Salmonella Typhi, Klebsiella Pneumoniae and Shigella dysentariae), and for their fungicidal activity against Aspergillus niger and Candida albicans. and find out their minimal inhibitory concentration (MIC).

II. EXPERIMENTAL

Some of the compounds synthesized were screen for antibacterial and antifungal activities to evaluate their efficiencies against animal and plant pathogenic microorganisms. The antimicrobial screening carried out by agar-well diffusion method. In this method the antimicrobials are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameters of zone of inhibition can be measured in millimeters.

MEDIA USED

1. Nutrient Agar Medium

Composition

Yeast extract	--	1.5gms
Beef extract	--	1.5gms
Peptone	--	5.0gms
NaCl	--	5.0gms
Agar powder	--	20.0gms
Distilled water	--	1000ml
pH	--	7.4 + 0.2 (at 25^0c)

2. Nutrient Broth Medium

Compostion

Yeast extract	--	1.5gms
Beef extract	--	1.5gms
Peptone	--	5.0gms
NaCl	--	5.0gms
Distilled water	--	1000ml
pH	--	7.4 + 0.2 (at 25^0c)

3. Potato Dextroxe Agar-

Composition

Potato infusion form	--	200gms
Dextrose	--	20gms
Agar	--	15gms
Distilled water	--	1000ml
pH	--	5.6 ± 0.2 (at 25^0C)

Table 1. Showing the media used and pH of media for selected microbes

Sr. No.	Microorganisms	Media used	pH of media
	Fungi ⇨	⇩	⇩
1	Candida albicans	Sabroud's Dextrose Agar	5.6
2	Aspergillus niger	Potato Dextrose Agar	5.6
	Bacteria ⇨	⇩	⇩
3	Salmonella typhi	Nutrient Agar	6.8
4	Shigella dysenteriae	Nutrient Agar	6.8
5	Escherichia coli	Nutrient Agar	6.8

6	Staphylococcus aureus	Nutrient Agar	6.8
7	Klebsiella pneumoniae	Nutrient Agar	6.8

SLANT PREPARATION

Nutrient agar medium was dissolved in distilled water and was sterilized by auto claving. About 5 ml of molten media was transferred specially in previously-sterilized test tubes. The test tubes were then plugged tightly and placed in a slanting position to cool and solidify.

STOCK CULTURE

Culture was grown on nutrient agar slants by incubating them for 24 hours at 37^0C.

CULTURE DILUTION (SUB – CULTURING)

One loopful of stock culture was added to 5 ml of nutrient broth medium for inoculation. The inoculated broth was incubated for 24 hr at 37^0C. For all experimental purpose 24 hr fresh diluted cultures of both the organisms were used.

PREPARATION OF SAMPLE SOLUTION

An antibacterial activity is usually tested by making aqueous solution samples. However, compounds used in the present study were insoluble in water. Hence, to study antimicrobial activity their dilutions were prepared by using 70% dioxane. Thus, 70% dioxane was taken and tested as control.

To check the potency of compounds, the solutions were prepared with 10µ gm/ml, 20µ gm/ml, 30µ gm/ml, concentration. 1ml of each solution was added to 5 ml of nutrient broth solution containing organisms to be tested. Tubes with organisms and medium with solvent were used as controls. These tubes were kept for incubation at 37^0C for 24 hr. Most of the compounds under study exhibited total inhibition of the test cultures within 24 hr of incubation. The tube containing compounds showing inhibition (antimicrobial activity) was clear and the tube, which was kept as control where no compound was added showed growth.

AGAR- WELL DIFFUSION METHOD

In this method, the antimicrobials are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameters of zone of inhibition can be measured in millimeters. This method required following reagents

1. MULLER HINTON AGAR MEDIUM (1 L)

The medium was prepared by dissolving 33.9g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured on to 100 mm petriplates (25-30ml/plate) while still molten.

2. NUTRIENT BROTH (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000 ml distilled water and boiled to dissolve the medium completely. The medium was 44 dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3.CHLORAMPHENICOL DISC (STANDARD ANTIBACTERIAL AGENT)

Petri plates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20 µl of the solution were added.The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Chloramphenicol disc was used as a positive control.

All the microorganisms were inoculated in nutrient broth and incubated for overnight to observe their growth. The optical density (O.D.) was maintained at 1.0 (600 nm). The petri plates were prepared with the selective agar Medias for each culture of the species. The plates were spread with the respective culture of each species. These plates were incubated at room temperature for half an hour. The wells were made using a borer. The wells were filled with the respective chemicals, volume 10µl, 20µl and 30µl to check the antimicrobial activity of that chemical.

The compounds, which showed antimicrobial activity, were further tested for their minimum inhibitory concentration by serial Dilution Method.

SERIAL DILUTION METHOD[18]

The compounds which showed antimicrobial activity were further examined to find out their MIC value. The same procedure was followed to find out their MIC value only the volumes were taken to be 5µl - 30µl depending upon the zone in which the specific chemical showed antimicrobial activity. Sterile conditions were strictly maintained to avoid any kind of contamination.

To determine the MIC of various compounds the following procedure (Serial Dolution Method) was followed

Nutrient broth was prepared by dissolving 13gms of dehydrated medium in 1 litre of distilled water. The pH of the medium was adjusted to 7.4. 5ml of the medium was distributed in each tube. All the tubes were sterilized at 121^0C for 20 minutes.

The appropriate amount to test compound was dissolved in the solvent 70% dioxane to give final concentration of 1 X 10^{-2} M.Various amounts of the above stock solution was aspectically added to the various nutrient broth tubes (viz. 0.5, 1.0, 1.2, 1.4, 1.6, 1.8, .0…..5.8, 6.0ml) Fresh culture of the test bacterium was inoculated in each tube (0.2 ml culture) The inoculum size of the test bacterium was adjusted to give approx. 10^7 cFu. All the tubes were incubated at 37^0C for 24 hrs. Uninoculated tube was kept as a control in which nutrient broth and 5 ml of the solvent was taken.

After 24 hr of incubation, all the tubes were observed for MIC against test bacterium. This was observed by the absence of visual turbidity in the tube receiving the highest dilution of the test compounds. To determine MIC of various test compounds against moduls (fungus) the following procedure was adopted.

Pottato dextrose broth was prepared as follows.

200 gms. of potato (peeled) was added to 1 litre of distilled water. It was steamed for 20 mins and volume adjusted to 1 litre. 20 gms of dextrose was added to this.

Appropriate amount of test compounds was dissolved in 70% dioxane methanol to give final concentration of 1 X 10^{-2} M. various amounts of the above stock solution was added aspectically to the potato dextrose broth tubes (viz. 0.5,1.0,1.2,…….., 6ml) Fresh fungal culture were incubated at 28^0C for 96 hr After 48 hr of incubation all the tubes were observed for the MIC of test compounds.

III. RESULTS AND DISCUSSIONS

MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC is the concentration of antimicrobial required to inhibit the growth of a particular bacterial isolate *in vitro*. Clinically the MIC is used to assign an organism to a susceptibility category (sensitive, intermediate, resistant). Dose regimens in common use generally produce plasma concentrations 2s - 4 x the MIC.

MINIMUM BACTERICIDAL CONCENTRATION (MBC)

MBC is rarely (if ever) determined clinically. MBC is the concentration required to kill a particular bacterial isolate *in vitro*. Experimentally diagnose the MBC is usually 2 to 4 x the MIC for the same isolate. Among the synthesized compounds only selected twelve compounds were studied for their antimicrobial activities. All the pathogens tested during analysis are human pathogens. The activities of compounds were tested against all the pathogens by agar well diffusion method.

In this method, the antimicrobials are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth.

IV. RESULTS AND DISCUSSIONS

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The activity against tested pathogens was given in following table

Table 2. showing the activity of compound against Aspergillus niger (media – Potato Dextrose Agar)

Sr No.	compounds	Size Of Inhibition Zone			MIC Value
		10µl	20µl	30 µl	
2	9d	10.5mm	11.5mm	11.5mm	1.32 mg/ µl

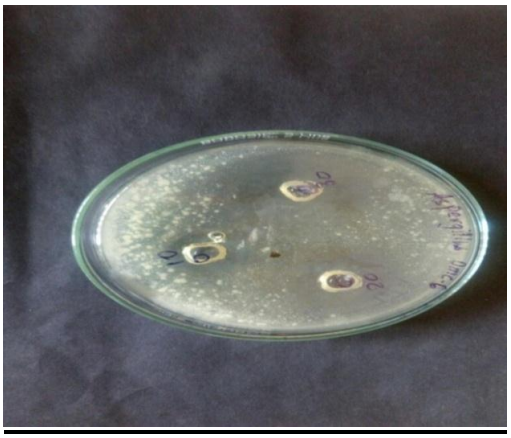

Figure 2. image of Size of inhibition zone against *Aspergillus niger*

BACTERIAL CULTURES

Table 3. showing the activity of compound against Salmonella typhi (media – Nutrient Agar)

Sr No.	compounds	Size of inhibition zone			MIC value		
		10µl	20µl	30 µl	10µl	20µl	30 µl
1	9d	6mm	8.5mm	18mm	1 mg/µl	2 mg/µl	3 mg/µl
2	9c	2mm	6.5mm	6.5mm	1.12 mg/µl	2.24 mg/µl	3.36 mg/µl

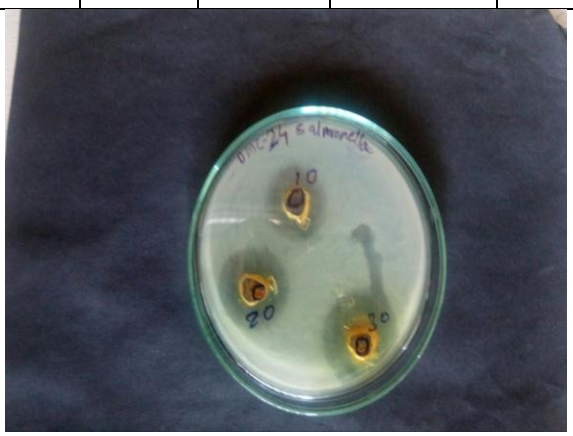

Figure 3. image of Size of inhibition zone against Salmonella typhi

Table 4. showing the activity of compound against Shigella dysenreriae (media – Nutrient Agar)

Sr No.	compounds	Size Of Inhibition Zone	MIC Value

		10µl	20µl	30 µl	10µl	20µl	30 µl
1	9c	5.5mm	3.5mm	5mm	-	-	3.36 mg/µl

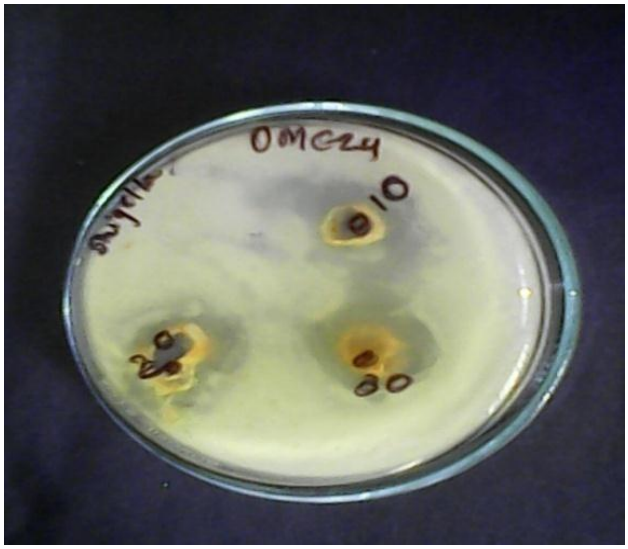

Figure 4. image of Size of inhibition zone against Shigella dysenteriae

Table 5. showing the activity of compound against Escherichia coli (Media – Nutrient Agar):

Sr No.	compounds	Size of Inhibition Zone			MIC Value		
		10µl	20µl	30 µl	10µl	20µl	30 µl
1	9c	2.5mm	3mm	2.5mm	-	-	3.36 mg/µl

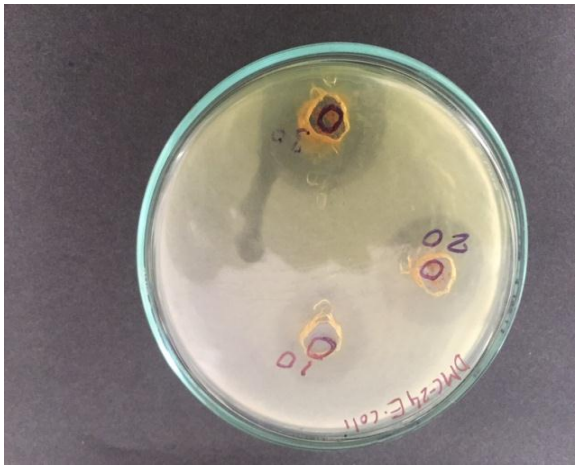

Figure 5. image of Size of inhibition zone against Escherichia coli

Table 7. showing the activity of compound against *Klebsiella Pneumoniae* (Media – NutrientAgar)

Sr No.	compounds	Size of Inhibition Zone			MIC Value		
		10µl	20µl	30 µl	10µl	20µl	30 µl
3	9c	2.5 mm	4 mm	4.5 mm		2.24 mg/µl	3.36 2.4 mg/µl
4	9d	2 mm	3 mm	5.5 mm	1 mg/µl	2 mg/µl	3 mg/µl

Figure 7. image of Size of inhibition zone against Klebsiella Pneumoniae

Table 8. Showing the activity of synthesized compound against different Fungal and Bacterial species.

Compound	**Activity**																				
	Candida			**Aspergillus**			**Salmonella**			**Shigella**			**E.coli**			**S.Aureus**			**Klebseilla**		
	10 µl	20 µl	30 µl	10 µl	20 µl	30 µl	10 µl	20 µl	30 µl	10 µl	20 µl	30 µl	10 µl	20 µl	30 µl	10 µl	20 µl	30 µl	10 µl	20 µl	30 µl
5a	Y	Y	**Y**	-	-	-	-	-	-	Y	Y	**Y**	-	-	-	-	-	-	-	-	-
5b	-	-	-	-	-	-	-	-	-	Y	Y	Y	Y	Y	Y	-	Y	-	-	-	-
5c	-	-	-	Y	Y	Y	Y	Y	Y	-	Y	-	-	-	-	-	-	-	-	-	-
5d	-	-	-	Y	Y	Y	Y	Y	Y	-	-	-	-	-	-	-	-	-	Y	Y	**Y**

V. CONCLUSION

The compound 9a shows the activity at the all concentrations like 10µl, 20µl and 30µl for the fungal species Candida albicans and Shigella dysenteriae but the synthesized compound 9a are inactive for the species Aspergillus niger, Salmonella typhi, , E. coli, Klebseilla pneumonia and Staphylococcus aurous at all concentrations.

The compound 9b shows remarkable activity at the concentrations like 20µl for Staphylococcus aureus and for the concentration 10µl, 20µl and 30µl it shows the activity against two pathogens Shigella dysenteriae, E. coli, but the synthesized compound 9b are inactive for the species Aspergillus niger, Salmonella typhi and Klebseilla pneumonia at all concentrations.

The synthesized compound 9c shows remarkable activity against the bacteria Salmonella typhi at all concentrrations; Salmonella typhi[19-28]is a gram negative bacteriaand it is causative agent of typhoid ranging from 7–14 days. The patient shows mild pyrexiz, which may become fatal fulminating disease. As bile is good culture medium for the bacteria it is multiplied abundantly in gall bladder and is discharged continuously into intestine where it involves the Teyer's patches and lymphoid follicles of the illium. This becomes inflamed and undergoes nausea and slough off, leaving behind ulcer complications, investinal perforation and haemorrhage.

The compound 9c also shows remarkable activity against the species Shigella dysentarie, E. col and Klebseilla pneumoniae at all concentration level. As the above synthesized compounds show remarkable antimicrobial activity against S. typhi, Shigella dysenteriae, E. col and Klebseilla Pneumoniae at minimum concentration, therefore these synthesized compounds can be used as an alternative drug for the treatment of disease caused by above microorganism

only their detailed study in pharmaceutical, biochemical, medicinal sciences. These drugs may replace the traditional drugs if they do not have toxic and other side effects.

The compound 9d shows versatile activity like antibacterial and antifungal, as it is active against both the pathogenic microorganism, this synthesized compound shows activity agaist Aspergillus niger,fungal species at all minimum concentrations.It also indicates remarkable activity for S.typhi and Klebseilla pneumoniae.

VI. ACKNOWLEDGEMENTS

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