ANTIFUNGAL ACTIVITY OF NEW NITROGEN HETEROCYCLES SYNTHESIZED UNDER MICROWAVE IRRADIATION

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Quinoline compounds possess a wide range of antibacterial, antifungal and antimutagenic properties. The present work involves synthesis of three heterocycles-4-Phenyl 3-vinyl quinolin-2-one (**1**), 4-phenyl-3-chloro-quinoline (**2**), 4-phenyl-3-vinyl quinoline-2-thione (**3**) utilizing microwave technology and testing their antifungal activity against 4 fungi namely *Aspergillus flavus*, *Rhizopus nigricans*, *Mucor hiemalis* and *Candida albicans*. Antifungal studies of compounds showed good fungal activity. The antimicrobial significance of the synthesized compounds is well evident from the study.

INTRODUCTION

Heterocyclic compounds occur widely in nature and in a variety of non-naturally occurring compounds. Quinoline compounds possess a wide range of antibacterial, antifungal and antimutagenic properties. QSAR studies have proved the pharmaceutical and biological importance of many quinoline compounds [1]. The present work involves synthesis of three heterocycles-4-Phenyl 3-vinyl quinolin-2-one (1), 4-phenyl-3-chloroquinoline (2), 4-phenyl-3-vinyl quinoline-2-thione (3) utilizing microwave technology and testing their antifungal activity against 4 fungi namely *Aspergillus flavus, Rhizopus nigricans, Mucor hiemalis* and *Candida albicans.*

EXPERIMENTAL

General : All microwave reactions were carried out in a domestic microwave oven (IFB model 179 MIS), output power 750 W Microwave frequency 2450 Hz. Laminar Airflow Cabit (Kemi), autoclave (Osworld "Autoclave Steam Sterilizer" JRIC-39) and incubator (Genuine) were used for antifungal activity. The general procedure for preparation of 3- vinyl quinoline-2 (1H) ones were adopted from earlier work [2-5]. The procedure was adopted for microwave synthesis aided by clay as solid support. The synthesized compounds were screened for their antifungal activity against 4 fungi *Aspergillus flavus, Rhizopus nigricans, Mucor hiemalis* and *Candida albicans.* The fungi were collected from Jebi laboratory, Pollachi.

Preparation of culture media for antifungal studies [6]

Composition of culture media

SDA medium (Sabouard Dextrose Agar)

Contents	g/litre
SDA	65 g
Distilled water	100 ml

Suspended 65.0 g of SDA in 100 ml distilled water. Heated to boiling to dissolve the medium completely. Sterilized by auto claving at 15 lbs, pressure (121°C) for 15 min.

Potato dextrose – agar solid medium

Content	g/ml
Potato	25g
Agar	2g
Dextrose	2g
Distilled water	100 ml

25 g of sliced potato was boiled in distilled water (50 ml). After proper boiling, the extract was decanted and was transferred into a 100 ml beaker and the solution was made up to the mark with distilled water. Then the solution was taken in a pot when 2 g of dextrose and 2 g of agar was added slowly to the solution with gentle heating and stirring with a glass rod. The medium was boiled for 15 minutes and then transferred into 250 ml conical flask. The conical flasks were closed with cotton plug. Then the medium in the conical flask were autoclaved for half an hour at 150°C and 15 psi. The sterilized medium was then used for culturing fungi.

Sterilization : The petri dishes were sterilized by means of hot air oven at 160°C for 1 hour. Melted nutrient agar for bacteria and SDA for fungal was poured into separate dishes to a uniform depth of 5 mm and refrigerated for solidification. The plates were then transferred to a incubator at 37°C for 10 to 20 minutes to dry off the moisture that develop on the agar surface.

Streak plate isolation method for determination of zone of inhibition of antifu l activity : The required amount of SDA medium was taken in a conical flask separately and was sterilized in autoclave (at 121°C and 15 Psi) for 15 min. Liquefied a tube of SDA and poured into petridish. The plate was rotated gently for uniform distribution of the medium. The inoculating loop was held at a 60°C angle in the hottest part of the Bunsen burner flame. The entire tube was heated were to redness. Allowed the loop to cool for 15 to 20 seconds before it touches the culture. Picked up the tube of culture with free hand, removed the plug or cap of the tube flame from the mouth of the tube by momentarily rotating it within the upper region of the flame. Removed a small amount of the culture from the tube with the sterilized inoculating loop and streaked the microorganisms in a plate following quadrant. The stocks solutions were prepared following the quadrant. The stock solutions were prepared by dissolving the compounds in ethanol. The process of inoculation was done under aseptic condition and the spores were inoculated in the medium and incubated for 5 days. A clear zone or ring is present on a SDA plate. The diameters of the zone of inhibition are measured. The antifungal activities were recorded by photographing the petri dishes.

Agar diffusion method for determination of zone of inhibition of MIC'S of compounds 1-3 : Potato dextrose agar was sterilized in a conical flask and cooled to 45 to

36°C was distributed by pipette (1 ml) into each petri dish and swirled to distribute the medium homogeneously. After solidification of the medium, the fungal inoculums was placed on the center of the petri plates. The paper discs were sterilized in autoclave and dried at 100°C in an oven. Then the discs were soaked with test compounds and this disc was inoculated in the solid medium. All the plates were incubated at room temperature for 3 days. The inoculated plates were incubated at (25 ± 22°) C. After three to five days of incubation, the diameters of fungal mycelial growth were measured.

RESULTS AND DISCUSSION

Microwave enhanced synthesis of compounds 1-3 : Compound 1 was synthesized by procedure adopted from Shanmugam *et al*, 1976 [3]. Compounds 2 and 3 (90 and 86% yield respectively) were synthesized via microwave irradiation aided by clay as solid support. One of the most useful, advantages of MORE (Microwave – Induced Organic Reaction Enhancement) is the solvent free synthesis which will reduce the drastic "solvent pollution" and will be therefore eco friendly green approach. The work up of the reaction was also found to be more convenient than the normal procedure. There was a drastic reduction in the time of synthesis of compounds 2 and 3 (8 and 4 min respectively) portraying the successfulness of microwave synthesis. Characterizations of the compounds were done with Co-IR and by comparison of melting point with literature values.

Antifungal studies : In the disc diffusion technique, a concentration gradient of the drug in a nutrient medium is prepared and the growth of the bacteria, seeded in the medium after an inoculation period is observed. The clear zone of growth inhibition is noted around the disc due to diffusion of drug and growth of bacteria.

The diameter of the inhibition zone denotes the relative susceptibility of the test micro organisms to a particular anti microbe [8].

Inhibition zone disc	Type of anti microbe
> 13 mm	Highly sensitive or susceptible
8.13 mm	Moderately sensitive or intermediate
< 8 mm	Resistant

The term susceptible implies that an infection caused by the strain tested may be expected to respond favourably to the indicated anti microbial agent for that type of infection and pathogen. Resistant strain are not inhibited completely by the therapeutic concentration. Intermediate implies that the strain may respond to unusually high concentration of the agent, resulting from high dosage.

The synthesized compounds were screened for their antifungal activity against *Aspergillus flavus*, *Mucor hiemalis*, *Rhizopus nigricans* and *Candida albicans* by streak plate isolation method using ethanol as a solvent. The solution of compounds (10 µg/ml) were compared with standard drug flucanozole. In the streak plate isolation method, a concentration gradient of the drug in a SDA medium was prepared and the growth of fungus, seeded in the medium after inoculation period is observed. The clear zone of growth inhibition was noted around the disc due to diffusion of drug and growth of fungi. From the screening results (Table 1) it was observed that compounds showed resistance to all the fungi and were found to inhibit twice when compared with the standard flucanozole (10 mm).

Minimum inhibitory concentration of compounds against *Aspergillus flavus* : The minimum inhibitory concentration of the synthesized compounds were tested against *Aspergillus flavus*. Concentration of 0.05 µg / ml to 3 µg / ml were used in the study. It was found that all the three compounds showed good inhibition at higher concentrations (Table 2).

Calculation of inhibition of antifungal activity by streak plate isolation method : The percentage of zone of inhibition of the test fungi was calculated by a formula.

$$I = \frac{C - T}{C} \times 100$$

The results are tabulated (Table 3).

Table 1. Zone of inhibition observed against Fungus by the test compound

Fungus	1 (mm)	2 (mm)	3 (mm)	Control (mm). Flucanozole
Aspergillus flavus	4	5	3	10
Rhizopus nigricans	4	5	2	10
Mucor hiemalis	4	2	2	10
Candida albicans	1	2	1	10

Table 2. Minimum inhibitory concentration of compounds against *Aspergillus Flavus*

	Zone of inhibition (mm) obtained for compound		
0.1 mg / ml	1	2	3
0.05	4	5	1
0.1	4.5	6	3
0.15	5	8	6
0.2	6	9	10
0.25	7	9.5	12
0.3	8	10	14

Table 3. Percentage of inhibition of Fungai

Fungi	Compound		
	1	2	3
Aspergillus flavus	40	50	30
Rhizopus nigricans	40	50	20
Mucor hiemalis	40	20	20
Candida albicans	10	20	20