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ANTIFUNGAL ACTIVITY OF PYRIMIDO BENZOTHIAZOLE DERIVATIVES BY DISC DIFFUSION METHOD

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ABSTRACT

2-substituted derivatives of 3-Cyano-4-imino-2-methylthio-8-nitro-4H-pyrimido [2,1-*b*] [1,3] benzothiazole obtained by the multicomponent reaction of 2-amino-6-nitro benzothiazole and bis methylthio methylene malononitrile on refluxed independently with aryl amines / phenols / heteryl amines / compounds containing active methylene group in the presence of 5 ml of dimethyl formamide with a pinch of anhydrous K_2CO_3.

KEYWORDS: 2-amino-6-nitrobenzothiazole, bis methylthio methylene malononitrile, hydrazine hydrate, dimethyl formamide, potassium carbonate.

INTRODUCTION

The antifungal activity[1-14] of synthesized compounds can be determined by screening them against the fungal species using microbial method (assay). The basic principle of microbial assay lies in the comparison of the inhibition of growth of fungi by known concentration of test compounds with that of known concentration of standard antifungal agent (Fluconazole) having known activity. Generally, two types of methods were used for determination of antifungal activity.

1) Agar plug method

Principle

The fungicidal effect of the compound can be assessed by the inhibition of mycelia growth of the fungus and is observed as a zone of inhibition near the disc or the wells.

Reagents

1. Potato Dextrose Agar medium: The commercially available (HiMedia) potato dextrose agar medium (39g) was suspended in 1000ml of distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 lbs pressure (121ºC) for 15 minutes.

2. Fluconazole (Standard antifungal agent)

Procedure: Potato Dextrose Agar medium was prepared and poured on to the petriplates. A fungal plug was placed in the center of the plate. Sterile discs immersed in the solution of newly synthesized compounds were also placed in the plates. Fluconazole was used as antifungal control. The antifungal effect was seen as crescent shaped zones of inhibition.

2) Spore Germination Assay

Principle

Lacto phenol cotton blue stains the fungal cytoplasm and provides a light blue background, against which the walls of the hyphae can readily be seen. It contains four constituents: phenol which serves as a fungicide, lactic acid as cleaning agent, cotton blue to stain the cytoplasm of the fungus and glycerol to give a semi-permeable preparation.

Reagents

Lacto phenol cotton blue stain

Phenol crystals	(20g)
Cotton blue	(0.05g)
Lactic acid	(20ml)
Glycerol	(20ml)
Distilled water	(20ml)

The stain was prepared by dissolving the chemicals with gentle heating for complete dissolution.

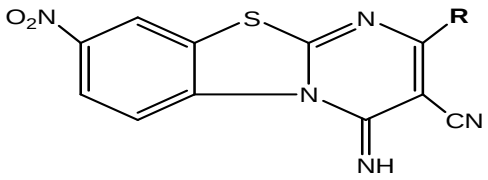
Procedure: Aliquots of spore were prepared by mixing loopful of fungal spores in sterile distilled water. 25µl of spore suspension was added to 10µl of the tested compound solution and placed in separate glass slides. Slides with 25µl of spore suspension alone served as the controls. Slides were then incubated in moist chamber at 25 ^{0}C for 24 hours. Each slide was fixed in lacto phenol cotton blue stain. The mold was mixed gently with the stain using two

teasing needles. A cover slip was placed on the preparation and examined under the phase contrast microscope (Kozo XJS500T, Japan) for spore germination.

MATERIAL AND METHODS

1) 3-Cyano-4-imino-2-substituted-8-nitro-4H-pyrimido [2,1-*b*][1,3]benzothiazole

A mixture of 2-amino-6-nitro benzothiazole **(II-64)** [0.195 gm, 0.001 mole], bis methylthio methylene malononitrile **(II-52)** [0.170 gm, 0.001 mole] independently reacts with aryl amines / phenols / heteryl amines / compounds containing active methylene group was refluxed in the presence of 5 ml of dimethyl formamide and a pinch of anhydrous potassium carbonate (0.2 gm) for six hours. The reaction mixture was cooled to room temperature and poured in ice cold water. The separated solid product was filtered, washed with water and recrystallized from DMF-ethanol mixture to give substituted derivatives of **(II-65).**



(II-65)

Antifungal activity by Disc diffusion method

In this method the sensitivity of synthesized compounds is measured by determining the zone of inhibition after placing paper disc dipped in solution of compounds. These results were compared with the zone of inhibition produced after placing disc dipped in solution of standard antibiotic.

Method for Antifungal activity

The organisms selected for antifungal activity are *Aspergillus Niger* & *Penicillium sp* species. These activity are performed by Department of Microbiology, Vai. Dhunda Maharaj College, Degloor an affiliated College to Swami Ramanand Teerth Marathwada University, Nanded.

Antifungal activity by well diffusion method

The *in-vitro* antifungal activity by agar well diffusion method was standardized using Fluconazole. This method is based on diffusion of antifungal component from reservoir hole to the surrounding inoculated Potato dextrose agar medium, so that the growth of fungus is inhibited as zone around the hole. Two fungi were selected viz. *Aspergillus niger* and *Penicillium s p.*

Antifungal activity by Well diffusion method of 3-Cyano-4-imino-2-methylthio-8-nitro-4H-pyrimido [2,1-*b*] [1,3] benzothiazole **(II-65)** and its 2-substituted derivatives.

Table 1: Antifungal Activity of 2-substituted derivatives of compound II-65.

Comp. Nos.	R	Diameter in mm of zone of inhibition in mm	
		Aspergillus Niger	***Penicillium sp***
II-66a	p-chloroanilino	21 mm	11 mm
II-66b	p-nitroanilino	---	---
II-66c	p-hydroxyanilino	13 mm	15 mm
II-66d	*p*-toluidino	18 mm	12 mm
II-67a	4'-nitro phenoxy	17 mm	14 mm
II-67b	4'-carboxylicphenoxy	---	---
II-67c	phenoxy	19 mm	17 mm
II-67d	4-methyl phenoxy	17 mm	15 mm
II-68a	malononitrile	---	---
II-68b	α-ethyl acetoacetyl	---	09 mm
II-68c	α-acetyl acetone	17 mm	19 mm
II-69a	piperazino	08 mm	---
II-69b	morpholino	10 mm	19 mm
II-69c	piperidino	---	---
Std.	Fluconazole	23 mm	21 mm
	DMSO	---	---

Note: '---' denotes no activity antifungal activity.

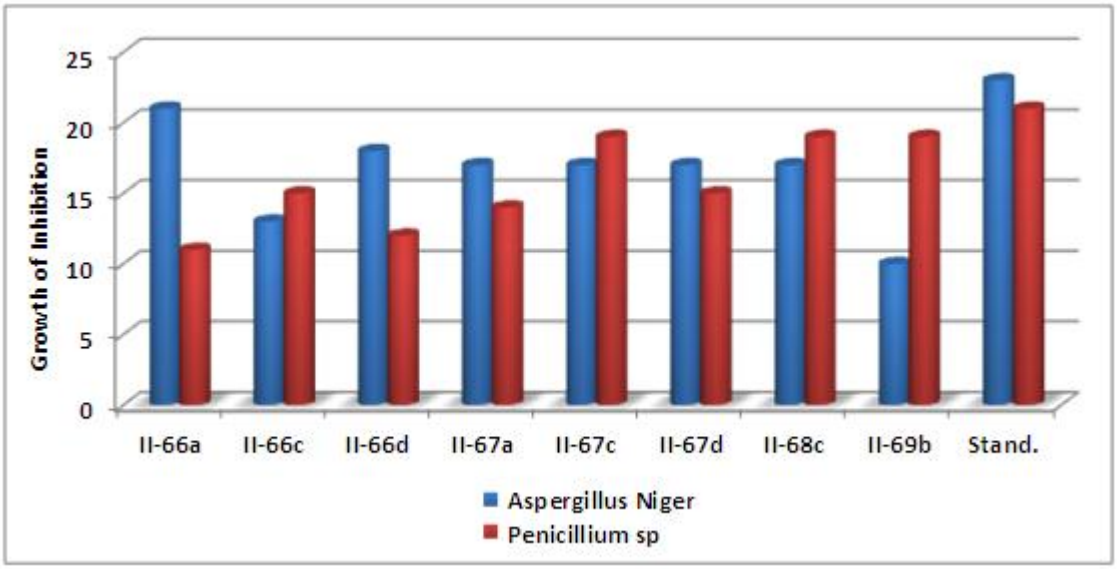


Figure 1: Antifungal Activity of Test Compounds by Well Diffusion Method.

RESULTS AND DISCUSSION

The screening for antifungal activity of newly synthesized compound 3-Cyano-4-imino-2-methylthio-8-nitro-4H-pyrimido [2,1-*b*] [1,3] benzothiazole **(II-65)** and its 2-substituted derivatives have been studied against *Aspergillus Niger* and *Penicillium sp* species by well

diffusion method. Compounds **II-66a, II-66d, II-67c, II-67d, II-68c** shows good antifungal activity against *Penicillium sp* species and compounds **II-67c, II-68c, II-69b** shows good antifungal activity against *Aspergillus Niger* species.

CONCLUSION

Two moieties are fused and screened for antifungal studies they showed a broad spectrum of antifungal activity. They showed good activity against *Penicillium sp* and *Aspergillus Niger* species. 3-Cyano-4-imino-2-methylthio-8-nitro-4H-pyrimido [2,1-*b*] [1,3] benzothiazole and its 2-substituted derivatives are responsible for antifungal activity, but it is interesting to note that benzothiazole moieties when fused with other moieties showed a broad spectrum antifungal activity. Hence in search of new generation of antibiotics it may be worthwhile to explore the possibility in this area by fusing different moieties and increase potency.

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