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Research Article

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Synthesis, Characterization and Antifungal Activity of 3-Substituted Triazipino [3,4-b] [1,3] Benzothiazole

Anil Chidrawar*

Department of Chemistry, Degloor College, Degloor, S.R.T.M.U, Nanded, Maharashtra, India

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ABSTRACT

A simple and efficient method have been used for the synthesis of 3-Substituted derivatives of 4-Cyano-5-imino-3-methylthio-9-nitro-2H-1,2,4-triazipino [3,4-b] [1,3] benzothiazole have been prepared through one Step Multicomponent reaction by heating a mixture of 2-hydrazino-6-nitro benzothiazole (I) and bis methylthio methylene malononitrile (II) independently with aromatic amines / phenols / heterylamines / compounds containing active methylene group respectively in the presence of dimethyl formamide and catalytic amount of anhydrous potassium carbonate. All these newly synthesized compounds were screened for antifungal activity.

Keywords: 2-Hydrazino-6-nitro benzothiazole; Aromatic amines; Phenols; Heterylamines

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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. Compounds containing thiazepines are well known for their varied biological activities [15-21] like analgesics, antihistamines, edrenolytics, neuroleptics and anti HIV. 1,4-Benzodiazepines [22-23] and thiazolo triazepines [24,25] and benzothiazole triazepines [26] exhibit psycho sedative, tranquilizing and CNS depressant activity.

EXPERIMENTAL SECTION

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 (39 g) 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.

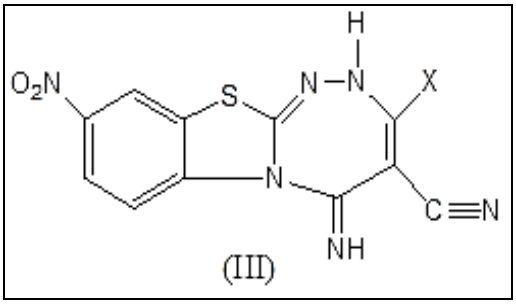
Spore Germination Assay
Principle:
Lacto phenol cotton blue stains the fungal cytoplasm and provides a light blue background, against which the walls of the hyphae can readily be seen. It contains four constituents: phenol which serves as a fungicide, lactic acid as cleaning agent, cotton blue to stain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

Reagents:
Lacto phenol cotton blue stain
Phenol crystals (20 g)
Cotton blue (0.05 g)
Lactic acid (20 ml)
Glycerol (20 ml)
Distilled water (20 ml)
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. 25 µl of spore suspension was added to 10 µl of the tested compound solution and placed in separate glass slides. Slides with 25 µl of spore suspension alone served as the controls. Slides were then incubated in moist chamber at 25°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.
All melting points were determined in open capillary tube and were uncorrected. IR spectra were recorded with potassium bromide pellets technique, ^{1}H NMR spectra were recorded on AVANCE 300 MHz Spectrometer in DMSO using TMS as internal standard. Mass spectra were recorded on a FT VG-7070 H Mass Spectrometer using EI technique at 70 eV. All the reactions were monitored by Thin Layer Chromatography.

MATERIAL AND METHODS

Synthesis of 4-Cyano-5-imino-3-methylthio-9-nitro-2H-1,2,4-triazipino [3,4-*b*] [1,3] benzothiazole (III)
A mixture of 2-hydrazino-6-nitro benzothiazole (I) [0.210 gm, 0.001 mole] and bis methylthio methylene malononitrile [0.170 gm, 0.001 mole] (II) was refluxed in the presence of 5 ml of dimethyl formamide and anhydrous potassium carbonate (0.2 gm) for five 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 ethanol to get 0.215 gm of crystalline solid of (III).
Yield: 64% M.P: 274°C, IR:(KBr/cm^{-1}): 3445 (=NH), 3250 (-NH), 2210 (CN), 1545 and 1335 (-NO_2), ^{1}H-NMR: (DMSO) : δ 2.80 (s 3H SCH_3), δ 9.10 (s 1H -NH), δ 9.50 (s 1H =NH), δ 7.70 - 8.50 (m 3H Ar-H). EI-MS: (m/z:RA%): 333 (M+1). ^{13}C NMR (DMSO) : δ 15 ($S\underline{C}H_3$), δ 61 (C_4), δ 115 (C-$\underline{C}$N), δ 117 (C_7, Ar-C), δ 118 (C_8 Ar-C), δ 126 (C_{10} Ar-C), δ 127 (C_{12} Ar-C), δ 138 (C_9 Ar-C), δ 153 (C_{13} Ar-C), δ 154 (C_{14}), δ 164 (C_5), δ 178 (C_3). Elemental analysis: $C_{12}H_8N_6O_2S_2$, Calculated: (%) C 43.37, H 2.43, N 25.29, O 9.63, S 19.30 Found (%): C 43.33, H 2.41, N 25.22, O 9.60, S 19.25.



(III)

Synthesis of 4-Cyano-5-imino-3-substituted-9-nitro-2H-1,2,4-triazipino [3,4-*b*] [1,3] benzothiazole (III)

A mixture of 2-hydrazino-6-nitro benzothiazole (I) [0.210 gm, 0.001 mole] and bis-methylthio methylene malononitrile (II) [0.170 gm, 0.001 mole] was refluxed in the presence of of dimethyl formamide (5 ml) and a pinch of anhydrous potassium carbonate (0.2 gm) was refluxed independently with one mole equivalent of aryl amines / phenols / heteryl amines and compounds containing active methylene group for six hours. The progress of reaction was monitored on TLC. After completion of reaction, the reaction mixture was cooled to room temperature and poured on ice cold water. The separated solid product was filtered, washed with water and recrystallized from ethanol to give respective products.

Method for Antifungal Activity

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.

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* sp (Figure 1).

Organisms Selected for Antifungal Activity

The organisms selected for antifungal activity are *Aspergillus Niger* & *Penicillium sp* species. Antifungal activity by Well diffusion method of 4-Cyano-5-imino-3-methylthio-9-nitro-2H-1,2,4-triazipino[3,4-*b*][1,3]benzo thiazole (III) and its 3-substituted derivatives (Figure 2).

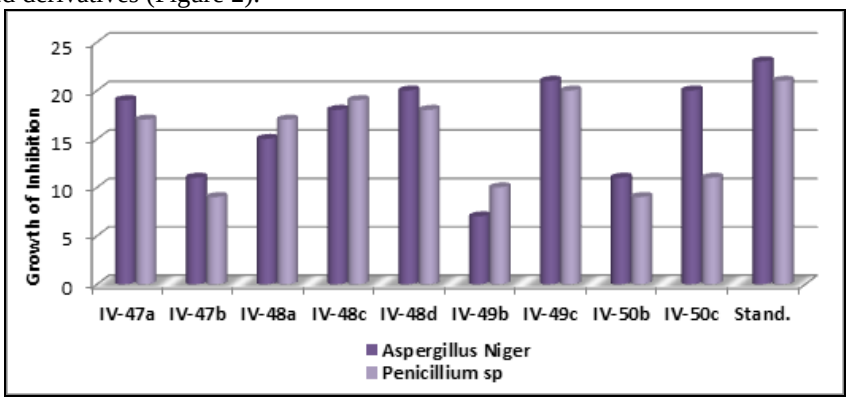


Figure 1: Antifungal activity of test compounds by well diffusion method

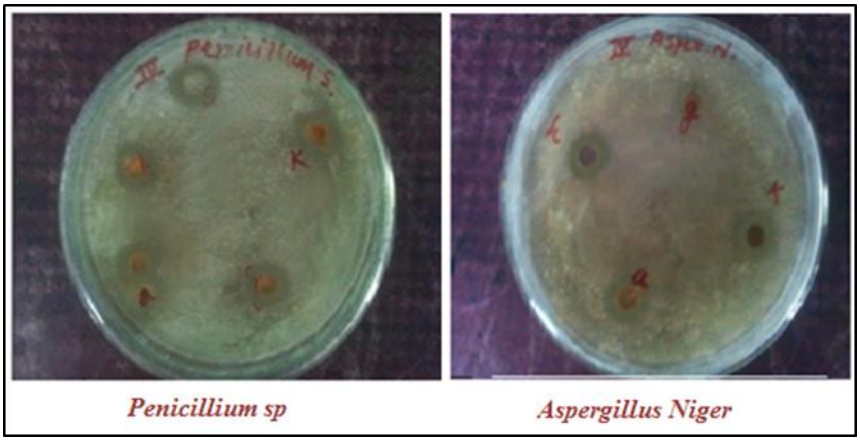


Figure 2: Antifungal activity of the test compounds with zone of inhibition

Table 1: Antifungal activity of compound III and its 3-substituted derivatives

Comp. Nos.	R	Diameter in mm of zone of inhibition in mm	
		Aspergillus niger	***Penicillium* sp**
IV-47a	p-chloroanilino	19 mm	17 mm
IV-47b	p-nitroanilino	11 mm	09 mm
IV-47c	p-hydroxyanilino	---	08 mm
IV-47d	*p*-toluidino	08 mm	---
IV-48a	4'-nitro phenoxy	15 mm	17 mm
IV-48b	4'-carboxylicphenoxy	10 mm	---
IV-48c	phenoxy	18 mm	19 mm
IV-48d	4-methyl phenoxy	20 mm	18 mm
IV-49a	malononitrile	---	07 mm
IV-49b	α-ethyl acetoacetyl	07 mm	10 mm
IV-49c	α-acetyl acetone	21 mm	20 mm
IV-50a	piperazino	14 mm	---
IV-50b	morpholino	11 mm	09 mm
IV-50c	piperidino	17 mm	11 mm
Std.	Fluconazole	23 mm	21 mm
	DMSO	---	---

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

RESULTS AND DISCUSSION

The screening for antifungal activity of newly synthesized compound 4-cyano-5-imino-3-methylthio-9-nitro-2H-1,2,4-triazepino [3,4-b] [1,3] benzo- thiazole (IV-46) and its 3-substituted derivatives have been studied against *Aspergillus niger* and *Penicillium sp* species by well diffusion method. The preliminary screening showed that, compounds are exhibited the zone of inhibition in the range of 7 mm to 23 mm in diameter for *Aspergillus niger* species and from 7 mm to 21 mm for *Penicillium sp species.*

Compounds IV-47b, IV-47d, IV-48a, IV-48b, IV-49b, IV-50a, IV-50b exhibited the zone of inhibition in between 7 mm to 16 mm in diameter shows poor antifungal activity, compounds IV-47a, IV-48c, IV-48d, IV-49c, IV-50c exhibited the zone of inhibition in between 17 mm to 23 mm in diameter shows good antifungal activity and compounds IV-47c, IV-49a do not shows antifungal activity against *Penicillium sp* species. The results of antifungal activity are shown in Table 1.

Compounds IV-47b, IV-47c, IV-49a, IV-49b, IV-50b, IV-50c exhibited the zone of inhibition in between 7 mm to 16 mm in diameter shows poor antifungal activity, compounds IV-47a, IV-48a, IV-48c, IV-48d, IV-49c exhibited the zone of inhibition in between 17 mm to 23 mm in diameter shows good antifungal activity and compounds IV-47d, IV-48b, IV-50a do not shows antifungal activity against *Aspergillus Niger species.* The results of antifungal activity are shown in Table 1.

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. 4-cyano-5-imino-3-methylthio-9-nitro-2H-1,2,4-triazepino [3,4-b] [1,3] benzo- thiazole (IV-46) and its 3-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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