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Synthesis & Evaluation of Antifungal Activity of Novel 1-(5, 5-Diphenyl Imidazolidone-2'-yl)-5- Thio-1, 2, 4-Triazolidin-3-one Derivatives

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ABSTRACT

A broad spectrum of pharmacological properties has been demonstrated by the 1,2,4-triazole. Many of the existing drugs have developed resistance and newer drugs are required with better activity profile. Very few drugs are available in the treatment of fungal infections that too have serious toxic effects. In the present investigation, an attempt was made to synthesize the new potential antifungal compounds (i.e. newer analogues of 4 – aryl / alkyl – 1 - (5, 5-diphenyl imidazolidone - 2'- yl) - 5 - thio-1, 2, 4-triazolidin-3-ones) using novel route from 2-thio-5, 5-diphenyl imidazolidone by reaction with solution of hydrazine hydrate followed by ethyl chloroformate and aryl/alkyl isothiocynate. It was observed that all the compounds exhibited activity against Candida albicans. Minimum inhibitory concentration of the said compounds was determined by broth dilution technique. The activity of all the synthesized compounds was compared against Fluconazole (Standard 1) and Amphotericine B (Standard 2).

Key Words: Antifungal compounds, 1, 2, 4-triazole, MIC, *Candida albicans,* Amphotericine B, Fluconazole

INTRODUCTION

Traditionally living organisms are either plants or animals but proper position of the fungi is still obscure. During recent years there has been increasing attention to view that fungi and heterotropic thallophytes are neither plants nor animals and possibly there merit classification is in distinct kingdom. The term fungus is a general term that includes a number of diverse forms such as molds and yeasts. Fungi are heterotropic, eukaryotic microorganisms that are distinguished from algae by lack of photosynthetic ability. Fungi differ from bacteria by greater size and possession of such intracellular structures as nuclear membrane and mitochondria; cells of fungi pathogenic for animals have rigid cell wall containing chitin and polysaccharide. Fungal cell membranes, other than those of Pneumocystis, contain ergosterol and zymosterol whereas animal cell membrane has cholesterol.

Fungal infections and their consequent mortality have been rising in frequency over the past decade[1]. A candida species infection rises continuously during the 1980's and aspergillosis, an infection observed among increasing numbers of immunocompromised patients, is associated with an overall mortality of 55% [2]. Among bone marrow transplants recipient's mortality due to aspergillosis is greater than 80% despite the empiric and directed use of amphotericin B[3]. Observations over 20 years have shown that dermatoses of varied nature are common in children below 16 years who form about 20% of the outpatient in skin clinic.

The development of new antifungal compounds for use in treatment of human mycoses begins with in vitro estimation of the antifungal spectrum of the proposed agent. The antifungal chemotherapeutic agents include drugs belonging to various classes, organometallic compounds, sulphur containing compounds, phenolics and diamidines, imidazole based compounds and antibiotics are being used to combat fungal infections. Many remedies have been used against fungal infections and research still continues which would lead one to conclude that an ideal antifungal agent has not yet found[4].

A broad spectrum of pharmacological properties has been demonstrated by the triazole nucleus[5-9]. Many of the existing drugs have developed resistance and newer drugs are required with better activity profile. Only few drugs are available in the treatment of fungal infections that too have serious toxic effects. Therefore safe and more effective drugs are required to be developed. 4-aryl-1- benzothiazole-2'-yl)-5-thio-1,2,4-triazolidine-3-one (3-hydroxy) group of compounds have been shown to possess anticancer activity. In view of these isosterically related benzimidazole type of compounds may also prove as potential anticancer agents. Triazole molecules have antibacterial[10,11], antifungal[12], anthelmintic[13] and anti cancer[11] activity.

The "azoles" represent a class of versatile antifungal agents with an apparently unique mechanism of action. Early members of this class, such as Clotrimazole and miconazole are highly substituted imidazoles. However structure activity relationship studies reveals that the imidazole ring could be replaced with the isosteric 1, 2, 4-Triazole ring without adversely affecting the antifungal properties of the molecule[14].

Considering the various activities of 1, 2, 4-triazole, the present research work was oriented towards the synthesis and evaluation of biological activity of newer analogs of "4 aryl/alkyl-1- (5,5-diphenylimidazolidone-2'-yl)-5-thio-1, 2,4-triazolidine-3-ones".

MATERIALS AND METHODS

Melting points were determined in open glass capillaries and using melting range apparatus and were uncorrected. IR spectra (V_{max} in cm^{-1}) were recorded on a Perkin-Elmer-1600 series FTIR, ^1H NMR spectra on 300MHz NMR VXRO spectrometer using DMSO-d_6 as solvent (chemical shifts in δ, ppm). TLC in various solvents showed that the compounds were homogeneous. Compound 1 and aryl/alkyl isothiocynate prepared by the literature methods[8, 9].

Synthesis of 2-thio5, 5-diphenyl imidazolidone (1)

5.3gm (0.025 moles) of benzil, 3.5gm (0.045 moles) of thiourea, 15ml of 30% aqueous NaOH solution and 75ml of ethanol were taken in 250ml round bottom flask attached with a reflux condenser. The reaction mixture was boiled under reflux on an electric heating mantle for 2hrs. The contents were then cooled to room temperature and poured into 125ml of water with continuous stirring and allowed to stand for 15 minutes. The by product obtained was removed by suction filtration and filtrate was treated with Concentrated HCl, which was further cooled in ice water. The product obtained was immediately filtered under suction filtration. The crude product was recrystalized using ethanol.

Synthesis of 2-hydrazino-5, 5-diphenyl imidazolidone (2)

A mixture of 2 – thio – 5, 5 – diphenyl imidazolidone (0.4M) and hydrazine hydrate (0.4M) was refluxed at 80 ºC using ethanol as solvent till the evolution of hydrogen sulphide gas was ceased. Absence of hydrogen sulphide gas was checked by lead acetate paper. On cooling, the product was precipitated out and was recrystallized from ethanol and further absence of sulphur was checked by sodium fusion test.

Synthesis of 2-Carbethoxy-(5, 5-diphenylimidazolidone-2'-yl) hydrazine (3)

A solution of 2 – hydrazine – 5, 5 – diphenyl imidazolidone (0.02M) in water and pyridine was cooled in ice bath and then treated with ethyl chloroformate (0.02M) for 10 minutes with rigorous shaking. The product was collected and recrystalized from acetone water mixture.

Synthesis of Aryl/ alkyl isothiocynate (4)

137gm (110ml) of (1.5M) carbon disulphide and cold solution of 72gm (1.5M) sodium hydroxide in 160ml of water were placed in a one liter three necked flask surrounded by crushed ice, placed on magnetic stirrer fitted with reflux condenser, a thermometer and 250ml dropping funnel. This mixture was cooled to 10-15ºC. 1.5M of aryl/alkyl amine was added with stirring over period of 30 minutes, then stirring was continued and mixture was warmed gently on a steam bath for 1-2hrs to ensure complete reaction. The bright red solution was cooled to 35-40ºC and 1.8M of ethyl chloroformate was added over a period of 1hr with stirring. The stirring was continued for 30 minutes after all the ethyl chloroformate was added at that time the temperature was fallen

between to 30-40ºC. The aryl/alkyl isothiocynate was separated on top, was removed from the reaction mixture. The product was dried over 10gm of anhydrous sodium sulphate and distilled under reduced pressure.

Synthesis of 4-aryl/alkyl-1- (5, 5-diphenyl imidazolidone-2'-yl)-5-thio-1,2,4-triazolidin-3-ones(5):
A mixture of 2 – Carbethoxy – (5, 5 – diphenyl imidazolidone-2'-yl) hydrazine (0.02M) and aryl/alkyl isothiocynate in 8% acetonic sodium hydroxide was refluxed for 2hrs. The resulting reaction mixture was cooled to room temperature and poured drop wise on to crushed ice, it was then stirred till miscible with water, acidified by dilute HCl, and resulting solid was recrystalized by using dioxane water mixture. The % yield was calculated and the structure was confirmed for various compounds using spectral studies.

Evaluation of antifungal activity
The culture of *Candida albicans* was obtained from the microbiology department of Prin. K. M. K. College of Pharmacy, Mumbai. Sabourauds Dextrose Agar was used for growing *Candida albicans.* Stock cultures were preserved in Sabourauds Agar media. Culture and standardized by Spectrophotometric method using McFarland turbidity standard.[15]

Method
The microbial experiments were carried out in aseptic area under High Efficiency Particulate Air filters. Sabourauds dextrose broth was prepared. The medium was then poured in the test tubes, which were then sterilized by autoclave using 15lb pressure at 121°C for 30 minutes. Using sterile pipettes exact amounts of test solution was added. The tubes were then inoculated with 0.05 ml of standardized culture. The tubes were incubated at temperature 30-32°C and observed for growth. The lowest concentration that can inhibit the growth is reported. The activity of all the synthesized compounds was compared against Fluconazole (Standard 1) and Amphotericine B (Standard 2).

RESULTS AND DISCUSSION
The scheme of the synthesis is represented in figure-1.The Physical and spectral data of compounds 5a, 5b, 5c, 5d and 5e is shown in table-1. All the compounds were shown to exhibit activity against *Candida albicans*.The minimum inhibitory concentration of compounds (MIC) are shown in table-2. It was observed that the synthesized compounds were as active as that of Fluconazole and more active than Amphotericine B. The objective of microbiological testing was to measure the effect on the antifungal potency by varying the substituents at position 4 in the heterocyclic ring of the triazole analogues. P-amino phenyl substituted analogues showed highest activity where as compound possessing hydroxyl phenyl derivative gave lower activity

CONCLUSION
The newer congeners of 1, 2, 4 triazole which are synthesized in laboratory, were found to give comparable activity. These encouraging results in terms of potency and efficacy further permit the execution of detailed studies of these compounds.

Figure-1: Scheme of synthesis

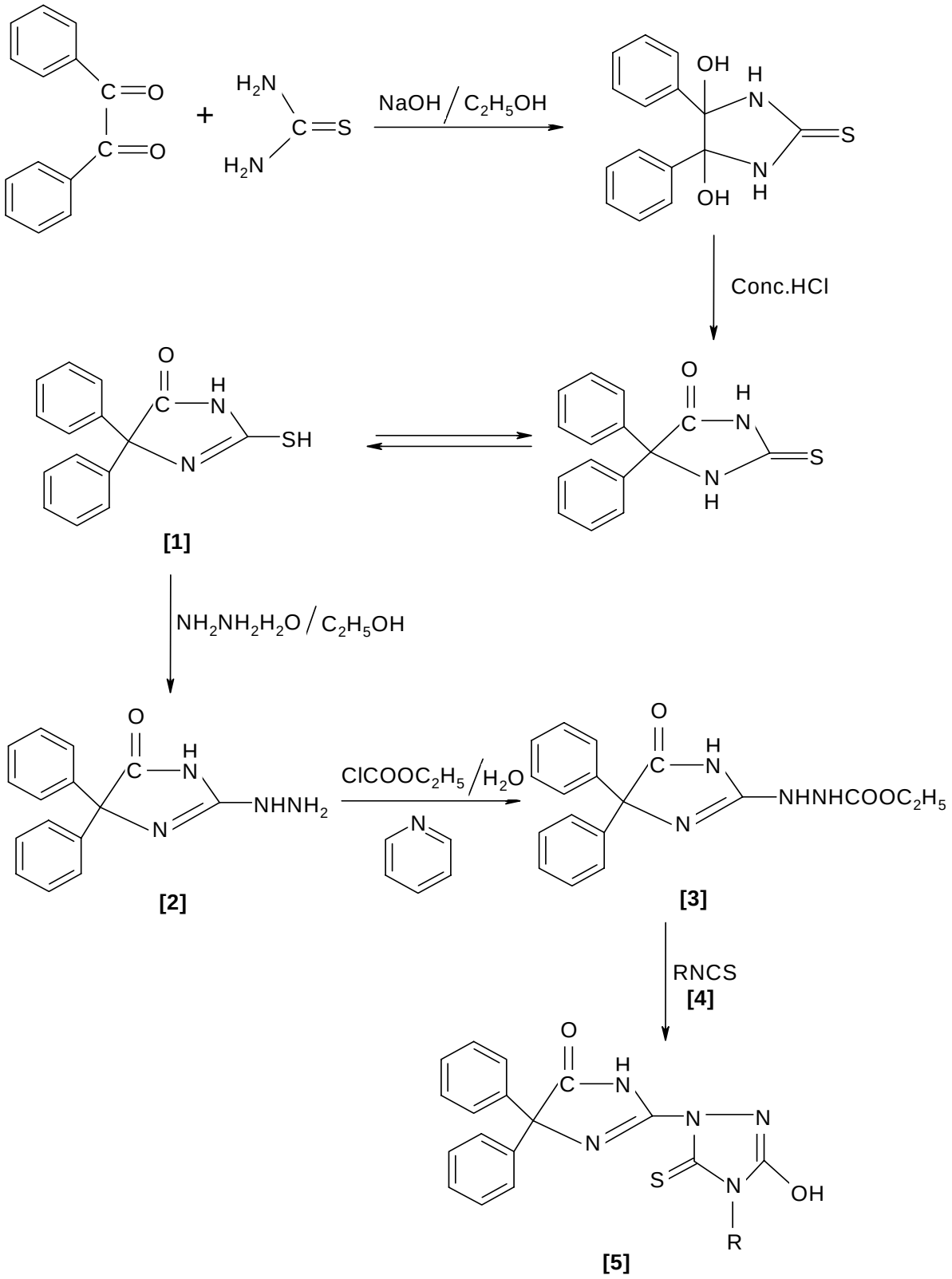

Table-1: Physical and spectral data of compounds 5a, 5b, 5c, 5d and 5e

Compound	R	Molecular Formula	m.p. (˚C)	Yield (%)	Calculated % N	IR (V_{max} incm^{-1})
5a*	p-$HOOCC_6H_4$	$C_{24}H_{17}N_5SO_4$	274	73.4	13.40	698, 746, 910, 1448, 1597,937, 1286, 1493, 1070, 1400, 1238, 1720
5b	p-$H_2NC_6H_4$	$C_{23}H_{18}N_6SO_2$	283	63.04	20.36	698, 745, 910, 1450, 937, 3277, 1597, 1286, 1493, 1070, 1402
5c	p-HOOC, m-HOC_6H_3	$C_{24}H_{17}N_5SO_5$	278	69.3	15.10	698, 746, 910, 1450, 937, 1402, 1597, 1286, 1493, 1070, 1236, 1720, 3205
5d**	m-HOC_6H_4	$C_{23}H_{17}N_5SO_3$	272	67.30	16.48	698, 746, 910, 1448, 937, 1400, 1597, 1286, 1493, 1070, 3205
5e	p-$H_3CCOHNC_6H_4$	$C_{25}H_{20}N_6SO_3$	180	73.88	18.20	684, 767, 1228, 1452, 3080, 3314, 1311, 1408, 1479, 1076, 1666, 2943

H-NMR* 7.2-7.5 (m, ArH), 9.3 (d, NH), 11.11 (s, ArCOOH), 2.4 (m, DMSO-d_6)
H-NMR** 7.2-7.5 (m, ArH), 9.3 (d, NH), 11.10 (s, ArOH), 2.4 (m, DMSO-d_6). MASS: M/Z at 165 for biphenyl

Table-2: Minimum inhibitory concentration (MIC) of the synthesized compounds

Sr. No.	Compound	MIC in µg/ml
1	5a	90
2	5b	100
3	5c	100
4	5d	110
5	5e	100
6	Solution of Standard 1	70
7	Solution of Standard 2	600

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