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Original Article

Pharmacology Section

Synthesis and Antifungal Activity of N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) Benzamide Derivatives: An in-vitro Study

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ABSTRACT

Introduction: Fungal infections are associated with morbidity and mortality globally and immense economic burden. With the emergence of multidrug resistant fungal strains, it is essential to develop new antifungal drugs.

Aim: To synthesize and evaluate the antifungal activity of N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus niger*.

Materials and Methods: An in-vitro study was conducted in the Department of Chemistry, Islamiah College, Vaniyambadi, Tamil Nadu, India from August 2022 to February 2024.

Novel N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides were synthesized by a simple procedure. Pyridine was added to 3-(4-(1,3-dioxolan-2yl) phenyl)-1,2,4-thiadiazol-5-amine and dichloromethane was added dropwise. The resulting mixture was washed with water, saturated brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The purification of crude residue was done by column chromatography. Their characteristics were confirmed by 1H and 13C Nuclear Magnetic Resonance (NMR) and Liquid Chromatography- Mass Spectrometry (LC-MS). They were screened for activity against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus niger* by disc diffusion method. A loopful of cells from fungal stock cultures was added to Sabouraud Dextrose Broth (SDB) containing test tubes and incubated at 25°C for 24 hours. The test compounds were assessed for their antifungal activity. Their activity was compared with fluconazole which was the standard drug. The experiments were conducted with the test compounds and control experiment was done with Fluconazole. The growth inhibition zones around the disks were measured in mm at 28°C after 2-3 days.

Results: Twelve N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (7a-7l) were synthesized with good yield. Compounds 7c and 7g had good activity against *Candida albicans.* Compounds 7f and 7g had moderate activity against *Aspergillus niger*, whereas Compounds 7d, 7f and 7h had moderate activity against *Cryptococcus neoformans*.

Conclusion: A series of N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (7a-7l) were synthesized. These compounds have moderate to good antifungal activity against *Candida albicans*, and moderate activity against *Aspergillus niger* and *Cryptococcus neoformans*.

Keywords: 1,2,4-thiadiazoles, *Aspergillus niger*, *Candida albicans*, *Cryptococcus neoformans*

INTRODUCTION

Fungal infections result in significant global morbidity and mortality, especially in individuals with compromised immunity or hospitalised patients with serious illnesses [1]. The global mortality associated with invasive fungal infections is estimated to be 1.5-2 million per year [2]. The economic burden of fungal infections is also immense. The most common fungal species causing Healthcare Associated Infections (HAI) are Candida, Aspergillus, Mucorales, Fusarium and molds [3,4]. *Cryptococcus, Candida, Aspergillus* and *Pneumocystis* are responsible for more than 90% of deaths due to fungal infections [5].

However, the epidemiology of fungal infections has undergone changes recently with the emergence of new pathogens, expansion of spectrum of existing species and multidrug resistance development [6]. *Candida albicans* is commonly associated with invasive fungal infections. But several non-albicans species such as *C. glabrata*, *C. tropicalis*, *C. krusei* etc., have emerged with rapid development of resistance to azoles, especially to fluconazole. Aspergillus species are common causes of invasive and chronic lung infections. There is emerging concern regarding development of azole resistance among Aspergillus species [7].

Cryptococcal infection begins as lung lesions which can progress to meningoencephalitis, disseminated cryptococcosis in Acquired Immunodeficiency Syndrome (AIDS) patients and recently, in severe COVID-19 infections. The treatment of cryptoccoal disease is restricted to amphotericin B, flucytosine and fluconazole. The treatment duration is long and these drugs, especially amphotericin B are highly toxic [8]. Therefore, it is essential to develop novel antifungal drugs which can be effective against these emerging infections.

Synthetic compounds comprising a heterocyclic ring have a pivotal role in the development of new antimicrobial drugs [9]. The 1,2,4-Thiadiazoles are heterocyclic compounds rich in heteroatoms that have an important place in medicinal chemistry programmes. Synthetic 1,2,4-thiadiazole compounds exhibit various biological activities such as anticancer, neuroprotective, muscarinic, cardioprotective etc., [10]. Many commercially available drugs like cephalosporins – cefozopran, ceftaroline and ceftobiprole, neurokinin-3 inhibitor – fezolinetant contain 1,2,4-thiadiazole [11]. The bio-isosteric replacement of the thiazole nucleus by thiadiazole moiety results in the synthetic compounds with activity similar to third and fourth generation cephalosporins [12]. In a previous study, the synthesis and antifungal activity of 2-(2-pyridyl)-2*H*-pyrazole-3-carboxamide compounds was reported against Fusarium oxysporum and *Candida albicans* [13]. In continuation of this work, the present study was planned with the following objectives: 1) To synthesize N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-

yl) benzamides; and 2) To evaluate their antifungal activity against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus niger*.

MATERIALS AND METHODS

An in-vitro study was conducted in the Department of Chemistry, Islamiah College, Vaniyambadi, Tamil Nadu, India from August 2022 to February 2024.

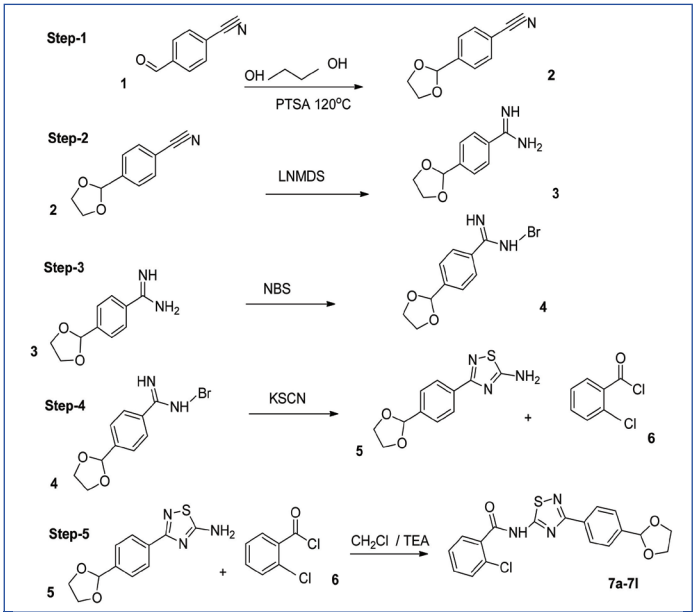
The study was approved by the institutional ethics committee of Islamiah College, Vaniyambadi, Tamil Nadu, India with reference no. IEC/IC/31/2022-23.

Chemicals and Reagents

Toluene, dry THF and Ethanol were procured from Spectrochem private limited, Mumbai, India [14]. All other study reagents were obtained from Sigma-Aldrich chemical co. Nitrogen atmosphere was employed to conduct all reactions. Rotavapor was used to remove excess solvents under reduced pressure. NMR spectra were estimated using Bruker AMX 400 instrument. 1H and 13C NMR spectra were estimated at 400 MHz and tetramethylsilane (TMS) was used as an internal reference. The Infrared Radiation (IR) spectra were estimated using Perkin-Elmer 1600 series FTIR spectrometer. LCMS data was obtained using Agilent 1200 series LC and Micro mass zQ spectrometer. Column chromatography was done using Silica gel with 230–400 mesh. The melting points were documented using Buchi melting point apparatus B-540. The Varian instrument VARIO EL3 series was employed to conduct the elemental analysis.

Synthesis of Novel N-(3-(4-(1, 3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (7a-7l)

Pyridine (0.096g, 1.2 mmol) was added to a mixture of 3-(4-(1,3-dioxolan-2yl) phenyl)-1,2,4-thiadiazol-5-amine (5) (0.1 g, 0.4 mmol) in dichloromethane (5 mL), which was cooled to 0°C and corresponding acid chloride (6) (0.07 g, 0.4 mmol) in dichloromethane was added dropwise. The resulting mass was stirred for 16hr at reaction time. Dichloromethane 50 mL was added to this mixture. It was washed with water, saturated brine solution, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The purification of crude residue was done by column chromatography using ethyl acetate and petroleum ether to obtain corresponding N-(3-(4-(1,3 dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (7a-7l). [Table/Fig-1] LCMS and NMR data was used to characterise the prepared compounds.



[Table/Fig-1]: Synthesis of Novel N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (7a-7l) Toluenesulfonic.
PTSA : p-Toluenesulfonic acid; LiHMDS: Lithium bis(trimethylsilyl) amide.: N-Bromosuccinimide; TEA: Triethylamine

Pharmacological Activity

Antifungal Activity: The antifungal activity of novel thiadiazole compounds (7a-7l) was tested against three fungal species- *Candida albicans (ATCC 10231), Aspergillus Niger (ATCC 16404)* and *Cryptococcus neoformans (ATCC 208821)* by disc diffusion method. The incubation of fungal stock cultures was done on Potato Dextrose Agar (PDA) at 37°C for one day. Their maintenance was done at 4°C on PDA slopes. A loopful of these cells was added to SDB containing test tubes. After incubation at 25°C for 24 hours, they were used for experiments. Desired optical densities were attained by adding fresh SDB. Similarly, the control experiments with Fluconazole were done. The growth inhibition zones around the disks were measured in mm at 28°C after 2-3 days [15].

STATISTICAL ANALYSIS

Descriptive statistics was used to summarise the data and data was compiled and tabulated using Microsoft excel (2013 version).

RESULTS

Synthesis and Yield of 1,2,4-thiadiazole Derivatives (7a-7l)

The various N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (7a-7l) were synthesised using the scheme described in [Table/Fig-1]. Their yields are shown in [Table/Fig-2].

Compound	Molecular formula	Halogen group	Melting points	Yield (%)
7a	$C_{18}H_{14}$ Cl N_3O_3S	Cl	163.1-164.3°C	73
7b	$C_{18}H_{14}$ F N_3O_3S	F	134 -135.8°C	80
7c	$C_{18}H_{14}$ Br N_3O_3S	Br	157.5-158.9°C	85
7d	$C_{19}H_{17}$ N_3O_4S	Cl	174.2-175°C	77
7e	$C_{17}H_{13}$ Cl N_4O_3S	Cl	129 -130.2°C	82
7f	$C_{16}H_{14}N_3O_3S_2$	-	181.-182.5°C	78
7g	$C_{18}H_{14}$ Cl N_3O_3S	Cl	129 -130.2°C	88
7h	$C_{18}H_{14}$ N_4O_5S	-	191.3-192.5°C	71
7i	$C_{18}H_{13}$ F N_4O_5S	F	222.3-223.6°C	83
7j	$C_{18}H_{13}$ F_2 N_3O_3S	F	180 - 181.4°C	81
7k	$C_{15}H_{15}$ N_3O_3S	-	156.3 -157.8°C	79
7l	$C_{19}H_{17}$ N_3O_3S	-	192.3 - 193.5°C	70

[Table/Fig-2]: Physical properties and yields of the 1,2,4-thiadiazole compounds (7a-7l).

Antifungal activity: The Thiadiazole derivatives (7a-7l) were evaluated for their activity against *Candida albicans, Aspergillus Niger* and *Cryptococcus neoformans* by disc diffusion method. Fluconazole was used as the reference antifungal drug. The zone of inhibition in mm indicated the antifungal effect of compounds 7a-7l [Table/Fig-3-6]. Majority of the thiadiazole derivatives had moderate to good activity against *Candida albicans*. Some compounds had moderate activity against *Aspergillus niger* and *Cryptococcus neoformans*.

Compound	*Aspergillus Niger*	*Candida Albicans*	*Cryptococcus neoformans*
7a	NZ	7	NZ
7b	NZ	6	NZ
7c	NZ	9	NZ
7d	NZ	NZ	8
7e	NZ	7	NZ
7f	7	8	8
7g	11	10	NZ
7h	NZ	NZ	6
7i	NZ	NZ	NZ

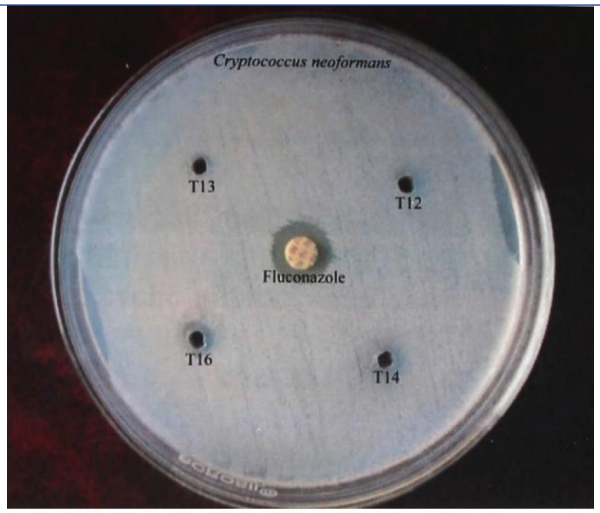
7j	NZ	NZ	NZ
7k	NZ	NZ	NZ
7l	NZ	NZ	NZ
Fluconazole	15	10	14

[Table/Fig-3]: Antifungal activities of the Thiadiazole derivatives (7a-7l). Zone of inhibition in mm.
Values displayed are the mean of three determinations with range less than 5% of mean; NZ: No zone of inhibition

a T12 -7a, T13-7b, T14-7c, T16 -7e

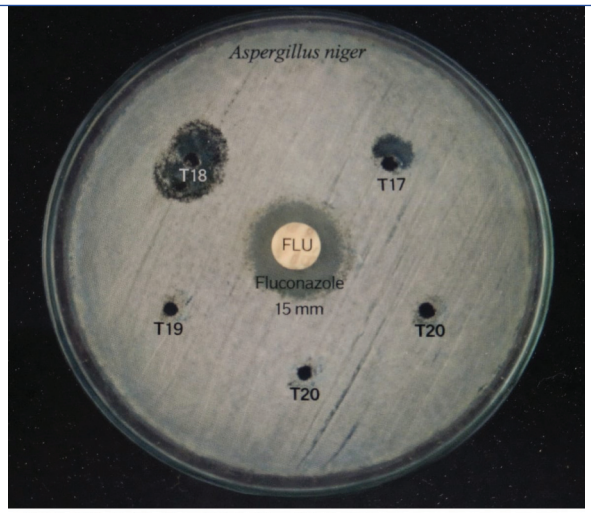
b T17-7f, T18-7g, T19-7h, T20-7d

[Table/Fig-4]: Zone of inhibition (mm) of 1,2,4-thiadiazoles in *Candida albicans*.



T12-7a, T13-7b, T14-7d, T16 -7f

[Table/Fig-5]: Zone of inhibition (mm) of 1,2,4-thiadiazoles in *Cryptococcus neoformans*.



T17-7f, T18-7g, T19-7h, T20-7d

[Table/Fig-6]: Zone of inhibition (mm) of 1,2,4-thiadiazoles in *Aspergillus niger*.

DISCUSSION

The emergence of multidrug resistant fungal infections is a challenge to public health globally and results in ineffective management and higher mortality [16]. With the availability of relatively few antifungal drugs whose efficacy is restricted by narrow spectrum of activity, toxicity, unfavourable drug interactions, high cost and resistance development, it is essential to develop novel compounds with antifungal activity [17]. In the present study, novel N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides were synthesized and screened for antifungal activity.

Twelve novel N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamides (7a-7l) were prepared by the scheme described in [Table/Fig-1]. and had good yield. The characteristics of these compounds were confirmed by LCMS and NMR data. The synthesis of these thiadiazoles is a cost-effective process which is important in clinical context because high cost of new drugs can be restrictive from patient's perspective. The activity of compounds 7a-7l was assessed by disc diffusion method against *Aspergillus Niger, Candida albicans* and *Cryptococcus neoformans.* These three fungal species were selected for studying the antifungal activity because they are responsible for more than 90% of fungal infection-related deaths along with *Pneumocystis species* [5].

Among the Thiadiazole compounds 7a-7l, compounds 7a, b, e, f had moderate activity against *Candida albicans*. Compound 7c had good activity against *Candida albicans* whereas compound 7g had activity comparable to Fluconazole. Only compounds 7f and 7g had moderate activity against *Aspergillus niger*. Compounds 7d, 7f and 7h had moderate activity against *Cryptococcus neoformans*. Compounds 7i-7l did not exhibit any antifungal activity.

The thiadiazole ring present in the structure of these compounds 7a-7h may be responsible for their antifungal activity. Although the Compounds 7i-7l contains the Thiadiazole moiety, they did not exhibit antifungal activity. This may be due to differences in their structures and positions of halogens and other substitution groups. Thiadiazoles are heterocyclic compounds containing nitrogen and sulfur and are extensively used as structural units

of biologically active molecules. They serve as intermediates in medicinal chemistry programmes. Thiadiazole exist in four isomer forms namely 1,2,5-thiadiazole, 1,3,4-thiadiazole, 1,2,3-thiadiazole and 1,2,4-thiadiazole [18]. Among these isomers, 1,3,4-thiadiazoles are extensively studied. Several studies have reported the antifungal activity of 1,3,4-thiadiazole containing compounds [19-21]. However, the present study is one among few studies which have reported the antifungal activity of 1,2,4-thiadiazole compounds. Eligeti R et al., have reported the antibacterial and antifungal activity of 1,2,4-thiadiazole containing compounds [22].

The availability of limited antifungal drugs and rapid development of resistance to them is a cause of concern [23]. The novel 1,2,4-thiadiazole compounds have the potential to be considered for future drug development. However, further studies to elucidate the structure activity relationship and mechanism of action of these compounds is essential.

Limitation(s)

Limitation of this study is that the antifungal activity of 1,2,4-thiadiazole compounds was studied against in only three fungal species.

CONCLUSION(S)

Novel N-(3-(4-(1,3-dioxolan-2-yl) phenyl)-1,2,4-thiadiazol-5-yl) benzamide derivatives (7a-7l) were synthesized by a simple and cost-effective process. These compounds have moderate to good activity against *Candida albicans* and moderate activity against *Cryptococcus neoformans* and *Aspergillus niger*.

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