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Anti-fungal Activities of Some 2-(p-Substituted Benzylidene)-3-(5-Alkyl-1,3,4-Thiadiazol-2-yl) Thiazolidin-4-Ones

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ABSTRACT

Reaction of thiosemicarbazide with alkyle or fatty acids in presence of concentrated sulphuric acid led to the formation 2-amino-5-alkyle-1, 3, 4-thiadiazole which on further reaction with different p-substituted benzaldehydes yielded the Schiff's base of N-(p-substituted benzylidene)-5-alkyle-1, 3, 4-thiadiazole-2-amines which is further reaction with thioglycollic acid in presence of small amount of zinc chloride in 1,4 dioxane as solvent gave title compounds. These compounds were characterized by spectral analysis and evaluated for their Anti-fungal activities and their minimum inhibitory concentration (MIC) were determined.

***Keywords**: Thiazolidinone, Schiff's base, Anti-fungal, Minimum inhibitory concentration (MIC).*

INTRODUCTION

The fungi are eukaryotic, non-vascular organism. These are filamentous or occasionally unicellular. These are mostly non-motile, but few are motile such as chytrides [1]. The fungi include both yeasts and molds and fleshy fungi. Yeasts are unicellular organism. Molds are multicellular filamentous organism such as mildews, rusts and smuts. The flashy fungi include mushroom, puffballs and coral fungi. Besides the great advantages to which fungi have been put to them to produce a number of

fungal diseases. Fungal diseases grouped into two types: (i) the superficial mycoses or dermatomycoses; (ii) the systemic mycoses. The superficial mycoses occur on the skin, hair, nails, and mucus membranes. Many of the fungi cause various forms of ringworm and the organisms that cause are commonly called the dermatophytes or ringworm fungi. Some common dermatophytes are *Trichophyton rubrum*, *Microsporum gypseum*, etc. The systemic, or deep, mycoses are serious fungal dieses. The organism invades subcutaneous tissues or the lungs, from which they may spread to other organs of the body where they become established and produce disease. *Candida albicans* is isolated from warm blooded animals. It is also present in human as a part of normal micro biota of mucus membrane. The diesis caused by this fungus is candidiasis, dieses of mucus membrane of the mouth, vagina and alimentary tract. *Aspergillus niger* is wide spread in nature such as found on fruits, vegetables etc. It is used for fermentation and production of citric acid, gluconic acid in industry. Direct contact of spore causes several local infections such as nasal sinuses and asthma, pneumonitis etc. [2-4].

Thiazolidinones are derivatives of thiazolidine which belong to important groups of heterocyclic compounds. Depending upon the presence of the carbonyl group at different position thiazolidinones are of different type such as 2, 4, and 5-position known as 2-thiazolidinones, 4-thiazolidinones, and 5-thiazolidinones. Among these thiazolidinone, 4-thiazolidinones shows greater biological activity [5]. Compounds containing thiazolidinone nucleus have been reported as anti-inflammatory [6,7], anti-tuberculosis [8] and antimicrobials [9-12]. These reports including our ongoing research program in the field of synthesis and antimicrobial activity of medicinally important compounds [7,8,13-19] inspired us to undertake the synthesis of some 2-(p-substituted benzylidene)-3-(5-propyl-1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones.

MATERIAL AND METHODS

In continuation of my work Anti-fungal activity of title compounds were screened against fungal strain *Candida albicans*, (MTCC 183) and *Aspergillus niger*, (MTCC 281) according to the procedure given below. The synthesis and characterization reported in previous paper.

Methodology

Anti-fungal activity was checked by using serial dilution method. All experiments were performed in triplicate.

Stock solution

Stock solution of the title compound and the standard drug having the concentration 10 µg/ml were prepared in dimethylsulphoxide. Further dilutions were made from this stock solution.

Media

The following media were used (Tables 1 and 2) [20].

Table 1: Sabouraud Liquid medium (SLM).

Ingredients	**Quantity**
Dextrose	40 grams
Peptone	10 grams
Water	1000 ml (q. s)

Table 2: Double strength Sabouraud Liquid medium (DSSLM).

Ingredients	Quantity
Dextrose	80 grams
Peptone	20 grams
Water	1000 ml (q. s)

The ingredients were mixed and boiled to prepare the solutions. The pH was adjusted to 5.6 ± 0.2 after sterilization.

Sterilization

The sterilization of culture media, culture tube, saline (NaCl, 0.9% w/v, in distilled water) and other materials was done by autoclaving at 15 lb/sq. inch pressure for 20 minutes.

Stock culture and inoculum

A loopful of fungal strain was transferred aseptically into the sterilized SLM and incubated at 25 ± 1°C for 48 hours for *C. albicans* and seven days for *A. niger*. This was taken as the sock culture. The fungal strain was subcultured by transferring aseptically a loopful of corresponding organism from the stock culture into the sterilized SLM and incubated. *C. albicans* culture was harvested by using sterilized saline and diluted suitably with sterilized saline solution to get the spore count about 1×10^7 colony forming unit per ml. Similarly, *A. niger* culture was harvested with sterilized saline solution containing 0.05% w/v of polysorbate 80 and adjusted the spore count to about 1×10^7 colony forming unit per ml with sterilized saline solution. An aliquot (0.1 ml) of this saline solution consisting of fungal strain was used for inoculation of culture tubes.

Determination of minimum inhibitory concentration (MIC) range

Anti-fungal activity evaluation of the synthesized compounds against *Candida albicans* and *A. niger* was performed by use of the Sabouraud's dextrose broth as media for assay. Clotrimazole was used as the standard drug for the present study. Serial two-fold dilution technique was used for the study of Anti-fungal activity [15]. A stock solution (10 µg/ml) of all the title compounds and standard drug was prepared in dimethyl sulfoxide. Sterilized DSSLM was used as a growth media. The stock solution was serially diluted by DSSLM aseptically to give concentrations of 5.0-0.01 µg/ml into a series of sterilized culture tubes. All the tubes were inoculated by fungal strain. The inoculum's size was approximately 10^6 colony forming units (CFU/ml). The inoculated tubes were incubated at 25 ± 1°C for 48 hours for *Candida albicans* and 7 days at 25 ± 1°C for *Asperagillus niger*. The growth in the tubes was observed visually for turbidity. The activity was then compared with that of the standard (Clotrimazole). The procedure was then repeated to confirm the MIC. The MIC for the title compounds and the standard drug, i.e., clotrimazole are presented in Tables 3 and 4.

Table 3: MIC range (µg/ml) of the synthesized compounds for Anti-fungal activity.

Compound	*A. niger*	*C. albicans*
1-40	0.75-0.45	0.75-0.45
Clotrimazole	0.31-0.16	0.16-0.08

The accurate MIC of standard drug and the title compounds for a particular fungal strain was determined.

Determination of accurate minimum inhibitory concentration (MIC) range

A similar method was used as in antibacterial activity using suitable temperature and incubation time for respective fungal strain.

Determination of minimum inhibitory concentration (MIC) of standard drug

Stock solution of standard drug was diluted appropriately in DMSO to get the solution having the following concentration ranges.

Table 4: MIC range (µg/ml) of the standard drug for Anti-fungal activity.

Standard drug solution	Conc. Range (µg/ml)
Series A	0.64-0.30
Series B	0.34-0.14

Similar procedure was adopted for preparation and inoculation of the tube as done for the synthesized compounds. In this way, the culture tube having the concentration ranges 0.32-0.15 µg/ml (against *A. niger*) and 0.17-0.07 µg/ml (against *C. albicans*) were obtained from the above series of standard drug solutions series A and B. The results of the MIC for the standard drug, clotrimazole, against the fungal strains used were found to be within the range as reported in the literature [21-29].

RESULTS AND DISCUSSION

Anti-fungal activities were carried out by serial dilution method of all the synthesized compounds against fungal strain *Aspergillus niger* and *Candida albicans*. The standard drug for Anti-fungal was clotrimazole. The results of Anti-fungal activity of N-(p-substituted benzylidene)-3-(5-alkyl-1, 3, 4-thiadiazol-2-yl)-thiazolidin-4-one are given in Tables 5 and 6.

The results showed that the compounds 3, 11, 19, 27 and 35 were more active against *A. niger* because of the presence of hydroxyl group at para position of phenyl ring. The compounds 7, 15, 23, 31 and 39 exhibited activity against both fungal strains probably due to the presence of fluoro group at para position of phenyl ring. The compounds 8, 16, 24, 32 and 40 which contain nitro group at para position were more susceptible to *C. albicans*. The compounds 6, 14, 22, 30 and 38 displayed moderate activity against *A. niger* may be due to the presence of bromo group. The compounds 2, 10, 18, 26, 34 and 4, 12, 20, 28, 36 indicated weak activity owing to the presence of p-methyl and methoxy groups respectively which are electron releasing groups.

Table 5: Anti-fungal activity of 2-(p-substituted benzylidene)-3-(5-alkyl-1,3,4-thiadiazol-2-yl) thiazolidin-4-one(1-20).

S. No.	R	R1	Minimum inhibitory concentration (MIC µg/ml)	
			A. niger **(MTCC No.281)**	***C. albicans*** **(MTCC No.183)**
1	CH_3	H	0.6	0.6
2	CH_3	CH_3	0.65	0.65
3	CH_3	OH	0.45	0.5
4	CH_3	OCH_3	0.7	0.7
5	CH_3	Cl	0.55	0.55

6	CH_3	Br	0.55	0.6
7	CH_3	F	0.45	0.45
8	CH_3	NO_2	0.5	0.5
9	CH_2CH_3	H	0.6	0.6
10	CH_2CH_3	CH_3	0.65	0.65
11	CH_2CH_3	OH	0.45	0.55
12	CH_2CH_3	OCH_3	0.7	0.7
13	CH_2CH_3	Cl	0.55	0.6
14	CH_2CH_3	Br	0.55	0.65
15	CH_2CH_3	F	0.45	0.5
16	CH_2CH_3	NO_2	0.55	0.55
17	$(CH_2)_2CH_3$	H	0.7	0.7
18	$(CH_2)_2CH_3$	CH_3	0.75	0.75
19	$(CH_2)_2CH_3$	OH	0.5	0.55
20	$(CH_2)_2CH_3$	OCH_3	0.7	0.7

Table 6: Anti-fungal activity of 2-(p-substituted benzylidene)-3-(5-alkyl-1,3,4-thiadiazol-2-yl) thiazolidin-4-one (21-40).

			Minimum inhibitory concentration (MIC µg/ml)	
S. No.	**R**	**R1**	***A. niger* (MTCC No.281)**	***C. albicans* (MTCC No.183)**
21	$CH_2CH_2CH_3$	Cl	0.55	0.6
22	$CH_2CH_2CH_3$	Br	0.55	0.65
23	$CH_2CH_2CH_3$	F	0.45	0.5
24	$CH_2CH_2CH_3$	NO_2	0.55	0.55
25	$CH_2(CH_2)_2CH_3$	H	0.7	0.7
26	$CH_2(CH_2)_2CH_3$	CH_3	0.75	0.75
27	$CH_2(CH_2)_2CH_3$	OH	0.5	0.55
28	$CH_2(CH_2)_2CH_3$	OCH_3	0.7	0.7
29	$CH_2(CH_2)_2CH_3$	Cl	0.55	0.6
30	$CH_2(CH_2)_2CH_3$	Br	0.55	0.65
31	$CH_2(CH_2)_2CH_3$	F	0.45	0.5
32	$CH_2(CH_2)_2CH_3$	NO_2	0.55	0.55
33	$CH_2(CH_2)_3CH_3$	H	0.7	0.7
34	$CH_2(CH_2)_3CH_3$	CH_3	0.75	0.75
35	$CH_2(CH_2)_3CH_3$	OH	0.5	0.55
36	$CH_2(CH_2)_3CH_3$	OCH_3	0.7	0.7
37	$CH_2(CH_2)_3CH_3$	Cl	0.55	0.6
38	$CH_2(CH_2)_3CH_3$	Br	0.55	0.65
39	$CH_2(CH_2)_3CH_3$	F	0.45	0.5
40	$CH_2(CH_2)_3CH_3$	NO_2	0.55	0.55
	Clotrimazole	0.3	0.1	

CONCLUSION

The work describes Anti-fungal studies of 2-(p-substituted benzylidene)-3-(5-alkyle -1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones against fungal strain *Candida albicans*, (MTCC 183) and *Aspergillus niger*, (MTCC 281) and their minimum inhibitory concentration (MIC) were determined. The results of Anti-fungal activity showed that compounds containing electron withdrawing groups were found to be more active than the compounds containing electron releasing groups. The methyl derivatives were more active.

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