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Antifungal activity of some 2-(5-aryl-1, 3, 4-oxadiazol-2-yl thio) acetic acid

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ABSTRACT

A series of 2-(5-aryl-1, 3, 4-oxadiazol-2-yl thio) acetic acid were screened for their in vitro antifungal activity against Candida albicans, Aspergillus niger and Aspergillus flavus using clotrimazole as the standard drug. Their minimum inhibitory concentration was also determined. All the title compounds showed antifungal activity against the selected fungal strains.

Keywords: 1, 3, 4-Oxadiazole, antifungal and minimum inhibitory concentration (MIC).

INTRODUCTION

Life threatening infections caused by pathogenic fungi are gradually more common, especially in immuno compromised patients suffering from cancer and AIDS. This is mainly due to the emergence of strains resistant to presently available antibiotics. However, there are only a limited number of antifungal drugs on hand to counter such infections, which leads to a strong need to develop new classes of compounds having antifungal activities [1]. Organic acids have been reported to possess antifungal activities [2]. Similarly, amongst the heterocyles, antifungal activity of azoles is also well documented [3]. In recent years, more attention has been given to the synthesis of 1, 3, 4-oxadiazole derivatives for the development of new antimicrobial agents. 1, 3, 4-oxadiazole derivatives possess diverse biological activities like antibacterial [4, 5], fungitoxic [6-8], insecticidal [9], herbicidal [10], anticancer [11], anti-inflammatory [12] etc. These reports including our earlier work on 1, 3, 4-oxadizoles [13-16] prompted us to study the antifungal activity of title compounds in which acetic acid functionality is attached as a side chain to 1, 3, 4-oxadiazole nucleus. The titled compounds were synthesized according to our earlier reports [13-15]. All the compounds were evaluated for their *in vitro* antifungal activity against *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 1344) and *Aspergillus flavus* (MTCC 277). Clotrimazole was used as the standard drug.

MATERIALS AND METHODS

Antifungal activity of the title compounds was carried out by using two fold serial dilution method [17]. All experiments were performed in triplicate.

Stock solution
Stock solutions of the title compounds and the standard drug *i.e.*, clotrimazole having the concentration 10 µg/mL were prepared in dimethyl sulfoxide (DMSO). Further dilutions were made from these stock solutions.

Media
The following media were used for this study [18].
1. Sabouraud Liquid Medium (SLM)

Ingredients	Quantity
Dextrose	40 g
Peptone	10 g
Water	1000 mL (q.s.)

2. Double Strength Sabouraud Liquid Medium (DSSLM)

Ingredients	Quantity
Dextrose	80 g
Peptone	20 g
Water	1000 mL (q.s.)

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

Sterilization
The sterilization of culture media, culture tubes, 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 h and 7 days respectively for *Candida albicans* and *Aspergillus* species (*A. niger* and *A. flavus*). This was taken as the stock culture. The fungal strain was sub-cultured by transferring aseptically a loopful of corresponding organism from the stock culture into the sterilized SLM and incubated as above. *C. albicans* culture was harvested by using sterilized saline solution and diluted suitably with the sterilized saline solution to get the spore count about 1×10^7 CFU/mL. Similarly *Aspergillus* species cultures were harvested with sterilized saline solution containing 0.05% w/v of polysorbate 80 and adjusted the spore count to about 1×10^7 CFU/mL with sterilized saline solution. An aliquot (0.1 mL) of this saline solution consisting of fungal strain was used for inoculation of the culture tubes.

Determination of the MIC range
A set of seven sterilized culture tubes was taken and 1.0 mL of sterilized DSSLM was transferred aseptically to Tube I and 1.0 mL of sterilized nutrient broth was transferred aseptically to the remaining six tubes. Different concentrations of all the compounds to be tested including standard drug were prepared by serial dilution method. To Tube I, 1.0 mL of stock solution was added aseptically and mixed well. From Tube I, 1.0 mL of the solution was transferred aseptically to the Tube II and so on to get the drug concentrations 5.0 µg/mL, 2.5 µg/mL,……. 0.078125 (0.08) µg/mL in Tube I to Tube VII respectively. A control tube was also prepared by transferring aseptically the 0.5 mL of sterilized DSSLM and 0.5 mL of solvent (DMSO). The culture tubes were inoculated by 0.1 mL of fungal culture in sterilized saline solution having microbial count about 1×10^7 CFU/mL so that the final microbial count in each culture tube would be about 1×10^6 CFU/mL. The tubes were incubated for 48 h at 25 ± 1 °C. After 48 h, the inoculated culture tubes were macroscopically examined for turbidity.

The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the MIC range for the compound. The MIC range for the title compounds and standard drug are given in table 1.

Table 1: MIC range (µg/mL) of 2-(5-aryl-1, 3, 4-oxadiazol-2-yl thio) acetic acid for antifungal activity

Compound	*C. albicans* (MTCC 227)	*A. niger* (MTCC 1344)	*A. flavus* (MTCC 277)
Title compounds(**1-15**)	0.63 < 0.31	0.63 < 0.31	0.63 < 0.31
Standard Drug (Clotrimazole)	0.16< 0.08	0.31 < 0.16	0.31 < 0.16

The accurate MIC of all the title compounds and standard drug for a particular fungal strain was determined by making further dilutions between the observed MIC range.

Determination of accurate MIC of title compounds

Aliquots of stock solution, 1.3 mL, 1.2 mL …… 0.6 mL were diluted to 10.0 mL with DMSO to get the solutions of the concentration of 1.3 µg/mL to 0.6 µg/mL respectively. An aliquot (0.5 mL) of each of the solution was transferred aseptically to different tubes having 0.5 mL of sterilized DSNB to get the solutions of the concentration range 0.65 µg/mL to 0.3 µg/mL. The tubes were inoculated with 0.1 mL of fungal culture (microbial count 1×10^7 CFU/mL) and incubated for 48 hours at25 ± 1 °C. After 48 h, the inoculated tubes were macroscopically examined for turbidity. The results are shown in table 2.

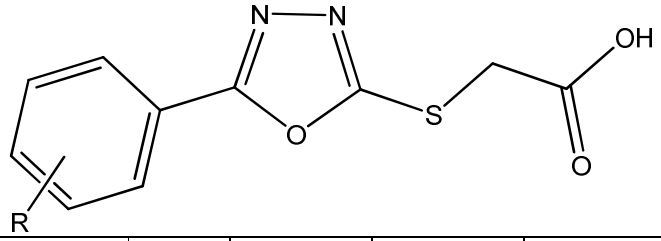
Determination of accurate MIC of standard drug

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

Standard drug solution	Conc. range (µg/mL)
Series A	0.32 – 0.15
Series B	0.17 – 0.07

The culture tubes were prepared and inoculated by adopting the similar procedure as used for the title compounds and culture tubes having the concentration ranges 0.32 – 0.15 µg/mL (against *A. niger* and *A. flavus*) and 0.17 – 0.07 µg/mL (against *C. albicans*) were obtained from the above series of standard drug solutions A and B respectively. The results are shown in table 2.

Table 2: Accurate MIC (µg/mL) of 2-(5-aryl-1, 3, 4-oxadiazol-2-yl thio) acetic acid for antifungal activity



Compound	R	*C. albicans* (MTCC 227)	*A. niger* (MTCC 1344)	*A. flavus* (MTCC 277)
1	H	0.50	0.60	0.60
2	2-CH_3	0.45	0.55	0.60
3	3-CH_3	0.50	0.60	0.60
4	4-CH_3	0.50	0.60	0.60
5	2-Cl	0.40	0.50	0.50
6	3-Cl	0.40	0.45	0.45
7	4-Cl	0.35	0.50	0.50
8	2-Br	0.40	0.50	0.45
9	3-Br	0.40	0.50	0.50
10	4-Br	0.35	0.45	0.50
11	2-OCH_3	0.50	0.60	0.60
12	4-OCH_3	0.50	0.55	0.55
13	3-NO_2	0.35	0.45	0.45
14	4-NO_2	0.35	0.45	0.45
15	2,4-$(Cl)_2$	0.35	0.45	0.45
Standard drug Clotrimazole	–	0.10	0.30	0.30

RESULTS AND DISCUSSION

All the title compounds **(1-15)** were evaluated for their *in vitro* antifungal activity against *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 1344) and *Aspergillus flavus* (MTCC 277) and their minimum inhibitory concentration (MIC) was determined. Perusal of tables 1 and 2 show that all the title compounds were found to be active against all the fungal strains used in this study. Out of the three fungal strains *Candida albicans* found to be

more susceptible against the tested compounds. The minimum inhibitory concentration (MIC) of the title compounds **(1-15)** was found to be 0.50-0.35 µg/mL against *Candida albicans* and 0.60-0.45 µg/mL for *Aspergillus niger* and *Aspergillus flavus* respectively. The title compounds showed no significant difference in their MIC values; however the compounds containing substituted phenyl groups exhibited more activity than the un-substituted phenyl group. The compounds having the phenyl group substituted with electron withdrawing groups like chloro, bromo or nitro displayed more activity than the compounds containing phenyl group substituted with electron releasing groups like methyl or methoxyl. The standard drug clotrimazole inhibited *Candida albicans* at a MIC of 0.10 µg/mL and *Aspergillus species* at MIC of 0.30 µg/mL respectively. The results of the MIC for the standard drug against the selected fungal strains were found to be within the range as reported in the literature [19, 20].

CONLUSION

Present study describes the in vitro antifungal activity of some 2-(5-aryl-1, 3, 4-oxadiazol-2-yl thio) acetic acid against *Candida albicans*,(MTCC 227) *Aspergillus niger* (MTCC 1344) and *Aspergillus flavus* (MTCC 277). Minimum inhibitory concentration of the title compounds was also determined. The results of antifungal activity showed that compounds containing electron withdrawing groups *e.g.*, chloro, bromo, or nitro were found to be more active than the compounds containing electron releasing groups such as methyl and methoxy. These results suggest that some more compounds having different aromatic, hetero-aromatic or aliphatic functionality on the **5** position and different carboxylic group on **2** position of the 1, 3, 4-oxadizole nucleus respectively should be synthesized and screened for their antifungal activity to explore the possibility of 2-(5-aryl-1, 3, 4-oxadiazol-2-yl thio) acetic acid as a novel series of antifungal drugs.

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