In vitro antifungal activity of 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate, a dihydropyrrrole derivative

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A novel compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate was isolated from the plant Datura metel L. The in vitro activity of this dihydropyrrrole derivative against Aspergillus and Candida species was evaluated by using standard methods approved by the National Committee for Clinical Laboratory Standards. The compound was found to be active against all the species tested, namely Candida albicans, Candida tropicalis, Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger. The MIC at which more than 90 % of growth was inhibited (MIC₉₀) by the compound ranged from 21.87 to 43.75 μg ml⁻¹ against various fungal species by microbroth dilution assay. Since the compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate has antifungal activity it can be explored further to develop new antimycotic drugs.

INTRODUCTION

Pathogens belonging to fungal genera Aspergillus and Candida are ubiquitous and global in distribution (Randhawa & Khan, 1987). These infections are increasingly recognized as an emerging threat to public health. In immunocompromised hosts, infections by Aspergillus, Candida, Histoplasma, etc., become invasive and disseminate from the primary site of infection to other parts of the body including the gut, kidney, spleen and brain.

Invasive aspergillosis is reported to be associated with a mortality rate of 55 % (Denning & Stevens, 1990). Mortality due to Aspergillus infection in bone marrow transplant recipients was observed to be as high as 80 % despite appropriate chemotherapy (Meyer, 1990). Cerebral aspergillosis presents the symptoms of acute meningitis and is always fatal.

Candida species have been found to be the fourth most prevalent group of pathogens and have been isolated from 8 % of patients with nosocomial bloodstream infections (Pfaller, 1994). They are also identified as critical pathogens in infections of wounds and other body fluids (Powderly et al., 1988). The change in mortality rate associated with disseminated candidiasis has been insignificant even after treatment with effective antifungal drugs (Pfaffer et al., 1999).

The drugs currently available for treatment of various fungal infections are primarily polyenes and theazole class of compounds. Amphotericin B, which is considered to be the drug of choice, has been found to be highly nephrotoxic and less effective in invasive aspergillosis (Powderly et al., 1988; Rex et al., 1995). Further, the development of fungal resistance against most of the available drugs has been observed (Pfaller et al., 1998a, b; Powderly, 1994). The increased occurrence of mycoses in immunocompromised patients and the development of resistance in fungi to current drugs has emphasized the need for developing new antifungal compounds with minimal adverse effects in humans.

Several bioactive molecules, including antifungals, from plants have been reported. 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate, a novel antifungal molecule, was isolated from the plant Datura metel L (Rajesh et al., 2001; Dabur et al., 2004). The results of preliminary experiments showed this molecule to have anti-Aspergillus properties. The present study deals with detailed investigations on the antifungal spectrum and potency of 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate against 10 clinical isolates of Candida and 19 clinical isolates of Aspergillus.

METHODS

Strains. Clinical isolates of Candida and Aspergillus, namely Candida albicans, Candida tropicalis, Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger, were obtained from the Microbiology Department,
Vallabhbhai Patel Chest Institute, Delhi. These were used along with the
standard strains procured from the Indian Type Culture Collection,
IARI, Delhi. Quality control strains of C. albicans (ITCC 4718), C.
tropicalis (ITCC 1634), A. fumigatus (ITCC 4517), A. flavus (ITCC 5192)
and A. niger (ITCC 5405) were included in each test as recommended by
the National Committee for Clinical Laboratory Standards (NCCLS).

**Antifungal agents.** The compound 2-(3,4-dimethyl-2,5-dihydro-1H-
pyrrol-2-yl)-1-methylethyl pentanoate was isolated from D. metel as
described previously (Rajesh et al., 2001; Dabur et al., 2004) and
characterized. The compound was dissolved in dimethyl sulphoxide
(DMSO) and diluted with distilled water. Amphotericin B was used as
the standard drug. Freshly prepared solutions of the test compound and
the standard drug were used in the study.

**Antifungal susceptibility tests.** Evaluations of the susceptibility of
*Candida* were made by the microbroth dilution method as per NCCLS
document M27-A (NCCLS, 1997). The fungi used as inocula were
grown overnight on Sabouraud dextrose agar (E. Merck) at 35 °C. Tests
were performed in RPMI 1640 (Gibco-BRL) buffered to pH 7.0 with
0.165 M morpholinepropanesulphonic acid (MOPS; Sigma). The
MIC<sub>90</sub> was considered to be the lowest concentration of the compound
that inhibited the visible growth of fungi. Effects of different media were
determined by using buffered RPMI 1640 supplemented with 20.0 g
glucose l<sup>-1</sup>, yeast nitrogen broth (pH 7.0; Difco laboratories) supple-
mented with 5.0 g glucose l<sup>-1</sup>, antibiotic medium 3 (Becton Dickson
Microbioogy systems) and Sabouraud dextrose broth (E. Merck). These
media were substituted for buffered RPMI 1640 as recommended by the
NCCLS (1997). Inoculum effects were determined as per NCCLS
(1997), except that strains were suspended to a turbidity equivalent to
that of a 0.5 McFarland standard in 0.9 % (w/v) NaCl and were further
diluted in 0.9 % NaCl to achieve the desired inoculum levels. Inoculum
densities were verified by determining the number of viable colonies per
millilitre on Sabouraud dextrose agar after serial dilutions in 0.9 %
NaCl.

The activity of 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-
methylethyl pentanoate against *Aspergillus* isolates was also determined
by the microbroth dilution method. Cultures were grown on Sabouraud
dextrose agar at 37 °C until sporulation. Stock spore suspensions were
prepared with yeast nitrogen broth (pH 7.0) supplemented with 5.0 g
glucose l<sup>-1</sup> and 25 % (v/v) glycerol, and were stored at 4 °C until use.
The c.f.u. ml<sup>-1</sup> was determined by plating serial dilutions on Sabouraud
dextrose agar plates.

Before inoculation for susceptibility tests, the spore suspensions were
diluted to achieve 2 × 10<sup>8</sup> to 1 × 10<sup>10</sup> c.f.u. ml<sup>-1</sup> in yeast nitrogen broth
with 0.5 % glucose (pH 7.0) and were incubated for 24 h at 37 °C
until germination of the spores. Serial twofold dilutions of the test compound
were made in yeast nitrogen broth plus 0.5 % glucose (pH 7.0) in 100 μl
volumes and were inoculated with 100 μl of the germinated spore
suspensions. The cultures were incubated for 72 h at 37 °C. The MIC<sub>90</sub>
was determined as the lowest concentration that inhibited visible fungal
growth.

**Time-kill analysis.** *C. albicans* ITCC 4718 was grown on Sabouraud
dextrose agar at 35 °C for 24 h. Isolated colonies were selected and
suspended in 0.9 % NaCl to a turbidity equivalent to that of 0.5
McFarland standard. Flasks were prepared that contained RPMI 1640
buffered with 0-165 M MOPS to pH 7-0 and four times the MIC<sub>90</sub>
of the test compound or no compound (growth control). The flasks were
inoculated with yeast suspension to a final concentration of approxi-
ately 10<sup>8</sup> c.f.u. ml<sup>-1</sup>. The cultures were incubated at 35 °C with
shaking for up to 24 h. At the defined time intervals, aliquots were
removed and the number of viable colonies per millilitre was deter-
mined on Sabouraud dextrose agar after serial dilution in 0.9 % NaCl.

### RESULTS AND DISCUSSION

**In vitro activity of the dihydropyrrrole derivative**

Nineteen strains of *Aspergillus* and 10 strains of *Candida* were employed
to evaluate the antifungal potential of the novel compound, 2-(3,4-
dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate. Its MIC<sub>90</sub>
was found to be in the concentration range of 21-87 to 43-75 μg ml<sup>-1</sup>
against all 29 strains of fungi. The growth of all the pathogenic fungal
strains, i.e. *C. albicans*, *C. tropicalis*, *A. fumigatus*, *A. flavus*
and *A. niger*, was inhibited by the compound. Antibiotic medium 3
was used to identify resistance, if any, in yeasts against the compound and amphotericin B as described by
Rex et al. (1995). The MIC<sub>90</sub> against the yeasts was found to
be 10-93 μg ml<sup>-1</sup> in antibiotic medium 3 (Table 1). Variations in susceptibility in the group of fungal strains tested
with 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate were observed; however, resistance against the
compound was not seen. It was observed that 2-(3,4-
dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pen-
tanoate had broad-spectrum and potent antifungal activity
against pathogenic fungi.

The fungicidal activity of 2-(3,4-dimethyl-2,5-dihydro-1H-
pyrrol-2-yl)-1-methylethyl pentanoate against clinical iso-
lates of *C. albicans* was analysed by time-kill analysis (Fig. 1). When the compound was tested at four times its MIC<sub>90</sub>, it

### Table 1. Effect of different test media on *in vitro* activity of 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-
methylethyl pentanoate

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of isolates</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (μg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>RPMI 1640</th>
<th>RPMI 1640 + 2 % glucose</th>
<th>YNB + 0.5 % glucose</th>
<th>Antibiotic medium 3</th>
<th>Sabouraud dextrose broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>7</td>
<td>21-87</td>
<td>21-87</td>
<td>43-75</td>
<td>10-93</td>
<td>43-75</td>
<td>≤10-93</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>3</td>
<td>21-87</td>
<td>ND</td>
<td>43-75</td>
<td>10-93</td>
<td>43-75</td>
<td>≤21-87</td>
</tr>
</tbody>
</table>
The MIC$_{90}$ of the compound against all the 19 strains of $C.$ albicans was approximately twofold higher in yeast nitrogen broth than in RPMI 1640. In contrast, the MIC$_{90}$ of 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylpentanoic acid was about twofold lower in antibiotic medium 3 and Sabouraud dextrose broth than in RPMI 1640. Rex et al. (1995) reported similar observations. Amphotericin B was found to be most active in antibiotic medium 3 and Sabouraud dextrose broth.

**Conclusion**

The novel compound 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylpentanoic acid isolated from $D.$ metel was found to have potent activity against all of the 29 pathogenic strains of fungi, i.e. isolates of $C.$ albicans, $C.$ tropicalis, $A.$ fumigatus, $A.$ niger and $A.$ flavus, tested in the present study, and the size of the inocula did not significantly affect the in vitro activity of the compound. The activity of the compound in antibiotic medium 3 against $C.$ albicans and $C.$ tropicalis was highest, the MIC$_{90}$ being 10.93 $\mu$g ml$^{-1}$. It was observed that 2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylpentanoic acid is fungicidal and resistance against this compound was not found in any of the strains tested in the present study.

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**REFERENCES**


