

INVITRO ANTIFUNGAL STUDIES OF TERBINAFINE

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(Received November 27, 1988; revised December 14, 1989)

Terbinafine a new orally active allylamine derivative was studied for its antifungal activity against dermatophytes and other filamentous fungi. Its activity was tested invitro against *Trichophyton rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. gallinae*, *T. longifuses*, *T. violaceum*, *T. semii*, *Microsporium canis*, *Epidermophyton floccosum*, *Aspergillus flavus*, *A. niger*, *Alternaria alternata*, *Drechslera rostrata*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Curvularia lunata* and *Penicillium* species at a comparable concentrations of clotrimazole and griseofulvin.

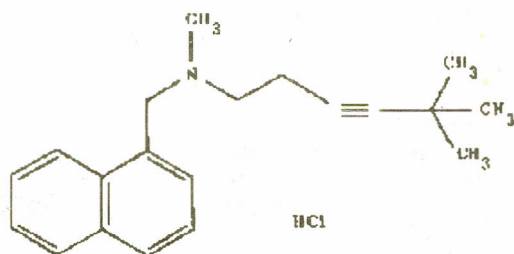
The minimum inhibitory concentration (MIC), with the exception of *T. gallinae*, ranged between 0.05–0.25 µg/ml against dermatophytes. This drug was found quite effective against *Aspergillus*, *penicillium* and dermatophytic fungi tested and superior to clotrimazole and griseofulvin. However, clotrimazole was found superior to terbinafine against *A. alternata*, *C. lunata*, *D. rostrata*, *F. moniliforme* and *F. oxysporum*.

Key words: Terbinafine, Antifungal, Minimum inhibitory concentration

Introduction

The allylamines are a newly developed class of antifungal agents with activity against a wide range of fungi pathogenic to man. These include a topical antimycotic drug, naftifine with a high efficacy against different forms of dermatophytes [4,5] and the more patent, systemically active terbinafine.

Terbinafine (E)-N-(6,6-Dimethyl-2-hepten-4-ynyl)-N-methyl-1-Naphthalenemethanamine hydrochloride is a recently described orally active allylamine derivative, structur-



Terbinafine [(E)-N-(6,6-Dimethyl-2-hepten-4-ynyl)-N-methyl-1-Naphthalenemethanamine Hydrochloride].

ally related to naftifine. An essential part of the molecule is a conjugated enyne group with trans-stereochemistry in the side chain.

The studies reported here were carried out to determine *in vitro* antifungal activity of terbinafine against dermatophytes and some other filamentous fungi. Terbinafine was found to be remarkably effective *in vitro* against a wide range of fungi (Table 1).

The research work was sponsored by Sandoz Pakistan Limited and was performed in collaboration with Dermatology Department, Jinnah Post Graduate Medical Centre, Karachi and Department of Microbiology, University of Karachi.

Materials and Methods

The antifungal spectrum of terbinafine was determined by agar incorporation and broth dilution method. The antifungal activity was compared with clotrimazole and griseofulvin.

Drugs. Terbinafine, clotrimazole and griseofulvin were dissolved in DMSO, methanol and mixture of methanol and 1N HCl respectively and stock solution of 10 mg/ml were prepared.

Preparation of Inocula. The cultures were grown on SDA (Oxoid) plates and harvested with sterile SD broth. Growth mascerated using overhead-derive homogenizer. Inocula of fungi (with exception to *Aspergillus* and *Penicillium* species) were adjusted by turbidimetry (final transmission read on adsorption was 65%). For *Aspergillus* and *Penicillium* the spore concentration was determined by hemocytometer and adjusted to 10⁶ spores/ml.

(A) Agar Dilution Method. Serial dilutions of terbinafine (0.01–5 µg/ml) were prepared in tubes containing 10ml Sabouraud dextrose agar with pH 6.5 (Oxoid). Each concentration of drug was used in triplicate. Three drug free slants of SDA (10ml) were added to each set of drug concentrations as control.

Slants of each concentrations in triplicate and three plain SDA slants were inoculated with 0.1 ml inoculum and incubated at 32° for 1–4 weeks according to the growth characteristics of the cultures. Each slant was examined and resulting MICs (minimal inhibiting concentration) were determined as the lowest concentration of drug which permitted no visible growth.

(B) Broth Dilution Method. The *in vitro* inhibitory effect and MIC was also determined by serial dilutions using Sabouraud dextrose broth (Oxoid) containing 2% dextrose with pH 6.5.

TABLE 1. ANTIFUNGAL ACTIVITY OF TERBINAFINE SF 86-327

Cultures	Media	Concentration of Terbinafine (SF 86-327) in µg/ml											
		0	0.01	0.05	0.1	0.25	0.5	0.75	1	2	3	4	5
<i>Trichopyhton rubrum</i> *	A	+++	+++	+	-	-	-	-	-	-	-	-	-
	B	++++	++++	+	-	-	-	-	-	-	-	-	-
<i>Trichophyton mentagrophytes</i> *	A	+++	++	-	-	-	-	-	-	-	-	-	-
	B	++++	++	-	-	-	-	-	-	-	-	-	-
<i>Trichophyton violaceum</i> ***	A	+++	++	+	-	-	-	-	-	-	-	-	-
	B	++++	++++	++	-	-	-	-	-	-	-	-	-
<i>Trichophyton tonsurans</i> *	A	+++	+++	++	++	-	-	-	-	-	-	-	-
	B	++++	++++	+++	++	-	-	-	-	-	-	-	-
<i>Trichophyton longifuses</i> ***	A	+++	+++	++	++	-	-	-	-	-	-	-	-
	B	++++	++++	+++	++	-	-	-	-	-	-	-	-
<i>Trichophyton gallinae</i> **	A	++++	+++	+++	+++	+++	++++	+++	++	+	-	-	-
	B	++++	++++	++++	++++	++++	++++	+++	+++	++	-	-	-
<i>Trichophyton semii</i> *	A	+++	+++	+++	++	-	-	-	-	-	-	-	-
	B	++++	+++	+++	++	-	-	-	-	-	-	-	-
<i>Microsporum canis</i> *	A	++++	++	-	-	-	-	-	-	-	-	-	-
	B	++++	+++	-	-	-	-	-	-	-	-	-	-
<i>Epidermophyton floccosum</i> **	A	+++	++	-	-	-	-	-	-	-	-	-	-
	B	++++	++	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus flavus</i> *	A	+++	+++	+++	++	+	+	+	-	-	-	-	-
	B	++++	++++	+++	++	++	+	+	-	-	-	-	-
<i>Aspergillus niger</i> *	A	++++	++++	+++	+++	+++	+++	++	+	-	-	-	-
	B	++++	++++	++++	+++	+++	+++	++	++	-	-	-	-
<i>Alternaria alternata</i> *	A	++++	++++	++++	++++	++++	++++	++++	+++	+++	++	+	-
	B	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	++	-
<i>Curvularia lunata</i> *	A	++++	++++	++++	++++	++++	++++	++++	++	++	+	+	-
	B	++++	++++	++++	++++	++++	++++	++++	+++	++	++	+	-
<i>Drechslera rostrata</i> *	A	++++	++++	++++	++++	++++	++++	++++	+++	+++	++	++	+
	B	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	++	+
<i>Fusarium moniliforme</i> *	A	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	++
	B	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++
<i>Fusarium oxysporum</i> *	A	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	++
	B	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++
<i>Penicillium spp.</i> *	A	++++	++++	++++	++++	++++	+++	+++	+++	+++	+	+	-
	B	++++	++++	++++	++++	++++	++++	++++	++++	++++	++	+	-

Medium:

A = Sabouraud dextrose agar (oxid)
 B = Sabouraud dextrose broth (Oxid)

Temperature:

A = 32°C incubator
 B = 32°C shaking water bath.

Incubation period:

* = 07 days (one week)
 ** = 14 days (two weeks)
 *** = 21 days (three weeks)
 **** = 28 days (four weeks)
 - = No growth
 + = Little growth
 ++ = Slight growth
 +++ = Moderate growth
 ++++ = Maximum growth

Serial dilutions of terbinafine were prepared in concentrations ranging from 0.01–20 µg/ml in 50 ml volume of medium in 100 ml flasks. These, along with drug free control (50 ml Sabouraud dextrose broth) were inoculated in triplicate with 0.5 ml adjusted inocula. The inoculated flasks were incubated in shaking water bath at 32° for 1–4 weeks according to the growth characteristics of the cultures. Each flask was examined to determine MIC. In broth dilution method, the MIC is the lowest concentration of drug at which all the three flasks of a particular dilution were visually negative for growth.

Antifungal activity of terbinafine was compared with identical concentrations of clotrimazole and griseofulvin by serial broth dilution method described above.

Results and Discussion

Antifungal activity of terbinafine was determined against nine dermatophytes and eight other filamentous fungi which also included two etiologic agents of eumycotic mycetoma (*C. lunata* and *F. moniliforme*). Results show that terbinafine possesses a remarkable antifungal activity against almost all the fungi tested (Table 1).

The MIC values with the exception of *T. gallinae* were found in the range of 0.05–0.25 µg/ml for the dermatophytic fungi tested. The MIC values for other filamentous fungi were also determined and found to be 1 µg/ml for *A. flavus*, 2 µg/ml for *A. niger*, 5 µg/ml for *C. lunata*, *A. alternata* and *Penicillium* species and 10 µg/ml for other plant pathogen/opportunistic fungi (Table 2).

The antifungal activity of terbinafine was also compared with identical concentrations of clotrimazole and griseofulvin. With the exception of *T. gallinae*, the effect of terbinafine on dermatophyte, *Aspergillus* and *Penicillium* species was found superior to the antibiotics used. However clotrimazole was found more effective than terbinafine on *A. alternata*, *C. lunata*, *D. rostrata*, *F. moniliforme* and *F. oxysporum* (Table 2).

TABLE 2. COMPARATIVE STUDIES OF ANTIFUNGAL ACTIVITIES OF TERBINAFINE WITH CLOTRIMAZOLE AND GRISEOFULVIN

Cultures	MIC in mg/ml Source	Terbinafine	Clotrimazole	Griseofulvin
<i>T. rubrum</i>	a	0.1	4	10
<i>T. tonsurans</i>	a	0.25	0.75	0.75
<i>T. mentagrophytes</i>	a	0.05	0.5	5
<i>T. semii</i>	b	0.25	10	15
<i>T. violaceum</i>	a	0.1	10	10
<i>T. gallinae</i>	a	3	0.75	20
<i>T. longifuses</i>	b	0.25	2	2
<i>M. canis</i>	b	0.05	0.05	0.5
<i>E. floccosum</i>	a	0.05	0.1	0.25
<i>A. flavus</i>	c	1	15	20
<i>Aspergillus niger</i>	c	2	20	20
<i>Alternaria alternata</i>	d	5	3	20
<i>Curvularia lunata</i>	d	2	1	20
<i>Drechslera rostrata</i>	d	10	5	20
<i>Fusarium moniliforme</i>	d	10	3	20
<i>Fusarium oxysporum</i>	d	20	20	20
<i>Penicillium spp.</i>	c	5	20	20

The increased incidence of fungal infections and non-availability of effective drugs to control them stimulated a vigorous search for anti-fungal antibiotics [1,2,6,7,10]. In recent years a number of synthetic and semi-synthetic anti-fungal compounds have been isolated [2,4]. Terbinafine provides one such example.

Antifungal activity of terbinafine has been tested against nine dermatophytes, two *Aspergillus*, two *Fusarium* and one each of *Alternaria*, *Curvularia*, *Drechslera* and *Penicillium* species. The MICs for dermatophytes with the exception of *T. gallinae* were in the range of 0.05–0.25 µg/ml. MIC value for *A. flavus*, *A. niger*, *T. gallinae* and *Penicillium* were 1 µg/ml, 2 µg/ml, 3 µg/ml and 5 µg/ml respectively. MICs for other fungi are shown in Table. 2

The antifungal activity of terbinafine was also compared with identical concentrations of clotrimazole and griseofulvin. With the exception of *T. gallinae*, the effect of terbinafine on dermatophyte, *Aspergillus* and *Penicillium* species was found superior to the antibiotics used. However clotrimazole was found more effective than terbinafine on *A. alternata*, *C. lunata*, *D. rostrata*, *F. moniliforme* and *F. oxysporum* (Table 2).

Terbinafine in concentration 5–10 µg/ml inhibited the growth of *C. lunata*, *F. moniliforme* and *F. oxysporum* which cause eumycotic mycetoma and also involved in eye infections. The only remedy in eumycotic mycetoma is the surgical excision [3,8,9]

Griseofulvin has been reported to be carcinogenic [11] hence the need for a safer oral antifungal drug. Terbinafine, so far, is free of this side effect and due to its better efficacy should prove useful in our armamentarium against various mycotic infections.

Acknowledgement. We are highly grateful to the Sandoz Pakistan Limited for sponsoring the project. We are also thankful to Mr. Sardar Abdul Jameel Khan, Senior Scientific Officer, Cereal Crop Research Institute PARC for supplying five cultures of filamentous fungi.

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