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# "In vitro" Antimycotic Activity of Fenticonazole (Rec 15/1476)

## Die antimykotische Wirkung von Fenticonazole (Rec 15/1476)

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**Key words:** Antimycotic activity - fenticonazole - dermatophytes - yeasts - moulds
**Schlüsselwörter:** Antimykotische Wirkung - Fenticonazole - Dermatophyten - Hefen - Schimmel

**Summary: The "in vitro" activity of Fenticonazole (Rec 15/1476) against a large variety of wild and collection fungi, in comparison with some trade drugs, was studied. The fungistatic and fungicidal activity of Fenticonazole was also studied against a certain number of yeasts variing the pH of the medium.**

**The results of the "in vitro" tests have demonstrated good activity of Fenticonazole against dermatophytes and against fungi responsible for deep infections.**

**The activity of Fenticonazole against yeasts is very good at acid pH while it decreases at alkaline pH.**

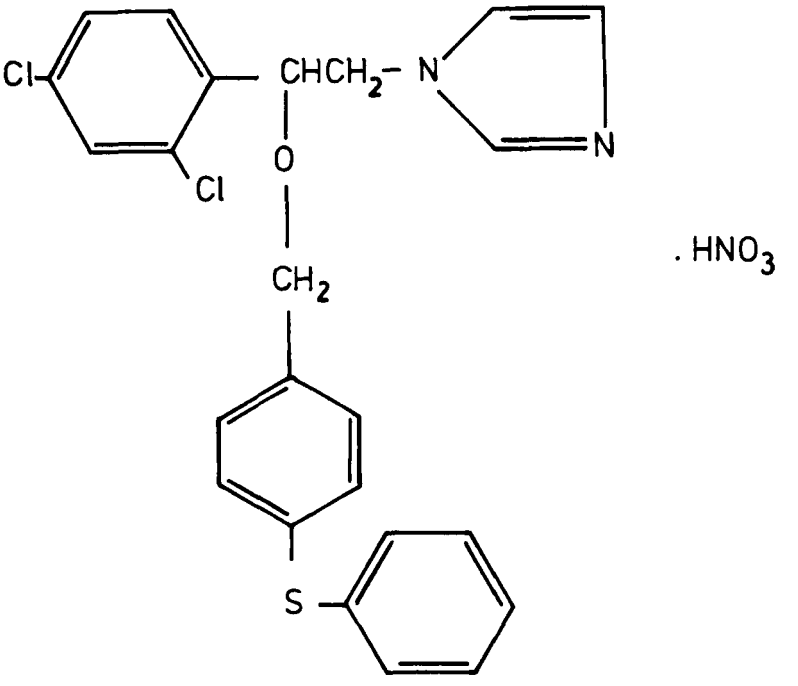
**Zusammenfassung: Die „in vitro" Wirksamkeit von Fenticonazole (Rec 15/1476) gegen verschieden pathogene Pilze wurde untersucht. Das Spektrum der fungiziden Wirkung ist sehr breit: Dermatophyten, Hefen und dimorphe Pilze werden stark gehemmt.**

**Fenticonazole zeigt "in vitro" die höchste Wirkung gegen Hefen bei pH 4–5.**

Among numerous new imidazole derivatives synthesized in Recordati Research Laboratories, the most active is Fenticonazole (Rec 15/1476), 1-2-(2,4-dichlorophenyl)-2-(4-phenylthiobenzyloxy)ethyl imidazole nitrate, a drug with satisfactory antibacterial action and a notable antimycotic action[1, 2, 3].

It is a white crystalline powder, odorless freely soluble in MeOH, $CHCl_3$ and DMF, pratically insoluble in $H_2O$ and $Et_2O$. UV spectrum exhibits a maximum at 252 nm ($a_M$ 14.000). Its molecular formula is $C_{24}H_{20}Cl_2N_2OS.HNO_3$ with molecular weight 518.417.

Its structural formula is




In order to verify the antifungal properties of Fenticonazole a series of "in vitro" experimental tests was carried out, in comparison with Miconazole, Econazole and Clotrimazole.

Then the fungistatic and fungicidal activity of Fenticonazole and of other trade compounds was studied against a certain number of yeasts changing the pH of the medium.

## Material and Methods

*Microorganisms*

24 NCPF strains, kindly supplied by Public Health Laboratory Service, London School of Hygene and Tropical Medicine, Keppel St. London Wc IE 7 TH, and 94 clinical isolates were studied. The clinical strains have been isolated from samples taken from patients hospitalized at the University Hospital of Messina (Italy) and identified by following methods:

a) Macroscopical morhology of the colonies grown in selective media.
b) Staining methods (cotton-blue, Gram and methyl-violet).
c) Cytochemical tests (Schiff, Gins, Sheenan and Story, Hotckiss, Piekarscki - Robinow).
d) Biochemical tests (fermentation and other biochemical properties).
e) Serological tests.
f) Filamentation test by Taschdjian[4].
g) Pathogenicity methods on rabbits (Candida) and on mice (Cryptococcus).
h) Chlamydospores formation according to Simonetti and al.[5].
i) Adsorption test according to Miura[6].
j) Enzymatic activities of some yeasts according to methods used in our previous studies[7-21].

*Culture media*

a) Sabouraud liquid medium (dextrose 4%, pH 5.80).

**Table 1**
MIC and MFC of Fenticonazole and of reference standards on fungi and yeasts.

| Strains | Values expressed in mcg/ml | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fenticonazole | | Miconazole | | Econazole | | Clotrimazole | |
| | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| N. 2 Trichophyton mentagrophytes NCPF London 296-297 | 0,31 | 1,25 | 0,61 | 20 | 0,80 | 10 | 0,61 | 5 |
| N. 20 Trichophyton mentagrophytes (Tinea capitis) | 0,28 | 0,96 | 0,70 | 5,30 | 0,60 | 4,26 | 0,45 | 4,10 |
| N. 3 Trichophyton rubrum NCPF London n. 233-234-235 | 0,33 | 1,10 | 0,55 | 4 | 0,58 | 3 | 8,40 | 37 |
| N. 12 Trichophyton tonsurans (Tinea capitis) | 0,25 | 1,80 | 8 | 32 | 4 | 50 | 4 | 36 |
| N. 3 Microsporum audouini NCPF London, 188-189-190 | 9,20 | 40 | 9 | 67 | 10 | 80 | 2,15 | 22 |
| N. 10 Microsporum audouini (Tinea capitis) | 8 | 27 | 10 | 62 | 10 | 60 | 1,90 | 20 |
| N. 1 Microsporum gypseum NCPF London, 261 | 8,80 | 89 | 12 | 78 | 10 | 60 | 5,60 | 36 |
| N. 15 Epidermophyton floccosum (Tinea corporis) | 1,10 | 8,40 | 0,40 | 20 | 1,30 | 6,7 | 4 | 26 |
| N. 2 Aspergillus niger NCPF London 2022-2023 | 37,50 | 80 | 22 | 94 | 18 | 73 | 10 | 45 |
| N. 3 Aspergillus fumigatus NCPF London, 2078-2079-2080 | 22,30 | 88 | 33,10 | 110 | 28 | 94 | 18,90 | 56 |
| N. 3 Aspergillus flavus NCPF London 2008-2009-2010 | 10 | 40 | 12 | 63 | 21 | 108 | 15,61 | 92 |
| N. 2 Candida albicans NCPF London 3152-3154 | 42 | 90 | 3,90 | 18 | 2,30 | 17,10 | 11,30 | 38 |
| N. 30 Candida albicans from vagina | 28,30 | 82 | 2,80 | 20 | 3,20 | 18 | 8,10 | 30 |
| N. 2 Cryptococcus neoformans Messina, I-II from liquor | 0,39 | 12,50 | 1,95 | 45 | 2,25 | 50 | 6,25 | 65 |
| N. 2 Cryptococcus neoformans NCPF London, Mac Way | 0,55 | 32,50 | 1,25 | 10 | 2,62 | 42,50 | 6,25 | 85 |
| N. 12 Geotrichum candidum from stools | 40 | 160 | 40 | 80 | 40 | 160 | 20 | 80 |
| N. 3 Torulopsis glabrata NCPF London, 3203-3204-3205 | 40 | 80 | 1,25 | 20 | 0,31 | 2,50 | 5 | 40 |

**Table 2**
MIC and MFC of Fenticonazole and of reference standards on some yeasts at various pH.

| Strains | pH | Values expressed in mcg/ml | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fenticonazole | | Miconazole | | Econazole | | Clotrimazole | |
| | | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| N° 2 Candida albicans NCPC London, 3153–3154 | 3,20 | 3 | 10 | 2 | 10 | 1,80 | 10 | 6,10 | 15 |
| | 3,62 | 3,16 | 12 | 2 | 10 | 1,94 | 10 | 6,90 | 20 |
| | 3,88 | 4,35 | 20 | 2 | 10 | 2,00 | 18 | 7,22 | 30 |
| | 4,13 | 5 | 20 | 2,20 | 15 | 2,20 | 33 | 8,00 | 40 |
| | 4,33 | 5,20 | 22 | 2,66 | 14 | 3,15 | 40 | 9,36 | 53 |
| | 4,66 | 10 | 45 | 3 | 18 | 3,90 | 50 | 10 | 60 |
| | 4,93 | 15 | 60 | 3,94 | 30 | 4 | 50 | 18,80 | 20 |
| | 5,32 | 20 | 68 | 4 | 20 | 4 | 55 | 19,40 | 30 |
| N. 30 Candida albicans from vagina | 3,20 | 4 | 10 | 2,33 | 10 | 2 | 10 | 7 | 18 |
| | 3,62 | 3,95 | 13 | 2,50 | 10 | 2 | 10 | 7 | 20 |
| | 3,88 | 4,82 | 20 | 2,80 | 10 | 2,30 | 19 | 8 | 32 |
| | 4,13 | 5 | 22 | 3,14 | 15 | 3 | 20 | 9 | 43 |
| | 4,33 | 5,60 | 24 | 3,70 | 18 | 4 | 40 | 11 | 56 |
| | 4,66 | 10 | 40 | 4,00 | 20 | 4 | 40 | 12 | 60 |
| | 4,93 | 18 | 63 | 4,15 | 40 | 4,70 | 52 | 13 | 70 |
| | 5,32 | 21 | 70 | 4,90 | 60 | 5,00 | 80 | 20 | 90 |
| N. 2 Cryptococcus neoformans NCPF London Mac Way | 3,20 | 0,12 | 2 | 0,32 | 1 | 0,40 | 5 | 1,80 | 10 |
| | 3,62 | 0,15 | 2 | 0,35 | 1 | 0,50 | 10 | 2,20 | 15 |
| | 3,88 | 0,20 | 4 | 0,40 | 2 | 0,50 | 10 | 3 | 30 |
| | 4,33 | 0,30 | 8 | 0,50 | 3 | 0,80 | 40 | 5 | 67 |
| | 4,66 | 0,33 | 8 | 0,90 | 10 | 1,30 | 40 | 5 | 70 |
| | 4,93 | 0,34 | 9 | 1,15 | 20 | 1,60 | 40 | 5 | 80 |
| | 5,32 | 0,38 | 10 | 1,80 | 38 | 1,60 | 40 | 6 | 80 |
| N. 2 Cryptococcus neoformans Messina from liquor I-II | 3,20 | 0,11 | 2 | 0,30 | 0,90 | 0,38 | 7 | 1,20 | 5 |
| | 3,62 | 0,14 | 2 | 0,30 | 0,90 | 0,43 | 9 | 2 | 8 |
| | 3,88 | 0,18 | 4 | 0,40 | 0,90 | 0,50 | 10 | 3 | 10 |
| | 4,13 | 0,30 | 8 | 0,40 | 0,90 | 0,55 | 10 | 5 | 20 |
| | 4,33 | 0,33 | 8 | 0,55 | 1 | 1,20 | 20 | 5 | 20 |
| | 4,66 | 0,33 | 9 | 0,70 | 1,4 | 1,20 | 40 | 5 | 50 |
| | 4,93 | 0,46 | 10 | 0,70 | 1,70 | 1,70 | 40 | 6 | 70 |
| | 5,32 | 0,50 | 18 | 0,80 | 8,90 | 1,70 | 40 | 6 | 82 |
| N. 12 Geotrichum candidum from stools | 3,20 | 6 | 17 | 8 | 34 | 10 | 40 | 6 | 12 |
| | 3,62 | 8 | 20 | 10 | 40 | 11 | 60 | 7 | 20 |
| | 3,88 | 8 | 20 | 10 | 40 | 12 | 65 | 7 | 20 |
| | 4,13 | 10 | 30 | 12 | 50 | 15 | 70 | 8 | 26 |
| | 4,33 | 20 | 60 | 20 | 60 | 18 | 75 | 10 | 30 |
| | 4,66 | 25 | 80 | 25 | 60 | 22 | 90 | 13 | 47 |
| | 4,93 | 30 | 88 | 30 | 65 | 30 | 100 | 18 | 60 |
| | 5,32 | 37 | 120 | 35 | 70 | 40 | 143 | 20 | 70 |
| N. 3 Torulopsis glabrata NCPF London, 3203-3204-3205 | 3,20 | 10 | 30 | 1 | 15 | 0,16 | 3 | 1,80 | 10 |
| | 3,62 | 12 | 30 | 1 | 15 | 0,19 | 4 | 3 | 15 |
| | 3,88 | 15 | 40 | 1 | 15 | 0,20 | 4 | 2 | 15 |
| | 4,13 | 20 | 50 | 1,40 | 20 | 0,23 | 4 | 2,50 | 18 |
| | 4,33 | 25 | 50 | 1,50 | 22 | 0,30 | 4 | 2,50 | 20 |
| | 4,66 | 30 | 70 | 1,70 | 30 | 0,40 | 4,10 | 3 | 22 |
| | 4,93 | 35 | 80 | 2 | 40 | 0,50 | 5 | 4 | 30 |
| | 5,32 | 35 | 80 | 2 | 40 | 0,52 | 5 | 4 | 30 |

b) Sabouraud liquid medium (dextrose 2%, pH 5.70).
c) Sabouraud dextrose agar (pH 5.80 at 45°C).
d) Dextrose and yeast extract agar for the production of Microsporum macroconidia.
e) Chlamydospore agar recommended for use in differentiating C. albicans from other species of Candida on the basis of chlamydospore formation.
f) Malt and yeast extract agar for the production of the asci.
g) Littman Oxgall broth and agar for primary isolation and luxuriantly grown of some dermatophytes.
h) Sabouraud dextrose agar + crystal violet (dil. 0.50 x $10^{-7}$) for the test of Miura.
i) Media for carbohydrate studies (dextrose, maltose, saccharose, levulose ecc.) in according with Lodder and all[22].
j) API 20 for identification of Candida.

*Drugs*

a) Fenticonazole (Rec 15/1476) kindly supplied by Recordati S.p.A., Milan - Italy, title 99,90%, chemical analysis bullettin n° N-4905.
b) Miconazole (lot. N.A. 29/1) by Janssen Farmaceutici s.r.l., Rome - Italy.
c) Econazole by Cilag-Chemie - Italiana.
d) Clotrimazole, pt 8043C by Bayer - Italia S.p.A., Milan - Italy.
All drugs were dissolved in polyethylenglycole 200 (Carbowax).

The results show that Fenticonazole has a high inhibiting activity on Trichophyton with values of MIC between 0.25 and 0.31 mcg/ml and with values of MFC between 0.96 and 1.80 mcg/ml. Fenticonazole proves to be more effective than the other products used as reference standards.

Also on Microsporum, Fenticonazole is very active and so on Epidermophyton and Aspergillus, with values which generally coincide with those of reference standards.

Fenticonazole shows, at least, a good activity against all the strains of Cryptococcus, Torulopsis, Geotrichum candidum and Candida albicans (Table 1).

Table 2 reports the microorganisms, the pH of the media and the fungistatic and fungicidal concentration expressed in mcg/ml of Fenticonazole, Miconazole, Econazole and Clotrimazole. The results obtained on such yeasts, using Sabouraud liquid medium at various pH (from 3.20 to 5.32 in Michaelis buffer), are very interesting.

Fenticonazole shows an appreciable increase of activity with lowering of pH both for fungistatic and fungicidal activity.

The MIC for C. albicans strains that in Sabouraud liquid medium at pH 5.8 varied around 42 mcg/ml, at acid pH arrived at 3 mcg/ml and the values of fungicidal activity from 90 mcg/ml to 10 mcg/ml.

Also the other yeasts show an activity comparable to that of C. albicans.

The reference standards, at lower pH, show a certain increase of fungistatic and fungicidal activity on yeasts.

**Conclusions**

In the experimental test carried out "in vitro" to study the antifungal activity on 118 pathogen moulds, Fenticonazole proves to be mainly effective on dermatophytes and shows an interesting activity against fungi responsible for deep infections.

It is also active against Cryptococcus neoformans and shows a good activity against C. albicans, Geotrichum candidum and Torulopsis glabrata.

It is also studied the activity of Fenticonazole against 51 yeasts at various pH of the medium. The fungistatic and fungicidal activity of the drug is very good at acid pH while it decreases at alkaline pH.

From the results reported above we can conclude that Fenticonazole has a high inhibitory activity on all fungal or yeast strains tested. The activity against certain strains of fungi res-

ponsible for many cutaneous infections in man, shows that Fenticonazole is suitable for tropical treatment of infections due to dermatophytes and Candida.

Further "in vivo" laboratory studies and clinical trials are essential, remembering the compound has a considerable "in vitro" activity against grampositive microorganisms[3].

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