Antifungal Activity of GBR-14206, a New Imidazole Derivative: 
In Vitro Studies

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Abstract

The antifungal activity of GBR-14206, a new imidazole derivative, was evaluated in 
comparison with those of clotrimazole (CTZ) and miconazole (MCZ) using an agar dilution 
procedure.

GBR-14206 showed a potent activity against a wide range of pathogenic fungi including 
those associated with deep-seated and subcutaneous mycosis; it inhibited some standard 
strains of C. albicans at concentrations of 12.5 µg/ml or less. Similarly, clinical isolates from 
various mycoses were also highly susceptible to GBR-14206 (MIC range; 0.0125-6.25 µg/ml). 
The most striking activity of GBR-14206 was displayed against Cryptococcus neoformans (MIC 
range; 0.0125-0.05 µg/ml), which was far superior to MCZ and CTZ. Against C. albicans, 
Trichophyton spp., and Microsporum spp., the activity of GBR-14206 was more moderate than 
MCZ and CTZ. Against Sporothrix schenckii, GBR-14206 had a lower mean MIC value than 
that of CTZ and was similar to MCZ. On the other hand, against Aspergillus spp., GBR-14206 
was less effective than MCZ and CTZ. Fungal susceptibility of GBR-14206 tended to be 
enhanced with increasing medium pH. The activity was also lowered by addition of calf 
serum.

Key words: antifungal activity, new imidazole derivative, GBR-14206

Introduction

Superficial fungal infections caused by dermatophytes and yeasts are common clinical prob-
lems. Some infections of the vagina and glabrous skin are effectively treated by topical therapy with 
a variety of agents. The newer imidazole antifungal drugs, clotrimazole and miconazole, possess a very broad 
spectrum of antifungal activity4,7,8 and resistance to them is rare7. Their proven efficacy has led to 
their widespread acceptance for topical use and therefore, to the development of a number of imi-
dazole compounds.

GBR-14206 is a new imidazole antifungal agent developed by Morishita Pharmaceutical Co., Ltd.. 
Imidazole antifungal agents generally fall into 2 
classes: polyarylmethylimidazole (e.g., clotrima-
zole) and phenethylimidazole (e.g., miconazole), 
while the structure of GBR-14206 is based on the

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stryrylimidazole skeleton, namely z-1-2-(2,4-dichlorophenyl)-3-methyl-1-pentenyl-1H-imidazole (Fig. 1).

The experiments presented here were designed to characterize the in vitro antifungal activity of GBR-14206 in comparison with those of the now widely used agents, miconazole and clotrimazole using an agar dilution procedure.

**Materials and Methods**

**Fungi**

The fungal strains used were obtained from the Institute for Fermentation, Osaka and clinical isolates from Kyoto Biseibutsu Kenkyujo.

All fungi were maintained on modified Sabouraud dextrose agar slant (0.4% glucose, 0.1% polypepton, 1.5% agar) with paraffin over-laying, and fresh cultures were grown as needed on Sabouraud dextrose agar at 27°C for 48 hr (yeast) or 7 days (other fungi). Monomorphic yeasts were harvested from growing cultures and washed and suspended in sterile saline. Spore suspensions of filamentous fungi were prepared by rubbing the surface of slant with a loop after the addition of sterile saline containing 0.1% (w/v) Tween 80, and filtered through two layers of gauze or left at room temperature to settle down large blocks of cell aggregates or mycelia. All suspensions thus prepared were adjusted to contain 1 × 10^6 total cells/ml. Total cell counting was done by hemocytometer (Burkel-Turk; depth, 0.1 mm).

**Compounds**

GBR-14206 was synthesized in the Research Laboratories of Morishita Pharmaceutical Co., Ltd.. Miconazole nitrate (MCZ) and clotrimazole (CTZ) were purchased from Sigma.

**MIC assay**

The test strains, 5 × 10^5 cells each, were spotted on Sabouraud dextrose agar (SDA, Nissui) supplemented with 1% of yeast extract (Difco) containing serially diluted compound and cultured at 27°C for 48 hr (yeast) or 7 days (other fungi). Then drugs (maximal concentration, 10 mg/ml) were dissolved in dimethylsulfoxide and added to the cultures. Controls were similarly set up with an equivalent amount of the solvent (final concentration: 1.0%). The MIC was defined as the lowest concentration of the drug preventing macroscopically visible growth. The effect of serum on MIC values was determined using 5 strains of Candida species. SDA with 10% calf serum was used.

**Results and Discussion**

Table 1 shows the antifungal activity of GBR-14206 against standard strains of Candida spp. GBR-14206 (MIC range; 0.78—12.5 µg/ml) showed good activities against all Candida spp. tested to the same degree as MCZ (MIC range; 0.20—6.25 µg/ml) but was somewhat inferior to CTZ (MIC range; 0.39—6.25 µg/ml).

As may be seen from the geometric means of MIC values (G-MIC) summarized in Table 2, GBR-14206 exhibited excellent activity against clinical isolates from a wide range of pathogenic fungi including those associated with deep-seated and subcutaneous mycosis. The MIC values were less than 6.25 µg/ml for most of 67 isolates. Among these results, the most striking activity of GBR-14206 was displayed against Cryptococcus neoformans (G-MIC; 0.03 µg/ml), which was almost 15 times more potent than MCZ (G-MIC; 0.52 µg/ml) and CTZ (G-MIC; 0.45 µg/ml). The activity of GBR-14206 (MIC range; <0.1—6.25 µg/ml) against C. albicans varied, compared with MCZ (MIC range; 3.13—6.25 µg/ml) and CTZ (0.78—3.13 µg/ml).

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (µg/ml) GBR-14206</th>
<th>MIC (µg/ml) MCZ</th>
<th>MIC (µg/ml) CTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans IFO-1060</td>
<td>12.5</td>
<td>6.25</td>
<td>3.13</td>
</tr>
<tr>
<td>C. albicans IFO-1594</td>
<td>3.13</td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>C. guilliermondii IFO-0566</td>
<td>6.25</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>C. pseudotropicalis IFO-1056</td>
<td>1.56</td>
<td>3.13</td>
<td>0.39</td>
</tr>
<tr>
<td>C. kruase IFO-1395</td>
<td>3.13</td>
<td>3.13</td>
<td>0.78</td>
</tr>
<tr>
<td>C. parapsilosis IFO-1396</td>
<td>0.78</td>
<td>0.20</td>
<td>0.39</td>
</tr>
<tr>
<td>C. stellatoidea IFO-1398</td>
<td>12.5</td>
<td>6.25</td>
<td>1.56</td>
</tr>
</tbody>
</table>

All organisms were read after 48 hr. Sabouraud dextrose agar (pH4.8) was used.
Table 2. *In vitro* antifungal activities of GBR-14206, MCZ and CTZ against clinical isolates of medically important fungi

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains</th>
<th>Geometric mean of MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GBR-14206</td>
</tr>
<tr>
<td>C. albicans</td>
<td>10</td>
<td>3.33 (0.10–6.25)*</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>7</td>
<td>0.03 (0.0125–0.05)</td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>10</td>
<td>1.92 (0.78–3.13)</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>10</td>
<td>1.67 (0.78–3.13)</td>
</tr>
<tr>
<td>Sporothrix schenckii</td>
<td>10</td>
<td>4.42 (3.13–6.25)</td>
</tr>
<tr>
<td>Microsporum spp.</td>
<td>9</td>
<td>1.82 (0.78–3.13)</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>11</td>
<td>1.29 (0.78–3.13)</td>
</tr>
</tbody>
</table>

Yeast were read after 48 hr and others after 7 days. Sabouraud's dextrose agar (PH5.3) was used.

*MIC range

Table 3. Influence of medium pH on antifungal activities of GBR-14206 and MCZ against C. albicans in buffered medium

<table>
<thead>
<tr>
<th>pH values</th>
<th>IFO-1060</th>
<th>IFO-1594</th>
<th>CAA-14*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GBR-14206</td>
<td>MCZ</td>
<td>GBR-14206</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>3.13</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>1.56</td>
<td>0.78</td>
<td>6.25</td>
</tr>
<tr>
<td>5</td>
<td>0.39</td>
<td>0.30</td>
<td>3.13</td>
</tr>
<tr>
<td>6</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>0.39</td>
</tr>
<tr>
<td>7</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>0.39</td>
</tr>
<tr>
<td>8</td>
<td>--**</td>
<td>--</td>
<td>&lt;0.10</td>
</tr>
</tbody>
</table>

Medium was adjusted to appropriate pH values with 0.02 M Briton-Robinson buffer as has been described (6).

Candida spp. were read after 48 hr.

*Clinical isolate

**No visible growth

µg/ml), and the G-MIC or GBR-14206 (3.33 µg/ml) was similar to MCZ (G-MIC; 4.07 µg/ml) and somewhat inferior to CTZ (G-MIC; 1.95 µg/ml). Against dermatophytes of Trichophyton spp. and Microsporum spp. (including 6 isolates of M. gypseum and 3 isolates of M. canis), GBR-14206 was almost equally effective at the MIC range of 1.67–1.82 µg/ml and was similar to MCZ (MIC range 1.18–2.68 µg/ml) and inferior to CTZ (MIC range; 0.34–1.10 µg/ml). Against Aspergillus spp. (6 isolates of A. flavus, 4 isolates of A. niger and 1 isolate of A. terreus), the activity of GBR-14206 (G-MIC; 1.29 µg/ml) was inferior to those of CTZ (G-MIC; 0.35 µg/ml) and MCZ (G-MIC; 0.39 µg/ml). On the other hand, against Sporothrix schenckii GBR-14206 and MCZ maintained excellent activity (G-MIC; 4.42 and 1.56 µg/ml, respectively), while CTZ (G-MIC; 25 µg/ml) was much less active.

All these results for MCZ and CTZ obtained in the present experiments were virtually consistent with previously published data⁴,⁷,⁸), although there were some variations in MICs probably because of different assay conditions employed. In general, GBR-14206 showed broad spectrum antifungal activity as did MCZ and CTZ and appeared to be slightly less active than CTZ against C. albicans and filamentous fungi. However, it should be noted that, to our knowledge, GBR-14206 exerted the most potent activity against Cr. neoformans among a series of imidazole derivatives reported²,⁴,⁶,⁸). In order to confirm the antifungal activity
of GBR-14206 against *Cr. neoformans* and to define it in more detail, further studies are in progress.

Antifungal activities of MCZ and several other imidazole derivatives have previously been found to be affected by the pH and the presence of serum in the medium\(^1\)^\(^3\)^\(^9\). Therefore, experiments were carried out to determine these effects for the antifungal activity of GBR-14206. As demonstrated with 3 strains of *C. albicans*, the antifungal efficacy of GBR-14206 tended to be lowered with decreasing pH values and this trend was more distinct than in MCZ (Table 3).

The effects of pH values on the antifungal activities of the imidazoles CTZ, MCZ and econazole (ECZ) have been reported previously\(^1\)^\(^3\)^\(^9\). Although the activity of each imidazole derivative depends on the pH value, the activity of GBR-14206 was affected more than MCZ and CTZ. However, *in vivo* pH values of the vagina, skin and many other mammalian tissues usually exceed pH 5, and therefore the activity of GBR-14206 measured in medium buffered over pH 5 should correspond more closely to the activity *in vivo*.

Additionally, with *Candida* spp., the activity of GBR-14206 was also lowered by the addition of calf serum, but to a lesser degree than MCZ (Table 4). This profile may offer some advantages in application for systemic use.

*In vitro* MICs of antifungal activities may not accurately reflect the potential clinical value of the compound. In order to evaluate more adequately the clinical potential of GBR-14206, *in vitro* data must be reinforced by data on therapeutic effects in animal models of mycosis. We recently succeeded in developing a lipid emulsion of GBR-14206 as an intravenous preparation, which stimulated further experimental investigations on the drugs efficacy against deep mycosis. The results of these studies are reported in a separate paper.

### References


