Sordarin derivatives represent a new class of antifungal agents that act as potent inhibitors of fungal protein synthesis and possess a broad spectrum of activity. The in vivo activity of GM193663 and GM237354 was studied in mouse models of disseminated candidiasis and aspergillosis and in a rat model of pneumocystosis. The pharmacokinetic behavior of both sordarin derivatives was studied in mice and rats. In all studies, compounds were administered by the subcutaneous route. After a subcutaneous dose of 50 mg/kg of body weight to mice, the maximum level in serum, area under the concentration-time curve, half-life, and clearance for GM193663 and GM237354 were 51.8 and 23 µg/ml, 79.5 and 46 µg · h/ml, 0.8 and 0.85 h, and 21 and 25 ml/h, respectively. Systemic candidiasis and aspergillosis were established in CD-1 male mice infected with Candida albicans or Aspergillus fumigatus. For systemic candidiasis, compounds were given three times per day for seven consecutive days at 15, 30, 60, or 120 mg/kg/day. GM193663 and GM237354 showed dose-related efficacy against C. albicans, with 50% effective doses, 1 month after infection, of 25.2 and 10.7 mg/kg/dose, respectively. In experimental infections with A. fumigatus, GM237354 was given three times per day at 30, 60, or 120 mg/kg/day for five consecutive days. Animals treated with GM237354 demonstrated irregular responses. The survival of animals treated with GM237354 was 0, 30, and 0% at 30, 60, and 120 mg/kg/day, respectively. The therapeutic efficacy of GM193663 and GM237354 against Pneumocystis carinii pneumonia (PCP) was studied in an experimental P. carinii pneumonia (PCP) rat model. After a subcutaneous dose of 10 mg/kg given to rats, the maximum level in serum, area under the concentration-time curve, half-life, and clearance of GM193663 and GM237354 were 6.6 and 7.2 µg/ml, 8.5 and 11.8 µg · h/ml, 0.7 and 0.8 h, and 230 and 133 ml/h, respectively. To induce spontaneous PCP, rats were chronically immunosuppressed with dexamethasone. Infected animals were treated twice daily for 10 days at 0.2, 2, or 10 mg/kg/day. The therapeutic effect was estimated by the reduction in the number of cysts in the lungs of treated versus untreated animals. GM193663 and GM237354 significantly reduced the mean (± standard deviation) log number of cysts from 7.6 ± 2 in the untreated group to 4.7 ± 0.2 and 4.6 ± 0.1, respectively, when the drugs were administered at a dose of 2 mg/kg/day. The log number of cysts was also reduced in infected animals given lower doses of the compounds (0.2 mg/kg/day). In summary, GM193663 and GM237354 are new sordarin derivatives that may potentially play a major role in the treatment of candidiasis and PCP. Further testing with Aspergillus in other animal models is warranted.
Microorganisms. C. albicans 4711E and A. fumigatus 48238E obtained from the Glaxo Wellcome culture collection (Glaxo Wellcome Laboratories, Greenford, United Kingdom) were used to produce lethal systemic infections in mice. PCP was induced with immunosuppression in spontaneously infected Wistar rats, as described below.

Animals. Male CD-1 mice (age, 6 weeks; weight, approximately 25 g; Charles River France Inc., Lyon, France) were used in the pharmacokinetic studies and in the mouse protection tests. Female Wistar rats (age, 6 weeks; weight, approximately 150 g; Iffa-Credo France Inc., Lyon, France) were used in the PCP studies. These animals develop spontaneous P. carinii infection after corticosteroid treatment (1). Mice and rats were housed in cages of 10 and 5 animals per group, respectively, with food and water available ad libitum. The research complied with European legislation and with company policy on the care and use of animals and with related codes of practice.

Pharmacokinetic studies. GM193663 and GM237354 were administered once subcutaneously at a dose of 50 and 10 mg/kg of body weight to mice and rats, respectively. In the case of mice, blood samples were taken by cardiac puncture at 0, 0.25, 0.5, 0.75, 1.5, 2, 2.5, and 3 h postadministration. Three animals were sacrificed at each sampling time by cervical dislocation. Groups of three rats each were sampled from the end of the tail (19) at 0, 0.25, 0.5, 0.75, 1.5, 2, 2.5, and 3 h postadministration. Blood samples were allowed to clot for at least 2 h, then centrifuged to obtain the serum, and finally frozen at −70°C until analysis. Concentrations of sordarin derivatives in serum were determined by the agar diffusion bioassay method, using C. albicans 2005 as the indicator organism. The medium for the bioassay was prepared by supplementing yeast nitrogen base agar (Difco, Detroit, Mich.) with 10% d-glucose (Sigma-Aldrich S.A., Madrid, Spain) and 6% sodium citrate (Merck, Darmstadt, Germany). Then, C. albicans 2005 was added to yield a final concentration of 5 × 10^5 CFU/ml. Supplemented yeast nitrogen base agar (100 ml) with microorganisms was poured into square plastic Nunc (Nalge Nunc International) bioassay plates (245 by 245 mm). The agar was allowed to settle to room temperature for 1 h, and 5-mm-diameter wells were cut using a 36-well template. Wells were loaded with 20 μl of fluids. Standard curves were generated from pooled mouse or rat serum using concentrations of 0.625, 1.25, 2.5, 5, and 10 μg/ml. Each standard sample was assayed in triplicate, while unknown samples were loaded in duplicate. Plates were incubated overnight at 35°C, and the inhibition zone was measured with a digital caliper (Mitutoyo Ltd., London, United Kingdom). The lower limit of detection was <0.625 μg/ml. Finally, samples were quantitatively analyzed and pharmacokinetic parameters were derived by means of a previously described method (2), with slight modifications. Drug efficacy against P. carinii was determined by comparing the P. carinii cyst burden of lungs in the treatment groups with those in the controls. All results were expressed as the log_10 number of cysts per gram of lung.

RESULTS

The molecular structures of the new sordarin derivatives are displayed in Fig. 1. These compounds are structurally related and have different types of fused rings at position C-3 and C-4 of the sugar moiety of the sordarin molecule. GM193663 contains a 3', 4'-fused dioloxane ring, while GM237354 contains a 3', 4'-fused tetrahydrofurane ring with an exomethylene group.

Pharmacokinetic studies. Concentrations of GM193663 and GM237354 in the serum of mice and corticosteroid-treated rats administered a single subcutaneous dose of 50 and 10 mg/kg, respectively, are shown in Fig. 2. The maximum concentration in serum (C_max) area under the concentration-time curve for serum (AUC), elimination half-life (t_1/2), and clearance (CL) for sordarin derivative compounds are shown in Table 1. In mice, the peak concentration of GM193663 (51.8 μg/ml) was twofold higher than that of GM237354 (23.0 μg/ml). The AUC was also twofold greater for GM193663 (79.5 μg·h/ml) than for GM237354 (46.0 μg·h/ml). However, similar t_1/2 and CL values were obtained for both sordarin derivatives. After subcutaneous administration to immunosuppressed rats, the C_max (6.6 and 7.2 μg/ml), AUC (8.5 and 11.8 μg·h/ml), and t_1/2 (0.7 and 0.8 h) values were similar for both compounds. However, the CL of GM193663 (230 ml/h) was significantly higher than that of GM237354 (133 ml/h).

In vivo antifungal activities. The therapeutic efficacy of GM193663 and GM237354 was studied in mouse models of disseminated candidiasis and aspergillosis and in a rat model of pneumocystosis.

FIG. 1. Chemical structures of sordarin derivatives.
(i) Systemic candidiasis in mice. MICs of GM193663 and GM237354 for *C. albicans* 4711E were 0.015 and 0.001 mg/ml, respectively (E. Herreros, personal communication). *C. albicans* 4711E infection was lethal, with untreated control mice dying by days 5 to 10. A summary of MICs, mean and median survival times, and *P* values, from comparisons to the control group, for GM193663 and GM237354 against *C. albicans* is provided in Table 2. There was a significant improvement in survival in mice treated with sordarin derivatives at any administered dose compared to survival of untreated controls. The ED$_{50}$s of sordarin derivatives were calculated from the survival rates at the end of the experiment (30 days after infection). Infections caused by *C. albicans* were more effectively treated with GM237354 than with GM193663. The ED$_{50}$ of GM237354 was 10.7 mg/kg/dose and was at least two-fold more effective than GM193663 (ED$_{50}$ 25.2 mg/kg/dose). When GM237354 was administered at 60 and 120 mg/kg/day, 90 and 100% of the treated mice survived for 30 days postinfection, respectively (Fig. 3 and 4).

(ii) Systemic aspergillosis in mice. GM237354 demonstrated limited in vitro activity against *A. fumigatus*, and the MIC for *A. fumigatus* 48238E was 64 mg/ml (18). With the exception of one mouse, all mice inoculated with *A. fumigatus* and untreated died by day 6 after infection (Fig. 5). The survival rates of animals treated with GM237354 at 30, 60, and 120 mg/kg/day were 0, 30, and 0%, respectively. Mice treated with GM237354 at 60 mg/kg/day showed a significant improvement in survival in comparison to untreated control mice (*P* = 0.04); however, this was not true for mice treated with GM237354 at 30 or 120 mg/kg/day (Table 3).

(iii) PCP in rats. GM193663 and GM237354 proved to be highly potent inhibitors of *P. carinii* protein synthesis, and both compounds showed 50% inhibitory concentrations of <0.008 mg/ml. A concentration of 0.008 mg of GM193663 or GM237354 per ml produced an inhibition of protein synthesis of 70 and 95%, respectively (18). Corticosteroid-treated rats showed physical signs of PCP (e.g., loss of weight, cyanosis, etc.) immediately before starting the antifungal treatment;

### Table 1. Pharmacokinetic parameters after subcutaneous administration of GM193663 and GM237354 to mice and rats

<table>
<thead>
<tr>
<th>Species</th>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>$C_{\text{max}}$ (μg/ml)</th>
<th>AUC (μg·h/ml)</th>
<th>$t_{1/2}$ (h)</th>
<th>CL (ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>GM193663</td>
<td>50</td>
<td>51.8</td>
<td>79.5</td>
<td>0.8</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>GM237354</td>
<td>50</td>
<td>23</td>
<td>46</td>
<td>0.85</td>
<td>25</td>
</tr>
<tr>
<td>Rat</td>
<td>GM193663</td>
<td>10</td>
<td>6.6</td>
<td>8.5</td>
<td>0.7</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>GM237354</td>
<td>10</td>
<td>7.2</td>
<td>11.8</td>
<td>0.8</td>
<td>133</td>
</tr>
</tbody>
</table>

*a* Values are means for three animals.
GM237354 was administered subcutaneously every 8 h (t.i.d.) at 5, 10, 20, or 40 mg/kg/dose for five consecutive days. Untreated animals showed no mortality, whereas treated animals showed a decrease in the number of cysts per gram of lung tissue. The therapeutic efficacy of GM237354 was estimated by the reduction in the number of cysts from the lungs of treated versus untreated rats. GM237354 administered at a dose of 2 mg/kg/day significantly reduced the log number of cysts per gram to 4.7 ± 0.2 (99.99% reduction of lung cyst burden, compared to untreated control group). The therapeutic efficacy of GM237354 was estimated by the reduction in the number of cysts from the lungs of treated versus untreated rats. GM237354 administered at a dose of 2 mg/kg/day significantly reduced the log number of cysts per gram to 4.7 ± 0.2 (99.99% reduction of lung cyst burden, compared to untreated control group). The therapeutic efficacy of GM237354 was estimated by the reduction in the number of cysts from the lungs of treated versus untreated rats. GM237354 administered at a dose of 2 mg/kg/day significantly reduced the log number of cysts per gram to 4.7 ± 0.2 (99.99% reduction of lung cyst burden, compared to untreated control group). The therapeutic efficacy of GM237354 was estimated by the reduction in the number of cysts from the lungs of treated versus untreated rats. GM237354 administered at a dose of 2 mg/kg/day significantly reduced the log number of cysts per gram to 4.7 ± 0.2 (99.99% reduction of lung cyst burden, compared to untreated control group).

The growing population of immunocompromised patients receiving immunosuppressive or anticancer therapy has resulted in an increased incidence of opportunistic mycoses. Deep-seated infections due to C. albicans are an important cause of infection in the immunocompromised population, and treatment for these infections is still limited to a few agents, including several liposomal amphotericin B formulations and, mainly, azole derivative compounds (2). Invasive aspergillosis is a life-threatening infection increasingly recognized in immunocompromised patients (8, 21), and pulmonary pneumocystosis has also become problematic in certain clinical settings (14).

Sordarin derivatives belong to a new family of antifungal compounds characterized by a novel mechanism of action. Dominguez et al. have identified elongation factor 2 of C. albicans as the primary target of this new class of antifungals (10, 11). Recently, Herreros et al. demonstrated the in vitro activity of several members of this new family against a wide range of pathogenic yeasts and filamentous fungi, including P. carinii (18). Moreover, in their article, Herreros et al. reported that modifications at position 19 resulted in a marked effect on the in vitro activity of sordarin derivatives (18).

The therapeutic potential of this new family of antifungal agents has been assessed on the basis of in vitro activities, pharmacokinetic behavior, and in vivo activity, as it is well known that sordarin derivatives were administered subcutaneously every 12 h (t.i.d.) at 5, 10, 20, or 40 mg/kg/dose for five consecutive days. The mean log of cysts in treated versus control group by log rank test, with a mean (± standard deviation) log number of cysts per gram of lung tissue of 7.6 ± 0.4 at the end of the experiment. The therapeutic effect of GM193663 and GM237354 was estimated by the reduction in the number of cysts from the lungs of treated versus untreated rats. GM193663 administered at a dose of 2 mg/kg/day significantly reduced the log number of cysts per gram to 4.7 ± 0.2 (99.99% reduction of lung cyst burden, compared to untreated control group). The therapeutic efficacy of GM193663 administered at 10 mg/kg/day (log 4.8 ± 0.3 cysts/g) was similar to that obtained with 2 mg/kg/day. A reduction in the number of cysts was observed in infected animals treated with 0.2 mg of GM193663/kg/day, although the results were not statistically significant. GM237354 was more potent than GM193663 and showed dose-related efficacy against PCP. At a dose of 0.2 mg/kg/day, GM237354 reduced the log number of cystic forms of P. carinii to 5.8 ± 0.9 per gram (99.8% reduction). In rats administered GM237354 at 2 and 10 mg/kg/day, the cyst levels were reduced 99.98 and 99.99%, respectively, relative to those in untreated control animals. Table 4 shows the results obtained after treatment with the sordarin derivatives.

### TABLE 3. Efficacy of sordarin derivatives against systemic aspergillosis in mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>No. of survivors/total no. of mice</th>
<th>Survival (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1/10</td>
<td>4.7 ± 1.12 3.0 3–15</td>
<td></td>
</tr>
<tr>
<td>GM237354 (64)</td>
<td>10 0/10 4.0 ± 0.21 4.0 3–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 3/10 8.2 ± 1.47 6.0 3–15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 0/10 4.9 ± 0.55 4.0 3–8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*GM237354 was administered subcutaneously every 8 h (t.i.d.) for five consecutive days.

### TABLE 4. Efficacy of sordarin derivatives against pneumocystosis in rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Log no. of cysts/g of lung</th>
<th>Reduction in log</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.6 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM193663</td>
<td>0.1 6.7 ± 0.9</td>
<td>0.9 89.81</td>
<td>2.9 99.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 4.7 ± 0.2*</td>
<td>2.9 99.90</td>
<td>2.8 99.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 4.8 ± 0.3*</td>
<td>2.8 99.86</td>
<td>1.8 99.82</td>
<td></td>
</tr>
<tr>
<td>GM237354</td>
<td>0.1 5.8 ± 0.9*</td>
<td>2.8 99.86</td>
<td>1.8 99.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 4.6 ± 0.1*</td>
<td>2.8 99.86</td>
<td>1.8 99.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 3.4 ± 0.2*</td>
<td>2.8 99.86</td>
<td>1.8 99.82</td>
<td></td>
</tr>
</tbody>
</table>

Sordarin derivatives were administered subcutaneously every 12 h for 10 consecutive days. *P value of <0.05 for mean log of cysts in treated versus control group by Student-Newman Keuls multiple comparison procedure. Values are means ± standard deviations.
that the final outcome of any anti-infective treatment is a consequence of in vitro activity and pharmacokinetic properties (12). GM193663 showed a MIC of 0.015 μg/ml for the C. albicans strain used in the murine model, which was 1 order of magnitude higher than the MIC of GM237354, 0.001 μg/ml. After subcutaneous administration of 50 mg/kg, pharmacokinetic studies in mice showed that GM193663 reached higher concentrations in serum than did GM237354 (51.8 and 23 μg/ml, respectively). In addition, the AUC of GM193663 was twofold higher than the AUC of GM237354 (79.5 and 46 μg · h/ml, respectively). In mice with systemic infection caused by C. albicans 4711E, the therapeutic efficacies (ED₅₀s) of GM193663 and GM237354 were 25.2 and 10.7 mg/kg/dose, respectively. These results were consistent with the in vitro data obtained and with the different pharmacokinetic profiles of GM193663 and GM237354. The results of these studies clearly demonstrate that sordarins show in vitro and in vivo activity against C. albicans.

Sordarins also have been evaluated in invasive aspergillosis in mice. GM237354 has demonstrated limited in vitro activity against Aspergillus spp. (18) and, consequently, limited therapeutic efficacy in treating systemic aspergillosis in mice. In addition, animals treated with GM237354 demonstrated an irregular response (the survival of animals treated with GM237354 was 0, 30, and 0% at 30, 60, and 120 mg/kg/day, respectively). However, these results are consistent with results obtained in other studies, such as those obtained by Oakley et al. in a murine temporary-neutropenia model of invasive aspergillosis. The survival rates of animals treated with GM237354 in that experiment were 0, 10, 40, and 0% for animals treated with 20, 40, 80, and 160 mg/kg/day (K. L. Oakley, P. E. Verweij, G. Morrissey, J. Morrissey, and D. W. Denning, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-61, 1997).

In spite of the limited anti-Aspergillus in vivo activity displayed by GM237354, sordarin derivatives have demonstrated in vitro and in vivo activities against other filamentous fungi. Clemons and Stevens recently demonstrated that sordarins (GM193663, GM211676, and GM237354) were equivalent or superior to fluconazole in the treatment of experimental systemic coccidioidomycosis in mice (7). In addition, Graybill et al. demonstrated that sordarin derivatives were effective at doses as low as 2 mg/kg in a murine model of histoplasmosis (17). Moreover, these authors noted that on a milligram-for-milligram basis, sordarins may be less potent than amphotericin B but they are more potent than fluconazole. Furthermore, sordarins can be given orally, unlike amphotericin B (17). P. carinii remains an important pathogen in AIDS patients and other immunocompromised individuals (14). Although the combination of trimethoprim and sulfamethoxazole has been used for prophylaxis and treatment of PCP for 25 years, the high frequency of adverse reactions to these drugs and a lack of efficacy in some patients has emphasized the need for new, safe, and effective drugs. The sordarin derivatives tested, GM193663 and GM237354, were very effective in the treatment of experimental PCP in rats (2 mg/kg/day). The therapeutic efficacy showed by sordarins against P. carinii may be related to the observed high in vitro activity and pharmacokinetic properties. GM193663 and GM237354 proved to be highly potent inhibitors of P. carinii protein synthesis, with both compounds having 50% inhibitory concentrations of <0.008 μg/ml. Furthermore, good agreement between in vitro parameters and in vivo outcome has been demonstrated recently, when PCP in rats was treated with sordarin derivatives (3). In addition, the two sordarins evaluated achieved significantly higher serum drug concentrations. Subcutaneous absorption of GM193663 and GM237354 was rapid, reaching peak concentrations in serum of 6.6 and 7.2 μg/ml, respectively, with half-lives of 0.7 and 0.8 h, respectively. The activity displayed a dose-related behavior, with the highest reduction obtained when higher doses were administered.

In addition to the above considerations, the in vitro toxicity profiles of the sordarin derivatives demonstrated the low toxicity of this new family of antifungals. In fact, these results have been confirmed by preliminary rodent toxicity tests demonstrating the favorable therapeutic index of these compounds (15).

We conclude that sordarins are effective in the treatment of lethal systemic candidiasis and PCP in rats and showed a limited protective effect in a murine model of lethal disseminated aspergillosis. The protective effect shown by GM193663 and GM237354 against a variety of experimental infections may be explained by integrating their in vitro antifungal activities and pharmacokinetic behaviors. Further studies to more accurately investigate the relationships between the in vitro and in vivo activities are in progress.

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REFERENCES


