Activities of Sordarins in Experimental Models of Candidiasis, Aspergillosis, and Pneumocystosis

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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 therapeutic efficacy of GM193663 and GM237354 against Pneumocystis 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 for 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 ± 6 to 4.7 ± 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.

Sordarin derivatives are a new class of antifungal agents that target the yeast protein elongation cycle (6, 10, 11). Previously reported sordarin derivatives have demonstrated potent broad-spectrum antifungal activity in several in vitro studies (18), and earlier research indicated that sordarin derivatives possess promising activity in several animal models of infection (15). In order to better understand the potential use of this novel class of compounds, we investigated the pharmacokinetic behavior and therapeutic properties of GM193663 and GM237354 as representatives of sordarin derivatives. To this effect, the in vivo efficacy of these new compounds has been evaluated in systemic candidiasis and aspergillosis in mice and in a pneumocystosis model in rats.


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MATERIALS AND METHODS

**Antifungal agents.** GM193663 and GM237354 (Fig. 1) were synthesized at the Glaxo Wellcome Research Centre in Madrid, Spain, and were provided as sodium salt powders. Immediately before each experiment, compounds were dissolved in sterile deionized water to reach the appropriate concentrations.
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 tail of the rat (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 × 10⁸ CFU/ml. Supernatant 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 pharmaco-kinetic parameters were derived for a one-compartment model using WinNonlin version 1.1 software (Scientific Consulting, Inc., North Carolina).

In vivo antifungal activities. Therapeutic efficacy tests were performed with the most important fungal opportunistic pathogens: *C. albicans, A. fumigatus,* and *P. carinii.*

(i) Systemic infections in mice. For inoculation in mice, *C. albicans* or *A. fumigatus* was grown on Sabouraud dextrose agar (Difco) plates at 30°C for 48 h or on agar slants at 30°C for 5 days, respectively. After incubation, cells or conidia were harvested, washed in sterile saline, and suspended and adjusted in sterile saline to a final concentration of 10⁷ cells/ml. The inoculum size was verified by quantitative culture of serial 10-fold dilutions on Sabouraud dextrose agar plates. Animals were infected by injection of 200 μl of the suspension into a lateral tail vein. After infection, the mice were randomized in groups of 10 for controls or for treatment with the antifungals. Compounds were administered subcutaneously three times a day (t.i.d.), starting 1 h postinfection. For systemic candidiasis, *GM193663* and *GM237354* were administered at doses of 15, 30, 60, and 120 mg/kg/day for seven consecutive days. For systemic aspergillosis, *GM237354* was administered subcutaneously t.i.d., at doses of 30, 60, and 120 mg/kg/day for five consecutive days. Control animals received subcutaneous injections of sterile water. Morbidity and mortality in each group following infection and treatment were monitored daily for up to 30 or 14 days after challenge with *C. albicans* or *A. fumigatus,* respectively.

(ii) Pneumocystosis in rats. PCP was established according to a previously described method (1). Briefly, animals were immunosuppressed with dexamethasone (Fortecortin; Merck Laboratories, Spain) at a concentration of 2 mg/liter in the drinking water for 9 weeks. These animals develop spontaneous *P. carinii* infection after corticosteroid treatment. Tetracycline (Terramicine; Pfizer Laboratories, Spain) at 1 g/liter also was added to the drinking water to minimize bacterial infections. All animals remained on immunosuppression with dexamethasone throughout the study. Before the start of treatment, two animals were sacrificed to microscopically confirm the presence of acute PCP, as previously described (20). The sordarin derivatives *GM193663* and *GM237354* were administered at doses of 0.2, 2, and 10 mg/kg/day by the subcutaneous route. The drugs were given twice a day for 10 consecutive days. Control animals were dosed with sterile water. Twenty-four hours after the last dose, all animals were sacrificed and an overdose of sodium pentobarbital (Euthatal, Noraxon, Spain). Lungs were aseptically removed and weighed. Parasite extractions were performed by means of a previously described method (1), with slight modifications. Lungs were cut into small pieces in sterile phosphate-buffered saline solution and homogenized using a Stomacher 400 blender (Pacisa S.A., Spain). Cell debris were removed by filtering the homogenate through sterile gauze. The filtrate was centrifuged at 2900 × g for 10 min, and the pellet was resuspended in phosphate-buffered saline. Quantification of *P. carinii* cysts was performed with toluidine blue-O (Sigma-Aldrich) staining. The number of cysts per gram of lung was determined by visual assessment under a light microscope (20 microscopic fields). 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 log₁₀ number of cysts per gram of lung.

Statistical analysis. (i) Systemic candidiasis and aspergillosis in mice. Statistical evaluation of differences in the survival rates (Kaplan-Meier plot) for mice with invasive candidiasis or invasive aspergillosis were performed by the log rank test. This test examines the decrease in survival rates over time as well as the final percentage of survival. *P* values of <0.05 were considered significant in these analyses. Also, cumulative mortality was used to calculate by probit analysis the amount of drug, in milligrams per kilogram of body weight per dose, required to prevent 50% of the lethality in infected mice at the end of the experiment (ED₅₀).

(ii) PCP in rats. The mean log number of cysts per gram of lung in treatment groups was compared with that in the lungs of untreated controls by the Student-Neuman-Keuls multiple comparison procedure. A *P* value of <0.05 was accepted as statistically significant.

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, C-4, and C-5 of the sugar moiety of the sordarin molecule. *GM193663* contains a 3, 4-fused dioxolane ring, while *GM237354* contains a 3, 4-fused tetrahydrafuran ring with an ethylene 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 (Cₘᵡₙₙ) area under the concentration-time curve for serum (AUC), elimination half-life (t½), 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* (25.0 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 t½ and CL values were obtained for both sordarin derivatives. After subcutaneous administration to immunosuppressed rats, the Cₘᵡₙₙ (6.6 and 7.2 μg/ml), AUC (8.5 and 11.8 μg h/ml), and t½ (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.
(i) Systemic candidiasis in mice. MICs of GM193663 and GM237354 for C. albicans 4711E were 0.015 and 0.001 µg/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 ED₅₀s 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 ED₅₀ of GM237354 was 10.7 mg/kg/dose and was at least two-fold more effective than GM193663 (ED₅₀, 25.2 mg/kg/dose). 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). 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 µg/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 µg/ml. A concentration of 0.008 µg/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;
however, no mortality was recorded in the control or treated groups throughout the experiment. Untreated animals showed high P. carinii infection levels before and after the treatment period, 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 (99.9% 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 also 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.

### DISCUSSION

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 assayed 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 for 10 consecutive days. 

### 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</td>
<td>3.0 3–15</td>
</tr>
<tr>
<td>GM237354</td>
<td>10</td>
<td>4.0 ± 0.21</td>
<td>4.0 3–5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8.2 ± 1.47</td>
<td>6.0 3–15</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>4.9 ± 0.55</td>
<td>4.0 3–8</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</td>
<td>6.7 ± 0.9</td>
<td>0.9</td>
<td>89.81</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4.7 ± 0.2*</td>
<td>2.9</td>
<td>99.90</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.8 ± 0.3*</td>
<td>2.8</td>
<td>99.86</td>
</tr>
<tr>
<td>GM237354</td>
<td>0.1</td>
<td>5.8 ± 0.9*</td>
<td>1.8</td>
<td>99.83</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4.6 ± 0.1*</td>
<td>3.0</td>
<td>99.98</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>3.4 ± 0.2*</td>
<td>4.2</td>
<td>99.99</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 a 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 0.008 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 therapeu tic 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. Clemens and Stevens recently demonstrated that sordarins (GM193663, GM211676, and GM237354) were equivalent or superior to fluconazole in the treatment of experimental systemic coccidiodomycosis in mice (7). In addition, Graybill et al. demonstrated that sordarins 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 treat ment of experimental PCP in rats (2 mg/kg/day). The thera peutic efficacy shown 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 invasive 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


