The Antidepressant Sertraline Provides a Promising Therapeutic Option for Neurotropic Cryptococcal Infections

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Therapeutic treatment for systemic mycoses is severely hampered by the extremely limited number of antifungals. The difficulty of treatment of fungal infections in the central nervous system is further compounded by the poor central nervous system (CNS) penetration of most antifungals due to the blood-brain barrier. Only a few fungistatic azole drugs, such as fluconazole, show reasonable CNS penetration. Here we demonstrate that sertraline (Zoloft), the most frequently prescribed antidepressant, displays potent antifungal activity against Cryptococcus neoformans, the major causative agent of fungal meningitis. In vitro assays, this neurotropic drug is fungicidal to all natural Cryptococcus isolates tested at clinically relevant concentrations. Furthermore, sertraline interacts synergistically or additively with fluconazole against Cryptococcus. Importantly, consistent with our in vitro observations, sertraline used alone reduces the brain fungal burden at an efficacy comparable to that of fluconazole in a murine model of systemic cryptococcosis. It works synergistically with fluconazole in reducing the fungal burden in brain, kidney, and spleen. In contrast to its potency against Cryptococcus, sertraline is less effective against strains of Candida species and its interactions with fluconazole against Candida strains are often antagonistic. Therefore, our data suggest the unique application of sertraline against cryptococcosis. To understand the antifungal mechanisms of sertraline, we screened a whole-genome deletion collection of Saccharomyces cerevisiae for altered sertraline susceptibility. Gene ontology analyses of selected mutations suggest that sertraline perturbs translation. In vitro translation assays using fungal cell extracts show that sertraline inhibits protein synthesis. Taken together, our findings indicate the potential of adopting this antidepressant in treating cryptococcal meningitis.
other organs are 20- to 40-fold higher than its serum concentra-
tion (31). Such levels could meet or exceed the MICs of sertraline against Cryptococcus isolates. In addition, abundant clinical data support the safety of long-term use of this antidepressant (6, 28, 29). Given its psychotropic nature and its potent fungidal activity against Cryptococcus, we hypothesized that sertraline could be uniquely suitable in treating systemic cryptococcosis.

The mechanism of action of sertraline in the mammalian nervous system has been well characterized: it blocks the 5-hydroxytryptamine (5-HT) transporter and inhibits the reuptake of 5-HT into the presynaptic cell (1). However, there is no conserved homolog of the 5-HT transporter in fungi. Thus, the underlying mechanisms responsible for the antifungal activity of sertraline remain mysterious. One report attributed the antifungal activity of sertraline to a nonspecific cytotoxicity due to its lipophilicity (37). However, findings of two recent studies suggested membrane organization and vesicle-mediated transport as its antifungal targets (27, 30). Sertraline was also shown to possess antitumor activity (15, 16, 32). The antitumor activity was attributed to the inhibition of the initiation of protein synthesis through its effects on the mTOR pathway (15). It is not clear if translation is also a target of sertraline in fungal cells, as homologs of certain relevant factors important for translational regulation in mammals do not exist in fungi. In addition to antifungal and antitumor activities, sertraline has been shown to exert antiviral activity (14), antibacterial activity (19, 20), antiparasite activity (23), and spermidical activity (10). The antiproliferation activity of sertraline against evolutionarily diverse organisms indicates its interference with some fundamental processes. Based on our genetic screen and evolutionarily diverse organisms indicates its interference with some fundamental processes. Based on our genetic screen and evolutionarily diverse organisms indicates its interference with some fundamental processes. Based on our genetic screen and evolutionarily diverse organisms indicates its interference with some fundamental processes. Based on our genetic screen and evolutionarily diverse organisms indicates its interference with some fundamental processes.

In vitro translation assay. Cryptococcus H99 cells from an overnight culture were inoculated into 2 liters of YPD medium to an optical density at 600 nm (OD600) of 0.05. The culture was incubated at 30°C with shaking for approximately 10 more hours to reach an optical density of 2. At this stage, the cells were collected, washed, and resuspended using the same procedure as previously described for the preparation of yeast cell extract (36). The resuspended cells were dripped directly into liquid ni-
tron by using a sterile Pasteur pipette to form small ice beads. Frozen cell beads were transferred into prechilled grinding vials of a Spex sam-
plePrep 6850 Freezer/Mill and powdered in the liquid nitrogen-filled tub using a setting consisting of 10 min of precooling followed by three 2-min grind cycles with 1 min of recooling between cycles. The powdered cells were transferred to prechilled 50-m1 polycarbonate centrifuge tubes, allowed to thaw on ice, and then centrifuged at 4°C for 15 min at 16,000 rpm.
TABLE 1 Sertraline, alone or with fluconazole, is potent against diverse Cryptococcus isolates

<table>
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<tr>
<th>Strain</th>
<th>Serotype</th>
<th>Geographic origin</th>
<th>SRT MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>FLC MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>MFC&lt;sub&gt;c&lt;/sub&gt;</th>
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<tr>
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<td>6 64</td>
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a (C), clinical strain; (E), environmental strain; (L), laboratory strain; (N), not available.

b MIC<sub>90</sub> (shown in micrograms per milliliter), the lowest drug concentration that resulted in a 90% decrease in absorbance.

c MFC (minimal fungicidal concentration) (shown in micrograms per milliliter), the lowest concentration at which at least 99% of cells were killed compared to the original inoculums. When fluconazole was used alone, the MFC was sometimes not achieved in the dose range tested, as indicated by “>”.

d FF CI, fractional fungicidal concentration index. FF CIs < 0.5, drug effects were synergistic; FF CIs between 0.5 and 1.0, drug effects were additive; FF CIs between 1.0 and 2.0, drug effects were indifferent; FF CIs > 2.0, drug effects were antagonistic (4).
Sertraline is fungicidal against both proliferative and quiescent Cryptococcus cells. (A) H99 cells were inoculated into RPMI media and cultured without any drug (control) or in the presence of fluconazole (FLC; 8 μg/ml) or sertraline (SRT; 10 μg/ml) or a combination of these two drugs. At the indicated time points, aliquots of cell suspensions were transferred and plated onto drug-free agar medium to determine CFU after 2 more days of incubation. Fungal cells proliferated rapidly in the absence of any drugs, but they were gradually cleared by any of the drug treatments. (B) H99 cells were inoculated into PBS buffer and cultured without any drug (control) or in the presence of fluconazole (8 μg/ml) or sertraline (8 μg/ml) or a combination of these two drugs. At the indicated time points, aliquots of cell suspensions were transferred and plated on drug-free medium to determine CFU after 2 more days of incubation. Fungal cells in PBS maintained viability during the tested period after 48 h of incubation (Fig. 1A). Sertraline was modestly more effective than fluconazole under these conditions (Fig. 1A). The drug combination showed the highest efficiency in clearing the yeast cells (Fig. 1A). Similarly, H99 cells in PBS buffer were rapidly cleared by sertraline (within 4 h after inoculation), indicating that sertraline is fungicidal and is potent against Cryptococcus cells regardless of whether cells are growing or not (Fig. 1B). In sharp contrast, fluconazole showed no effect on the viability of the fungal cells in PBS buffer (Fig. 1B). The lack of killing effect of fluconazole on quiescent fungal cells is consistent with the classification of fluconazole as a fungistatic drug: that is, it is effective in inhibiting fungal cell proliferation but ineffective in destroying cells that are not actively growing. The fungicidal effect of sertraline is not specific to Cryptococcus, as sertraline also killed S. cerevisiae BY4742 cells regardless of whether they were growing in RPMI medium or quiescent in PBS buffer (see Fig. S1 in the supplemental material). These results indicate that sertraline is able to kill fungal cells independently of whether they are proliferating.

To examine whether the susceptibility to sertraline is specific to the H99 strain or is a general trait of Cryptococcus, we further tested 23 genetically and phenotypically distinct Cryptococcus strains, including 13 serotype A (this serotype accounts for 95% of clinical cryptococcosis cases), 6 serotype D (serotype D represents fewer than 5% of clinical cases of cryptococcosis), 3 serotype A- serotype D hybrids, and 1 C. gattii strain (Table 1). These strains are clinical or environmental isolates from North America, Europe, or Africa. The MICs of sertraline were determined by the microdilution assay according to the CLSI standard. Compared to the wide range of inhibitory concentrations of fluconazole (from 1 to >64 μg/ml), all of these strains are very sensitive to sertraline, with a surprisingly narrow spectrum of inhibitory concentrations (≤10 μg/ml) (Table 1). Notably, there is no correlation between the levels of susceptibility to fluconazole and to sertraline among these strains, indicating that there is no cross-resistance between sertraline and fluconazole. Thus, it is unlikely that these two drugs share common mechanisms of action. These results imply the general susceptibility of Cryptococcus strains to sertraline irrespective of their level of susceptibility to fluconazole.

Sertraline interacts synergistically or additively with fluconazole against Cryptococcus in vitro. As fluconazole is the most commonly used antifungal in cryptococcosis treatment, drugs that can act additively or synergistically with fluconazole are more likely to be used clinically. In the time course assay described above, we observed that the combination of sertraline and fluconazole accelerated the clearance of proliferative Cryptococcus cells (Fig. 1A). To determine the interaction between sertraline and fluconazole against Cryptococcus, we compared the susceptibility of the 24 isolates to a combination of these drugs with their susceptibility to each drug alone using the disk diffusion halo assay. For all the strains tested, halos formed near disks containing the drug combination were larger and clearer than those formed near disks containing either fluconazole or sertraline alone (data not shown, but see Fig. S2 in the supplemental material). The results indicate possible synergistic or additive interactions between sertraline and fluconazole against Cryptococcus.

We next quantified the interaction between sertraline and fluconazole against Cryptococcus by using a microdilution assay to determine the fractional fungicidal concentration index (FFCI). Sertraline increases the antifungal activity of fluconazole against all the tested Cryptococcus strains, demonstrating a synergistic (FFCI < 0.5) or additive (FFCI between 0.5 and 1.0) (4) effect of the combination of the two drugs (Table 1). This is consistent with the results obtained by the disk diffusion method. Considering the divergence of the genetic backgrounds of the tested strains, these observations suggest the possibility that the combination of sertraline and fluconazole could be more effective at clearing different Cryptococcus isolates in cases of mammalian cryptococcosis.

Sertraline alone or in combination with fluconazole displays antifungal activity in a murine model of systemic cryptococcosis. As described earlier, sertraline exerts anti-Cryptococcus activity alone or in combination with fluconazole in vitro. To determine the efficacy of sertraline or its combination with fluconazole in vivo, we adopted a murine model of systemic cryptococcosis. We chose the intravenous model of cryptococcosis here, because our pilot experiments showed that organ fungal burden displayed relatively low variations among individual animals. To examine the drug efficacy in this model, we assigned mice into four groups: the control (PBS) group, the fluconazole treatment group, the sertraline treatment group, and the group receiving a drug combination. Given that it takes at least 5 to 7 days for sertraline to reach a steady state in the mouse brain (8), mice of the sertraline group and the drug combination group were given a 7-day pretreatment of sertraline prior to the infection by the Cryptococcus H99 reference strain. Based on previous studies, the serum level of sertraline in these animals was likely to be too low (55 to 250 ng/ml [34]) to
Sertraline antagonizes the growth-inhibitory effect of fluconazole on many Candida strains. To examine if the potency of sertraline alone or in combination with fluconazole can be extended to Candida species, we tested the MICs of sertraline against six strains that represent six different Candida spp.: C. albicans SC5314, C. glabrata PAT21S03, C. krusei DUMC132.91, C. parapsilosis MMRL1594, C. tropicalis MMRL2017, and C. lusitaniae 2-367. The MIC90s ranged from 12 to 24 μg/ml (Table 2), which are considerably higher than the MICs against Cryptococcus but are consistent with previous studies (13, 37). These MIC values are higher than the achievable blood or even organ levels of sertraline.

In striking contrast to the results obtained with Cryptococcus, sertraline had a significant antagonistic impact on the inhibitory effect of fluconazole for the majority of the tested Candida strains in vitro (Fig. 3). For example, 1-day incubation with fluconazole alone was able to effectively inhibit the growth of the C. tropicalis MMRL2017 strain at a concentration of 1.0 μg/ml. However, with the addition of sertraline (~1.0 to 6.0 μg/ml), fungal growth in the presence of fluconazole not only recovered but actually became more robust. The spectrum of the concentrations at which sertraline exerts an antagonistic effect with fluconazole unfortunately falls in the clinically relevant range. Similar antagonistic interactions between sertraline and fluconazole were also observed among three other Candida strains: C. albicans SC5314, C. glabrata PAT21S03, and C. parapsilosis MMRL1594 (Fig. 3). Sertraline did not decrease the inhibitory effect of fluconazole on C. lusitaniae 2-367 or on C. krusei DUMC132 (Fig. 3). Although synergistic or additive interactions between sertraline and fluconazole were reported in studies of some Candida strains (30), antagonistic interactions between fluconazole and sertraline are commonly observed among Candida and Aspergillus strains (this study and reference 7).

Sertraline interferes with translation in fungal cells. The broad antiproliferation activity of sertraline against many organisms indicates that the mechanism underlying its fungicidal activity may involve some fundamental cellular processes. To identify the potential fungal molecules and processes affected by sertraline, we screened the whole-genome deletion collection of S. cerevisiae for sertraline-sensitive or sertraline-resistant mutants. This mutant collection set is composed of a nonessential gene deletion set and a forward genetics collection set that contains all three orgs: (36) and an essential gene deletion in a haploid background and an essential gene deletion in a haploid background and an essential gene deletion in a haploid background and an essential gene deletion in a haploid background (35). After one round of initial

### TABLE 2 Sertraline is less potent against Candida strains

<table>
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<th>Strain</th>
<th>MIC90 (μg/ml)</th>
<th>MIC100a (μg/ml)</th>
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<td>48</td>
</tr>
<tr>
<td>Candida glabrata PAT21S03</td>
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<td>16</td>
</tr>
<tr>
<td>Candida krusei DUMC132.91</td>
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<td>Candida parapsilosis MMRL1594</td>
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<td>Candida lusitaniae 2-367</td>
<td>8</td>
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</table>

*a MIC100, the lowest drug concentration that resulted in a 100% decrease in absorbance.

FIG 2 Sertraline reduces the fungal burden alone or in combination with fluconazole in vivo. Brains (A), kidneys (B) and spleens (C) of mice from different treatment groups were dissected and homogenized. The suspensions were diluted serially, and the fungal burden was determined by calculating CFU. As shown in Fig. 2, the treatment with sertraline alone reduced the fungal burden in the brain (P < 0.05), with its efficacy comparable with that of fluconazole. Sertraline also reduced fungal burden in kidney, while fluconazole showed no apparent effect. Sertraline appeared to have a modest effect on fungal burden in spleen, but such effect was not statistically significant. The differences in the levels of drug efficacy for the three organs examined may reflect variations of drug concentrations at these organs. Importantly, the treatment with the combination of sertraline and fluconazole displayed the most potent efficacy in reducing fungal burden of all three organs examined (Fig. 2). Therefore, the data demonstrate that sertraline alone is efficacious against cryptococcosis and that the combination of sertraline with fluconazole is a more effective treatment than either drug alone due to their strong synergy in vivo.
screening and two rounds of additional screening to confirm the selected phenotype, 88 resistant and 36 sensitive strains were identified (see Table S2 in the supplemental material). Gene ontology analyses indicated that these genes are enriched for those with roles in intracellular vesicle transport and membrane organization (Fig. 4), which is consistent with the findings of two recent studies of the effects of sertraline on yeast (27, 30).

Interestingly, genes related to protein synthesis are highly enriched in the resistant group; the most sensitive mutant selected from our screen was strain \( \text{H9004} \) \( \text{tif3} \), in which an important translation initiation factor \( \text{Tif3} \) was disrupted. Since previous studies in mammalian cells have indicated that sertraline inhibits translation initiation (15), these data indicated that it was possible that sertraline also disrupts translation in fungi.

**Sertraline inhibits translation in a Cryptococcus cell-free system.** To determine the effect of sertraline on translation in Cryptococcus, we performed in vitro translation assays. In these assays, the luciferase mRNA was used as the template and the translation machinery was provided by the cell extract obtained from \( \text{C. neoformans} \) strain H99. As expected, translation in these Cryptococcus cell extracts was synergistically dependent on the mRNA 5′ terminal cap and 3′ terminal poly(A) tail, as with \( \text{Saccharomyces} \) cell extract (Fig. 5A) and \( \text{Neurospora crassa} \) cell extract (33, 36). This result verified that the Cryptococcus in vitro translation system faithfully recapitulated the dependence of cap and poly(A) for translation.

The effects of sertraline on translation of cap and poly(A) luciferase mRNA were assessed by measurement of both (i) the enzymatic activity of the luciferase produced by the in vitro translation and (ii) the level of [\(^{35}\text{S}\)] methionine incorporation into the luciferase polypeptide produced. We found that sertraline inhibited the translation efficiency in a dose-dependent manner using both detection methods (Fig. 5B and C). Luciferase enzyme activity dropped 50% compared to the control when the concentration of sertraline was increased to 0.1 mM (30.6 \( \mu \text{g/ml} \)). No enzyme activity above the background was detected in the presence of sertraline at 0.4 mM (122.4 \( \mu \text{g/ml} \)). The decrease in luciferase enzyme activity in the presence of sertraline was not due to direct interference of sertraline with the enzymatic activity of the synthesized luciferase, since, based on this measurement, addition of sertraline into the cell extract after translation was completed did not alter enzyme activity (Fig. 5B). The [\(^{35}\text{S}\)] methionine incorporation assay showed that sertraline affected the yield of luciferase polypeptide synthesized in this cell extract (Fig. 5C). Protein synthesis was also affected by sertraline in fungal extracts derived from the yeast \( \text{S. cerevisiae} \) and the filamentous fungus \( \text{N. crassa} \), although higher concentrations of sertraline were required to achieve a similar level of inhibition (see Fig. S3 in the supplemen-
shorten the duration of anticryptococcal therapy and reduce the
duration of time (25). In contrast, fluconazole, which is known to target the
enzyme Erg11 in the ergosterol biosynthetic pathway, did not show any inhibitory effect on protein synthesis in a similar concentration range in such assays (see Fig. S4 in the supplemental material). These data obtained in cell-free translation systems using fungal extracts show that sertraline interferes with fungal protein synthesis.

DISCUSSION
Sertraline offers a promising option for the treatment of cryptococcosis, especially cryptococcal meningitis. Here we provide evidence for the potent anticryptococcal activity of the antidepressant sertraline both in vitro and in vivo. Given the difficulties in developing antifungal drugs de novo, recent studies have explored existing clinical compounds for potential use as antifungals (39). Existing pharmaceutical and safety information concerning the use of these drugs in animals or in humans could greatly accelerate the investigation into their clinical use as antifungals. Consistent with the safety profile of sertraline for long-term use in patients, we did not observe any severe side effects due to sertraline administration during the treatment process in the animal studies presented here. Previous studies also showed the safety of sertraline administration in mice with a similar daily dose but for a much longer period of time (25).

The discovery that sertraline has anticryptococcal activity offers the potential for an additional choice for treating cryptococcosis. Our tests of 24 diverse Cryptococcus strains indicate a uniformly high sensitivity of this fungus to sertraline relative to other fungal species tested. Compared to fluconazole, sertraline showed a much narrower range of inhibitory concentrations against these diverse Cryptococcus isolates (Table 1), suggesting a lower probability of naturally occurring resistance to sertraline in existing Cryptococcus populations. The fungicidal nature of sertraline and its synergy with fluconazole against Cryptococcus, as previously observed in vitro (21) and in vivo in an insect model (30) and in this study shown in a mammalian model, could potentially shorten the duration of anticryptococcal therapy and reduce the risk of emerging drug resistance. During latent infection or during fluconazole treatment, Cryptococcus cells are likely dormant or grow slowly. Given that even dormant cells need transcription and translation (22), and that sertraline is capable of killing fungal cells under quiescent conditions, it is reasonable to speculate that sertraline might be useful to clear latent cryptococcal infections or to kill residual fungal cells unharmed by the fluconazole treatment. This could be tested by further investigation.

It is worth mentioning that we did not observe any differences in survival in any of the treatment groups (including the fluconazole-treated groups). This is most likely due to the rapid disease progression in this model. The A/J mouse used in this study is very susceptible to Cryptococcus infections, and H99 is one of the most virulent clinical isolates of Cryptococcus (38). We tried different inocula of the fungal cells (1 × 10³, 1 × 10⁴, and 1 × 10⁵ cells/mouse) in this intravenous infection model and found that a 10-fold decrease in the inoculum prolonged survival for an additional 1 to 2 days and that infected mice all succumbed to the diseases within 8 days. We speculate that better protection against cryptococcosis by sertraline or the drug combination might be observed in other animal models or in humans, or if cryptococcosis is caused by less virulent strains. Optimization of drug doses, the route and frequency of drug administration, and the duration of treatment warrants further investigation in order to assess the treatment outcomes.

One of the most valuable aspects of sertraline as a potential anticryptococcal drug is its superior ability to accumulate in the CNS relative to other antifungals. This is particularly critical in the treatment of cryptococcosis, given that Cryptococcus preferentially proliferates in the brain. Consistent with our expectations, the in vivo study presented here supports the hypothesis of the ability of sertraline, either alone or in combination with fluconazole, to reduce brain fungal burden.

Although sertraline demonstrates efficacy against cryptococcosis comparable to that of fluconazole based on the data presented here, the potential application of this drug to treat mycoses caused by fungal pathogens such as Candida or Aspergillus requires further investigation. The commonly observed antagonistic interaction between sertraline and fluconazole against Candida...
and *Aspergillus* strains is particularly concerning (this study and reference 7). It is possible that specific chemical modification of sertraline could increase its efficacy against these other fungi and abolish its antagonistic interaction with fluconazole. Such modifications would increase its value in the battle against systemic mycoses.

**The antifungal mechanisms of sertraline.** Sertraline displays extremely broad antiproliferation activity against evolutionarily diverse organisms (10, 12–15, 19, 20, 23). Recent studies indicate the influence of sertraline on membrane stability or vesicle transport in fungi (27, 30). The results of our *S. cerevisiae* mutant screens are consistent with these discoveries. Our mutant screens and the *in vitro* translation assays also indicated protein synthesis as another process interfered with by sertraline. Our finding regarding the inhibitory effect of sertraline on translation is consistent with a recent study on translation in tumor cells (15), even though the implicated mammalian factors in the mTOR pathway identified in that study, such as PDCD4 or REDD1, do not have obvious homologs in fungi. Inhibition of translation could possibly cause changes in other processes, as molecules involved in protein trafficking and membrane proteins were also significantly enriched in our screen (Fig. 4; see also Table S2 in the supplemental material). Sertraline’s impact on translation might more acutely affect protein synthesis from specific transcripts in *Cryptococcus* important for growth such that it does not need to completely inhibit protein synthesis for its strong anticryptococcal activity. Thus, while sertraline inhibits mammalian protein synthesis, it may be that qualitative, not quantitative, differences in sertraline’s effects on fungal protein synthesis are crucial for sertraline’s antiproliferation activity.

The possibility of emergence of fungal resistance to sertraline seems low based on the following observations. First, we noted during our genetic screen that *S. cerevisiae* gene deletion mutants selected for sertraline resistance showed an increase in MIC of only up to 50% compared to the wild type. Second, our repeated attempts to perform UV mutagenesis with *S. cerevisiae* and *C. neoformans* failed to yield any resistant strains with sertraline MICs greater than 14 μg/ml. Third, all natural *Cryptococcus* strains tested showed uniformly high sensitivity to sertraline. Such observations are dramatically different from what is known for azole drugs, as a more than 10- to 100-fold difference in fluconazole susceptibilities can be easily observed in both clinical and laboratory settings. This feature, although making it rather challenging to pinpoint the underlying fungicidal mechanisms of sertraline, could be advantageous for its clinical application, as the possibility of encountering sertraline-resistant *Cryptococcus* isolates and the risk of developing fungal resistance during therapy would be low.

**ACKNOWLEDGMENTS**

This work was supported by the American Heart Association (grant 08GI3540400 to X.L.), the Norman Hackerman Advanced Research Program (grant 01957 to X.L.), and National Institutes of Health (grant RO1 GM47498 to M.S.S. and grant RO1 AI097599 to X.L.).

We thank rotation students Lynn Dudinsky, Michael White, Rachana Gyawali, and Ying Fang for their contribution for initial observations; Hao Chen for his assistance on data processing with the Perl programing; Xiuyun Tian for her critical reading; and Wanqing Liao and Nan Zhou for their helpful discussions.

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