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EXPERIMENTAL THERAPEUTICS

A Second-Generation Fungerp Analog, SCY-247, Shows Potent *In Vitro* Activity against *Candida auris* and Other Clinically Relevant Fungal Isolates

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ABSTRACT Due to the increase of antifungal drug resistance and difficulties associated with drug administration, new antifungal agents for invasive fungal infections are needed. SCY-247 is a second-generation fungerp antifungal compound that interferes with the synthesis of the fungal cell wall polymer β-(1,3)-D-glucan. We conducted an extensive antifungal screen of SCY-247 against yeast and mold strains compared with the parent compound ibrexafungerp (IBX; formerly SCY-078) to evaluate the *in vitro* antifungal properties of SCY-247. SCY-247 demonstrated similar activity to IBX against all of the organisms tested. Moreover, SCY-247 showed a higher percentage of fungicidal activity against the panel of yeast and mold isolates than IBX. Notably, SCY-247 showed considerable antifungal properties against numerous strains of *Candida auris*. Additionally, SCY-247 retained its antifungal activity when evaluated in the presence of synthetic urine, indicating that SCY-247 maintains activity and structural stability under environments with decreased pH levels. Finally, a time-kill study showed SCY-247 has potent anti-*Candida*, -*Aspergillus*, and -*Scedosporium* activity. In summary, SCY-247 has potent antifungal activity against various fungal species, indicating that further studies on this fungerp analog are warranted.

KEYWORDS Ibrexafungerp, candidiasis, β-(1,3)-D-glucan, fungerps, triterpenoid class

Echinocandins are effective against *Candida* and *Aspergillus* species and are commonly the front-line antifungal agents used (1). Species of *Scedosporium* are also susceptible to echinocandins, especially when combined with granulocyte-macrophage colony-stimulating factor (GM-CSF) (2, 3). However, there has been an increasing amount of fungal species developing resistance to echinocandins, particularly in *Candida glabrata* and more recently *Candida auris* (4–6). Additionally, echinocandins are available only as intravenous formulations, which may be a disadvantage with regard to availability and ease of access (7).

Species of *Candida* and *Aspergillus* are the most common causes of invasive fungal infections (IFIs), and *Candida* species are the fourth leading cause of nosocomial bloodstream infections in the United States (8, 9). Invasive candidiasis and invasive aspergillosis have been reported to make up to 53% and 19% of IFIs, respectively (10). However, *Scedosporium* and *Fusarium* species are among the fungi increasingly reported to cause infections, especially in immunocompromised patients (11–13). Additionally, *Candida* species, especially *Candida albicans*, are the most common organisms causing fungal urinary tract infections (UTIs) (14, 15).

Due to the increasing resistance to echinocandins and their limitation regarding

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TABLE 1 MIC/MFC ranges for the *Candida* isolates tested against SCY-247 and IBX

	MIC (μg/ml) range against:		MFC (μg/ml) range against:		Cidal activity[c] against:	
Organism (*n*)	SCY-247	IBX	SCY-247	IBX	SCY-247	IBX
C. albicans (5)	0.25–1	0.125–1	8	0.5–8	4/5	3/5
C. auris (5)	0.25–0.5	0.25–0.5	4	8–16	5/5	3/5
C. glabrata (5)	0.5	0.5–1	0.5–1	1–2	5/5	5/5
C. kefyr (5)	0.125–0.5	0.125–1	0.25–4	0.5–2	5/5	5/5
C. krusei (6)	0.5–8	0.5–4	2–8	1–8	6/6	6/6
C. metapsilosis (5)	0.031–4	0.031–2	0.125–8	0.25–16	5/5	4/5
C. orthopsilosis (5)	0.125–0.5	0.125–0.5	0.25–8	0.5–8	3/5	3/5
C. parapsilosis (6)	0.125–0.5	0.125–0.5	4–8	0.5–16	3/6	2/6
C. tropicalis (5)	0.25–8	0.125–1	0.5–8	0.25–8	5/5	4/5
Total *Candida* sp. (47)	0.031–8[a]	0.031–4[a]	0.125–8[b]	0.25–16[b]	41/47	35/47

[a]MIC_{50} of 0.5, MIC_{90} of 1.
[b]MFC_{50} of 4, MFC_{90} of 8.
[c]No. of strains killed/no. of strains tested.

method of drug administration (intravenous [i.v.]), development of novel antifungals with oral bioavailability and broad spectrum of activity are needed to target potential resistant fungi. One new antifungal, SCY-078, was assigned the name "ibrexafungerp" (IBX) by the World Health Organization (WHO) International Nonproprietary Name (INN) group. The name ibrexafungerp included a new stem root, "-fungerp," which indicated that SCY-078 was unique and a first-in-class compound. Fungerps are β-1,3-glucan synthase inhibitors that interfere with the synthesis of the fungal cell wall polymer β-(1,3)-D-glucan. The mechanism of action is similar to that of echinocandins, but fungerps are structurally distinct and suitable for both oral and intravenous formulations. IBX has demonstrated *in vitro* and *in vivo* antifungal efficacy against *Candida* infections and has completed phase III testing for the treatment of vulvovaginal candidiasis (VVC). Additionally, IBX is currently in phase II and III clinical testing in patients with recurrent VVC (CANDLE; ClinicalTrials.gov identifier NCT04029116), refractory IFIs (FURI; NCT02244606), infections caused by *Candida auris* (CARES; NCT03363841), and invasive aspergillosis (SCYNERGIA; NCT03672292) (14–17). Although both oral and intravenous formulations of IBX are under development, IBX as an oral formulation has been the focus. Accordingly, progress on preclinical screening of additional members of the fungerp family is ongoing.

In this study, we conducted a primary antifungal screen of SCY-247 against yeast and mold strains by determining MIC for yeasts, minimum effective concentrations (MECs) for mold, and minimum fungicidal concentrations (MFCs). This primary screen showed that SCY-247 was active against different fungal isolates. We further examined the antifungal activity of SCY-247 against *C. albicans*, *C. glabrata*, and *C. auris* strains. We also explored the efficacy of this compound in combination with decreased pH conditions in the presence of synthetic urine (SU). Additionally, we explored the time-kill kinetics of SCY-247 against *Candida*, *Aspergillus*, and *Scedosporium* species.

RESULTS

MIC and MFC activity of SCY-247 against *Candida* isolates. Table 1 shows the MIC and MFC data for SCY-247 compared with those of SCY-078 (IBX) against different *Candida* species tested. IBX demonstrated an MIC range of 0.031 to 4 μg/ml against all *Candida* species. When available, strains that previously showed high MIC values to commercially available antifungals were used. This represents 27 of the 47 *Candida* isolates tested. SCY-247 showed a similar MIC range compared with that of IBX, with a range of 0.031 to 8 μg/ml for all *Candida* species tested. MIC_{50} and MIC_{90} values were the same for SCY-247 and IBX (0.5 and 1 μg/ml, respectively). Overall, IBX and SCY-247

TABLE 2 MIC/MFC ranges for various non-*Candida* yeast isolates tested against SCY-247 and IBX

Organism (*n*)	MIC (μg/ml) range against:		MFC (μg/ml) range against:		Cidal activity[b] against:	
	SCY-247	IBX	SCY-247	IBX	SCY-247	IBX
Coccidioides immitis (5)	<0.125–0.25	<0.125–0.25	N/A[a]	N/A		
Cryptococcus neoformans (5)	2	2	2	2–4	5/5	5/5
Geotrichum capitatum (5)	0.5–2	1–2	8–16	8–16	4/5	4/5
Histoplasma sp. (5)	<0.125–0.25	<0.125–0.25	N/A	N/A		
Kodamaea ohmeri (5)	0.125–0.5	0.125–0.5	4–8	8–16	4/5	1/5
Rhodotorula sp. (5)	1–4	1–8	8–16	16	5/5	4/5
Saccharomyces cerevisiae (5)	0.125–0.25	0.25–0.5	0.25–4	0.5–8	4/5	5/5
Trichosporon asahii (5)	2	2	8	8–16	5/5	5/5
All yeasts total					68/77	59/77

[a]N/A, not tested.
[b]No. of strains killed/no. of strains tested.

demonstrated similar MFC ranges and the same MFC_{50} and MFC_{90} values. Interestingly, SCY-247 demonstrated cidal activity against a greater number of isolates than IBX (41 and 35 isolates, respectively).

MIC and MFC activity of SCY-247 against non-*Candida* yeast isolates. Table 2 shows the MIC and MFC data for SCY-247 compared with those of IBX against the remaining yeast isolates tested. Overall, SCY-247 demonstrated similar MIC and MFC ranges for all isolates tested as those of IBX. SCY-247 demonstrated cidal activity against a greater number of yeast isolates than IBX (68 and 59 isolates, respectively).

MIC and MFC activity of SCY-247 against *Aspergillus* isolates. Table 3 shows the MIC and MFC data for SCY-247 compared with those of IBX against *Aspergillus* isolates. Nineteen of the 20 *Aspergillus* isolates tested previously showed high MIC values to commercially available antifungals. SCY-247 demonstrated similar MIC ranges and MIC_{50} and MIC_{90} values for all isolates tested as those of IBX; neither SCY-247 nor IBX demonstrated cidal activity against the *Aspergillus* isolates.

MIC and MFC activity of SCY-247 against *Fusarium* isolates. Table 4 shows the MIC and MFC data for SCY-247 compared with those of IBX against *Fusarium* isolates. All of the *Fusarium* isolates tested previously showed high MIC values to commercially available antifungals ($n = 10$). SCY-247 demonstrated similar MIC ranges and MIC_{50} and MIC_{90} values, as well as MFC ranges and MFC_{50} and MFC_{90} values, for all isolates tested as those of IBX. IBX and SCY-247 demonstrated cidal activity against all *Fusarium* isolates, for which an endpoint was determined.

MIC and MFC activity of SCY-247 against *Scedosporium* isolates. Table 5 shows the MIC and MFC data for SCY-247 compared with those of IBX against *Scedosporium* isolates. SCY-247 demonstrated slightly lower MIC ranges and MIC_{50} and MIC_{90} values, as well as MFC ranges and MFC_{50} and MFC_{90} values, for all isolates tested than those of IBX. However, these values were all within 2 dilutions. SCY-247 demonstrated cidal

TABLE 3 MIC/MFC ranges for the *Aspergillus* isolates tested against SCY-247 and IBX

Organism (*n*)	MIC (μg/ml) range against:		MFC (μg/ml) range against:		Cidal activity[e] against:	
	SCY-247	IBX	SCY-247	IBX	SCY-247	IBX
A. flavus (5)	0.063	<0.016 to 0.063	8 to >8	>8	0/5	0/5
A. fumigatus (5)	0.063 to 0.25	0.063 to 0.125	8 to >8	8 to >8	0/5	0/5
A. nidulans (5)	0.063 to 0.125	0.031 to 0.063	8 to >8	>8	0/5	0/5
A. terreus (5)	0.031 to 0.63	<0.016 to 0.031	8 to >8	>8	0/5	0/5
Aspergillus total (20)	0.031 to 0.25[a]	<0.016 to 0.125[b]	8 to >8[c]	8 to >8[d]	0/20	0/20

[a]MIC_{50}, 0.063; MIC_{90}, 0.125.
[b]MIC_{50} and MIC_{90}, 0.063.
[c]MFC_{50}, 8; MFC_{90}, >8.
[d]MFC_{50} and MFC_{90}, >8.
[e]No. of strains killed/no. of strains tested.

TABLE 4 MIC/MFC ranges for the *Fusarium* isolates tested against SCY-247 and IBX

	MIC (μg/ml) range against:		MFC (μg/ml) range against:		Cidal activity[f] against:	
Organism (*n*)	SCY-247	IBX	SCY-247	IBX	SCY-247	IBX
F. oxysporum (5)	8 to 16	8 to 16	8 to 32	16 to 32	5/5	5/5
F. solani (5)	1 to 8	16	8 to 32	32 to >64	5/5	4/4[e]
Fusarium sp. total (10)	1 to 16[a]	8 to 6[b]	8 to 32[c]	16 to >64[d]	10/10	9/9[e]

[a]MIC_{50} and MIC_{90}, 8.
[b]MIC_{50} and MIC_{90}, 16.
[c]MFC_{50}, 16; MFC_{90}, 32.
[d]MFC_{50}, 32; MFC_{90}, 64.
[e]Cidality indeterminable due to growth above the highest drug concentration.
[f]No. of strains killed/no. of strains tested.

activity against a greater number of *Scedosporium* isolates tested than IBX (7 and 4 isolates, respectively).

MIC and MFC activity of SCY-247 against remaining mold species. Table 6 shows the MIC and MFC data for SCY-247 compared with those of IBX against the remaining mold species. SCY-247 demonstrated similar MIC ranges, MIC_{50} and MIC_{90} values, MFC ranges, and MFC_{50} and MFC_{90} values for all isolates tested as those of IBX.

MIC and MFC determinations against *Candida auris*. Table 7 shows the MIC/MFC ranges (μg/ml) of SCY-247 compared with those of IBX against an expanded panel of *C. auris* isolates. SCY-247 demonstrated a MIC range and MIC_{50} and MIC_{90} of 0.06 to 1 μg/ml, 0.5 μg/ml, and 0.5 μg/ml, respectively. IBX showed similar results with a MIC range, MIC_{50}, and MIC_{90} of 0.06 to 2 μg/ml, 0.5 μg/ml, and 0.5 μg/ml, respectively.

SCY-247 demonstrated an MFC range, MFC_{50}, and MFC_{90} of 0.5 to 8 μg/ml, 4 μg/ml, and 4 μg/ml, respectively. IBX again showed similar results, with an MFC range, MFC_{50}, and MFC_{90} of 0.25 to 8 μg/ml, 4 μg/ml, and 8 μg/ml, respectively.

Table 8 shows the MIC and MFC values for SCY-247 compared with those of IBX against the individual *C. auris* isolates tested (μg/ml). For each strain tested, SCY-247 and IBX showed MIC and MFC values within 2 dilutions of each other. However, SCY-247 demonstrated cidal activity against 14 strains, while IBX demonstrated cidal activity against 7 of the *C. auris* isolates tested.

Effect of synthetic urine on the antifungal activity of SCY-247 on *C. albicans* and *C. glabrata*. The MIC values for SCY-247 against *C. albicans and C. glabrata* isolates at 50% growth inhibition showed no significant change in the presence of SU (Tables 9 and 10). Compared with the MIC values for SCY-247 in the presence of SU, the MIC values for SCY-247 against both *C. albicans* and *C. glabrata* isolates tested in RPMI media were within 2 dilutions, which is considered within the normal variation of MIC testing. Therefore, we did not observe a pH effect on MIC activity for SCY-247.

TABLE 5 MIC/MEC ranges for the *Scedosporium* isolates tested against SCY-247 and IBX

	MIC (μg/ml) range against:		MFC (μg/ml) range against:		Cidal activity[f] against:	
Organism (*n*)	SCY-247	SCY-078	SCY-247	SCY-078	SCY-247	SCY-078
S. apiospermum (5)	2 to 4	8	16 to >64	>64	3/5	0/0[a]
S. prolificans (5)	2 to 4	8	8 to >64	8 to >64	4/5	4/4[a]
Scedosporium sp. total (10)	2 to 4[b]	8[c]	8 to >64[d]	8 to >64[e]	7/10	4/4

[a]Cidality indeterminable due to growth above the highest drug concentration.
[b]MIC_{50}, 2; MIC_{90}, 4.
[c]MIC_{50} and MIC_{90}, 8.
[d]MFC_{50}, 16; MFC_{90}, >64.
[e]MFC_{50} and MFC_{90}, >64.
[f]No. of strains killed/no. of strains tested.

TABLE 6 MIC/MFC ranges for the remaining mold isolates tested against SCY-247 and IBX

Organism (*n*)	MIC (μg/ml) range against:		MFC (μg/ml) range against:		Cidal activity[b] against:	
	SCY-247	IBX	SCY-247	IBX	SCY-247	IBX
Acremonium sp. (5)	2 to 4	2 to 8	8 to 16	32 to 64	5/5	4/5
Fonsecaea pedrosoi (5)	4	8 to 16	8 to >64	16 to >64	4/5	3/3[a]
Paecilomyces sp. (5)	<0.125 to 4	<0.125 to 8	4 to >64	32 to >64	0/5	0/4[a]
Pseudallescheria boydii (5)	2 to 4	4 to 8	>64	>64	0/5	0/1[a]
Rhizopus oryzae (5)	8 to 16	32	16 to >64	>64	1/1[a]	0/0[a]
Trichoderma sp. (5)	1 to 4	2 to 8	64 to >64	>64	0/5	0/4[a]
All molds					27/61	20/45

[a]Cidality indeterminable due to growth above the highest drug concentration.
[b]No. of strains killed/no. of strains tested.

Time-kill kinetic study. SCY-247 showed a reduction in the log CFU/ml of *C. albicans* strains 11036 and 27884 at all concentrations and time points compared with the growth control (Fig. 1A and B). After 1 h of exposure to SCY-247, the growth of *C. albicans* 11036 was reduced to zero at SCY-247 concentrations of 2, 4, and 8 μg/ml, respectively. SCY-247 demonstrated fungicidal properties against *C. albicans* 27884 at all concentrations. Additionally, the growth of *C. albicans* 27884 in the presence of SCY-247 at all concentrations continued to decrease over time. An untreated growth control was included as a comparator to SCY-247 for all fungal species.

SCY-247 reduced the log CFU/ml of both *C. auris* 35653 and 35646 strains at all concentrations and time periods compared with the untreated growth control (Fig. 2A and B). After 1 h of exposure to SCY-247, both *C. auris* species showed no growth, demonstrating fungicidal activity.

Against the *Scedosporium apiospermum* 34114 strain, SCY-247 reduced the log CFU/ml at all concentrations and time points tested compared with the control and showed fungicidal activity after 24 h of incubation (Fig. 3A). SCY-247 treatment of *S. apiospermum* 34223 reduced the log CFU/ml of isolates and was fungicidal after 8 hours at concentrations of 8, 16, 32, 64, and 128 μg/ml (Fig. 3B).

Time-kill data for *A. fumigatus* 28385 treated with SCY-247 is shown in Fig. 4. Treatment of this strain showed larger reductions in CFU/ml at every SCY-247 concentration tested than those of the control strain. After 48 hours of incubation, SCY-247 showed fungicidal activity at concentrations of 4, 8, and 16 μg/ml.

DISCUSSION

Invasive fungal infections (IFIs) are a major cause of morbidity and mortality, especially among transplant and other immunocompromised patients, as well as those with extensive stays in intensive care units or undergoing extensive intra-abdominal surgeries. Despite a wide availability of newer classes of antifungals, IFIs are still common and mostly caused by *Candida* and *Aspergillus* species (10). Furthermore, IFI mortality rate is high (30% to 50%), especially in non-*Aspergillus* mold infections (18, 19). To address the need for novel effective antifungal agents to prevent IFIs, new analogs of IBX are currently being developed and tested against a broad spectrum of fungal species.

Our results show that the analog of ibrexafungerp SCY-247 has potent and broad-

TABLE 7 MIC/MFC ranges for SCY-247 and IBX against the *C. auris* isolates tested[a]

Compound	MIC (μg/ml)			MFC (μg/ml)		
	Range	50%	90%	Range	50%	90%
SCY-247	0.06–1	0.5	0.5	0.5–8	4	4
IBX	0.06–2	0.5	0.5	0.25–8	4	8

[a]*n* = 44.

TABLE 8 MIC and MFC values with fungicidal or fungistatic activity indicated for SCY-247 and IBX against the individual *C. auris* isolates[a] tested

	SCY-247			IBX		
Isolate identifier[b]	MIC[c]	MFC[c]	Antifungal Activity	MIC[c]	MFC[c]	Antifungal Activity
35364[Am,M]	0.125	1	Static	0.125	1	Static
35366[Am,M]	0.25	1	Cidal	0.5	1	Cidal
35367[Am,F,M]	0.5	1	Cidal	0.25	2	Static
35368[Am,C,F,M]	0.5	4	Static	0.5	8	Static
35370[Am, F,M]	0.5	8	Static	0.5	8	Static
35371[Am,F,M]	0.5	8	Static	0.5	8	Static
35372[Am,M]	0.5	4	Static	0.25	2	Static
35373[Am,C,F,M]	0.5	4	Static	0.25	4	Static
35374[Am,C,F,M]	0.5	4	Static	0.25	4	Static
35375[Am,F,M]	0.5	4	Static	0.25	4	Static
35376[Am,M]	0.5	2	Cidal	0.5	2	Cidal
35377[Am,C,M]	0.5	4	Static	0.25	2	Static
35378[C,M]	1	4	Cidal	0.5	4	Static
35379[Am]	0.5	4	Static	0.5	2	Cidal
35645	0.125	0.5	Cidal	0.125	2	Static
35646[C,F]	0.5	2	Cidal	0.25	4	Static
35647[F,V]	0.5	4	Static	0.5	4	Static
35648[Am,F,V]	0.5	2	Cidal	0.5	4	Static
35649[Am,F,V]	0.25	2	Static	0.25	4	Static
35650[Am,F,V]	0.25	4	Static	0.25	4	Static
35651[C,F]	0.5	2	Cidal	0.5	4	Static
35652[Am,C,F,V]	0.5	4	Static	0.5	1	Cidal
35653[An,C,F,V]	0.5	4	Static	0.5	8	Static
35654[An,F,V]	0.5	2	Cidal	0.25	4	Static
37101[Am]	0.5	4	Static	0.25	4	Static
37102[Am]	0.5	4	Static	0.5	4	Static
37103[Am]	0.25	2	Static	0.25	4	Static
37104[Am]	0.5	4	Static	0.5	4	Static
37432	0.5	2	Cidal	0.5	4	Static
37433	0.25	4	Static	0.25	4	Static
37434	0.25	2	Static	0.5	4	Static
38684	0.5	2	Cidal	0.5	4	Static
38883[Am]	0.5	4	Static	0.5	4	Static
39307[Am]	1	4	Cidal	2	8	Cidal
39308	0.5	4	Static	0.5	4	Static
39309	0.5	4	Static	0.25	4	Static
39414[Am]	0.25	4	Static	0.5	4	Static
39415[F]	0.125	2	Static	0.25	1	Cidal
39416[Am,C,M,F,V]	0.5	4	Static	0.5	4	Static
39417[AmF]	0.5	2	Cidal	0.5	4	Static
39418[AmF]	1	4	Cidal	0.5	4	Static
39419[Am,C,F,V]	0.5	4	Static	0.25	4	Static
39420[Am,F,V]	0.25	4	Static	0.5	8	Static
41527	0.06	0.5	Static	0.06	0.25	Cidal

[a]$n = 44$.
[b]Am, amphotericin B; An, anidulafungin; C, caspofungin; F, fluconazole; M, micafungin; V, voriconazole.
[c]μg/ml.

spectrum antifungal activity, as demonstrated by similar MIC and MFC values compared with those of IBX against a panel of clinically relevant yeast and molds. SCY-247 showed fungicidal activity against a large percentage of the yeast isolates tested (88%). However, both SCY-247 and IBX demonstrated mostly fungistatic activity against mold isolates, with MIC values lower against *Aspergillus* strains than those of the *Fusarium*, *Scedosporium*, *Acremonium*, *Fonsecaea*, *Paecilomyces*, *Pseudallescheria*, *Rhizopus*, and *Trichoderma* strains tested. Interestingly, SCY-247 demonstrated a higher percentage of cidality than IBX against this panel of yeast and mold isolates (88% and 44%, respectively).

Our findings further demonstrated that the 50% MIC endpoint for SCY-247

TABLE 9 MIC values for SCY-247 against the *C. albicans* isolates tested at 50% growth inhibition at neutral pH

Organism	Isolate identifier	SCY-247 50% endpoint (μg/ml) in presence of:		Δ in MIC (dilution)	Interpretation
		RPMI	SU[a]		
C. albicans	38382	0.125	0.06	−1	NV[b]
C. albicans	38397	0.125	0.125	No change	NV
C. albicans	38404	0.125	0.06	−1	NV
C. albicans	38405	0.125	0.06	−1	NV
C. albicans	38406	0.25	0.06	−1	NV

[a]SU, synthetic urine.
[b]NV, within the normal day to day variation of MIC testing.

against *C. albicans* and *C. glabrata* had no significant change in the presence of SU. This result suggests that SCY-247 is able to retain activity and structural stability against *Candida* species in the presence of SU that mimics the composition of urine and an environment with decreased pH levels, highlighting its potential effectiveness in fungal UTIs.

We further studied the *in vitro* activity of SCY-247 against strains of *C. albicans*, *C. auris*, *S. apiospermum*, and *A. fumigatus*. The time-kill curve study demonstrated that SCY-247 killed all fungi isolates tested (two *C. albicans*, two *C. auris*, two *S. apiospermum*, and one *A. fumigatus*) at various concentrations. High drug concentrations typically led to quicker fungicidal effects, except in *S. apiospermum* 34114. In *S. apiospermum* 34114 isolates, IBX showed a concentration-independent reduction in CFU/ml, as all concentrations tested showed an equivalent ability to inhibit growth. Our data suggest that SCY-247 has concentration- and time-dependent effects against different fungal species. Furthermore, SCY-247 shows broad-spectrum activity against yeasts and molds, as evidenced by its fungicidal activity against various strains of *Candida*, *Scedosporium*, and *Aspergillus*.

Given the numerous reports demonstrating the high resistance of *C. auris* to several antifungal agents, we chose to expand our cohort of *C. auris* isolates to include numerous clinical isolates previously demonstrated to be highly resistant to antifungals. SCY-247 showed elevated cidal activity against more *C. auris* strains than IBX, wherein cidal activity was observed against 14 versus 7 tested strains, respectively.

Echinocandins have been shown to be fungicidal *in vitro* and *in vivo* against most isolates of *Candida* species, especially of *C. albicans* (20, 21). In contrast, echinocandins show largely fungistatic properties against filamentous fungi such as *Aspergillus*, being unable to completely inhibit growth (22, 23). IBX, a first-in-class glucan synthase inhibitor, was developed in order to target both echinocandin-susceptible and echinocandin-resistant fungal species, as unlike echinocandins, IBX

TABLE 10 MIC values for SCY-247 against the *C. glabrata* isolates tested at 50% growth inhibition

Organism	Isolate identifier	SCY-247 50% endpoint (μg/ml) in presence of:		Δ in MIC (dilution)	Interpretation
		RPMI	SU[a]		
C. glabrata	32075	0.125	0.03	−2	NV[b]
C. glabrata	32232	0.06	0.03	−1	NV
C. glabrata	34870	0.125	0.03	−2	NV
C. glabrata	34901	0.06	0.03	−1	NV
C. glabrata	35164	0.06	0.03	−1	NV

[a]SU, synthetic urine.
[b]NV, within the normal day to day variation of MIC testing.

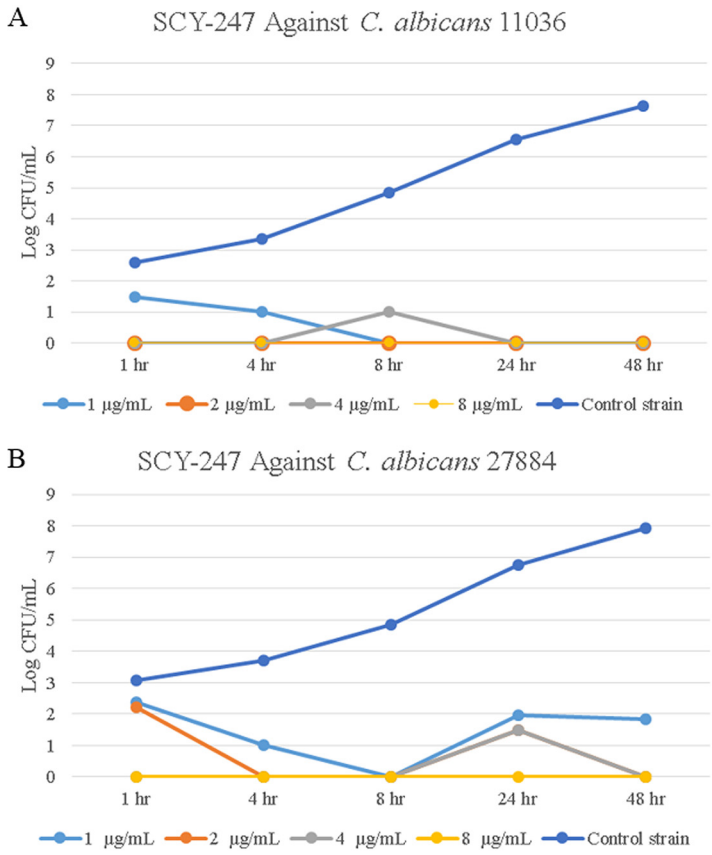

FIG 1 Growth of over 48 hours of *C. albicans* 11036 when exposed to SCY-247 at 1, 2, 4, and 8 μg/ml (A) and *C. albicans* 27884 when exposed to SCY-247 at 1, 2, 4, and 8 μg/ml (B).

antifungals are not inhibited by most common mutations occurring within the protein target *Fks* (24). IBX has shown *in vitro* and *in vivo* effectiveness against *Candida* and *Aspergillus* species, including azole-resistant and echinocandin-resistant strains (24–28). SCY-247 has a lower molecular weight than IBX, which may contribute to an improved profile with central nervous system penetration and suitability for effective and simple IV formulation.

In summary, our study demonstrates promising fungicidal properties of SCY-247. We showed that SCY-247 is a potent antifungal drug that could be an important addition to the repertoire of therapies to combat drug-resistant fungal infections. This is especially true for *C. auris* strains wherein high levels of resistance exhibited by this species of *Candida* have been a very problematic occurrence in health care facilities. The parent drug IBX has shown additive effects in combination with azoles, echinocandins, and amphotericin B versus *Candida* species and synergy in combination with azoles versus *Aspergillus* species (26, 29). Thus, larger *in vitro* and combination studies with this second-generation fungerp seem warranted.

MATERIALS AND METHODS

Minimum inhibitory concentration. MIC testing was performed according to the CLSI M27-A3 and M38-A2 standards for the susceptibility testing of yeasts and filamentous fungi, respectively (17, 30). For yeast isolates, incubation temperature and time were 35°C and 24 hours, respectively, and the inoculum size was 0.5 to 2.5 $\times$ 10^3 CFU/ml. For filamentous fungi, incubation times at 35°C were 24, 48, or 72 hours, and the inoculum size was 0.4 to 5 $\times$ 10^4 conidia/ml. RPMI was used throughout as the growth medium with the exception of *Cryptococcus* species, for which yeast nitrogen base (YNB) was used as described previously by Ghannoum et al., and the CLSI M27-A3 document (17, 31). The MIC endpoints were 50% and 100% inhibition compared with the growth control for yeasts, and MEC endpoints were used for molds, when available. The initial MIC range tested was 0.016 to 8 μg/ml; the range was increased to 0.125 to 64 μg/ml where needed to capture the MIC and MFC endpoints.

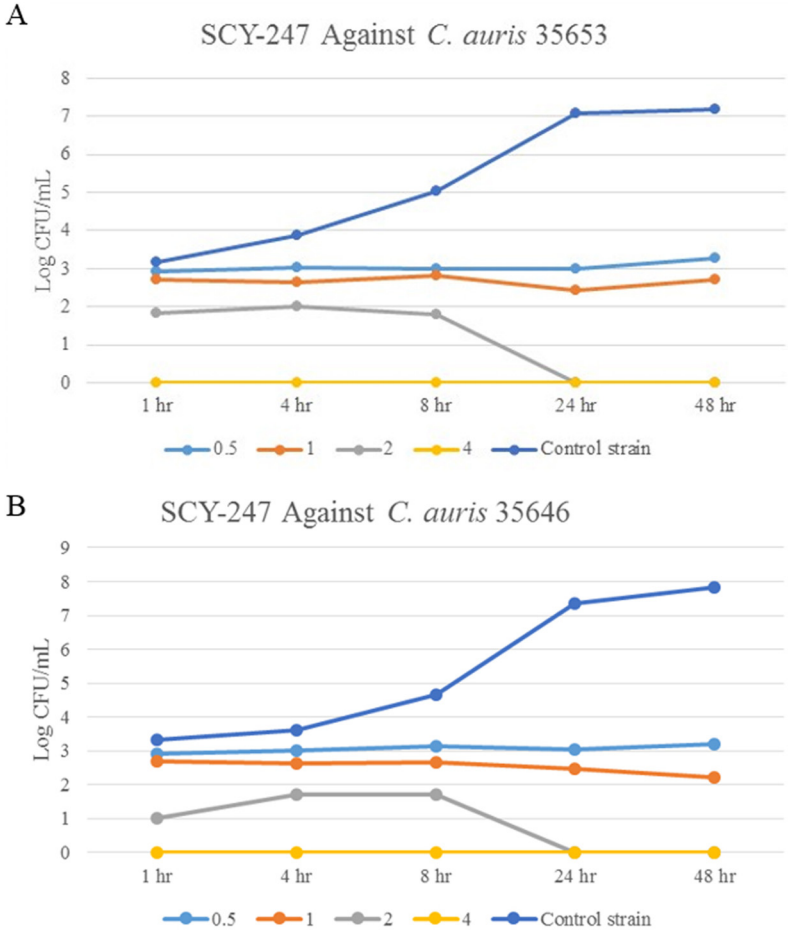

FIG 2 Growth of over 48 hours of *C. auris* 35653 when exposed to SCY-247 at 0.5, 1, 2, and 4 μg/ml (A) and *C. auris* 35646 when exposed to SCY-247 at 0.5, 1, 2, and 4 μg/ml (B).

Antifungal susceptibility tests against *Coccidioides* sp. and *Histoplasma capsulatum* were performed using a broth macrodilution method according to the Clinical and Laboratory Standards Institute M38-A2 standard. Isolates were adjusted by a spectrophotometer (80% to 82% transmittance) to a starting inoculum of 1×10^4 to 5×10^4 cells/ml and then added to tubes containing serial 2-fold dilutions of the antifungal agents (0.125 to 64 μg/ml). The assay was conducted in RPMI medium. The cultures were incubated at 35°C for 48 hours for *Coccidioides* sp. and at 30°C for 168 hours for *Histoplasma capsulatum*. MEC endpoints were used for evaluation.

Minimum Fungicidal Concentration. The MFC of SCY-247 was determined against the selected isolates tested above. Briefly, MFC determinations were performed according to the method previously described by Ghannoum and Isham (32). Specifically, the total contents of each clear well from the MIC assay were subcultured onto potato dextrose agar. To avoid antifungal carryover, aliquots were allowed to soak into the agar and then streaked for isolation once dry, thus removing the cells from the drug source. Petri dishes were incubated at 35°C for 48 hours, and the number of CFUs was determined. Fungicidal activity was defined as a ≥99.9% reduction in the number of CFUs from the starting inoculum count occurring within 4 dilutions of the MIC endpoint.

Effects of urine on SCY-247 antifungal activity. Five strains each of *C. albicans* and *C. glabrata* isolates were used to evaluate the effect of the addition of synthetic urine (SU) on the antifungal activity of SCY-247. Antifungal activity was assessed by determining the MIC according to the CLSI M27-A4 standard for *Candida* susceptibility testing. Incubation temperature and time were 35°C and 24 h, respectively, and the inoculum was 0.5×10^3 to 2.5×10^3 CFUs/ml. MIC determination was performed using SU medium, which consisted of $CaCl_2$ (0.65 g/liter), $MgCl_2$ (0.65 g/liter), NaCl (4.6 g/liter), Na_2SO_4 (2.3 g/liter), Na_3-citrate (0.65 g/liter), Na_2-oxalate (0.02 g/liter), KH_2PO_4 (2.8 g/liter), KCl (1.6 g/liter), NH_4Cl (1.0 g/liter), urea (25.0 g/liter), creatinine (1.1 g/liter), 5% yeast nitrogen base (YNB) medium (vol/vol), and 2% glucose (wt/vol). The pH was adjusted to 6.0, and the medium was filter sterilized by passing it through a 0.22-μm-pore filter. MIC determination was also performed in RPMI 1640 medium (pH of 7.0) for comparison. The MIC endpoints were 50% and 100% inhibition compared with the growth control.

Time-kill kinetics of SCY-247. Two strains of *C. albicans* and *C. auris*, four strains of *S. apiospermum*, and three strains of *A. fumigatus* were utilized to analyze the time-kill kinetics of SCY-247.

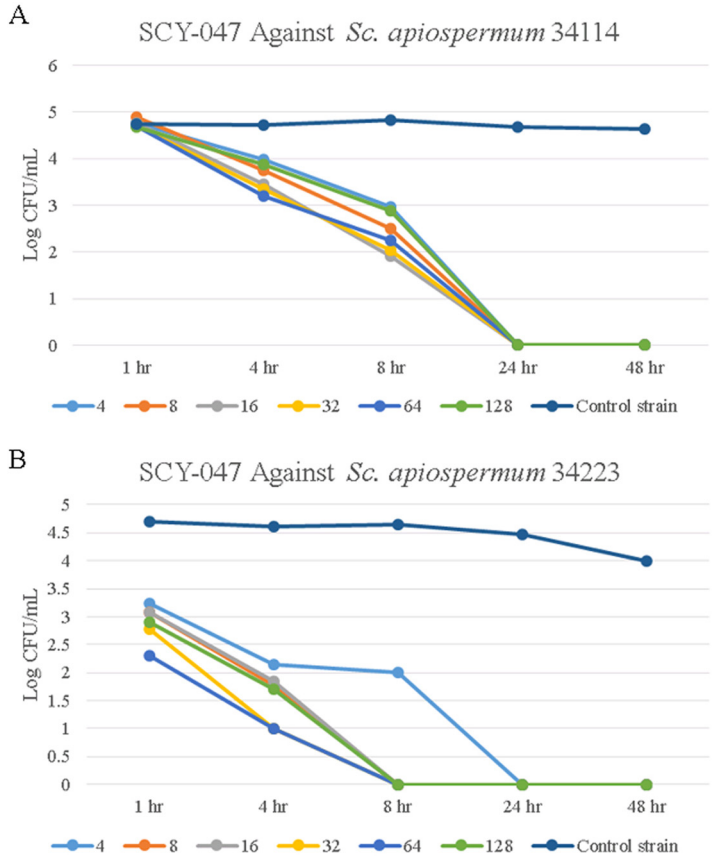

FIG 3 Growth of over 48 hours of *S. apiospermum* 34114 when exposed to SCY-247 at 4, 8, 16, 32, 64, and 128 μg/ml (A) and *S. apiospermum* 34223 when exposed to SCY-247 at 4, 8, 16, 32, 64, and 128 μg/ml (B).

Fungal cells were inoculated overnight in Sabouraud dextrose broth (SDB) at 37°C. Cells were harvested the following day, washed with phosphate-buffered saline (PBS), adjusted to 1×10^5 cells/ml, and suspended in 10 ml of SDB media containing 1, 2, 4, 8, 16, 32, 64, or 128 μg/ml of SCY-247. Tubes with the cells and SCY-247 were incubated for 1, 4, 8, 24, or 48 hours at 37°C. A sample tube with no drug served as a control. At each time point, 100-μl aliquots from each tube were removed, diluted serially with PBS, and spread onto Sabouraud dextrose agar (SDA) plates. CFUs were determined following 48 hours (yeast) or 2 to 4 days (molds) of incubation at 35°C. Results were calculated as log CFU/ml for each isolate and plotted against drug concentrations for predetermined time points to obtain the time-kill curve. To test the *Candida* strains, four concentrations for SCY-247 were chosen based on minimum fungicidal concentrations (MFCs) (0.0625×, 0.25×, 0.5×, and 1× MFC), resulting in different concentrations of SCY-247 being compared. Testing for the

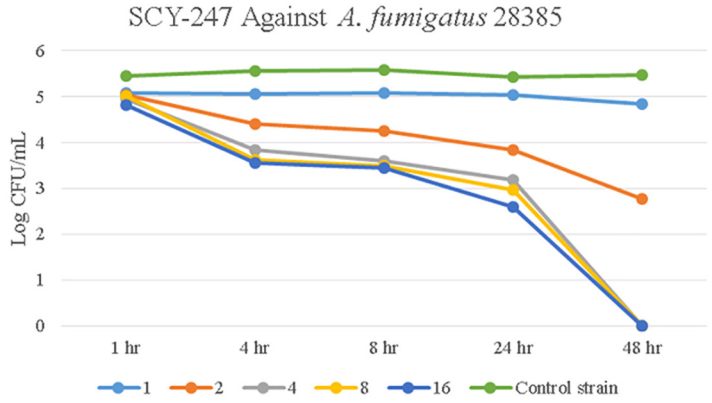

FIG 4 Growth of over 48 hours of *A. fumigatus* 28385 when exposed to SCY-247 at 1, 2, 4, 8, and 16 μg/ml.

Scedosporium and *Aspergillus* isolates was conducted using equivalent concentrations that included the MFC values for both agents.

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