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SUSCEPTIBILITY

In Vitro Activity of APX001A (Manogepix) and Comparator Agents against 1,706 Fungal Isolates Collected during an International Surveillance Program in 2017

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ABSTRACT Current antifungal agents cover a majority of opportunistic fungal pathogens; however, breakthrough invasive fungal infections continue to occur and increasingly involve relatively uncommon yeasts and molds, which often exhibit decreased susceptibility. APX001A (manogepix) is a first-in-class small-molecule inhibitor of the conserved fungal Gwt1 protein. This enzyme is required for acylation of inositol during glycosylphosphatidylinositol anchor biosynthesis. APX001A is active against the major fungal pathogens, i.e., *Candida* (except *Candida krusei*), *Aspergillus*, and hard-to-treat molds, including *Fusarium* and *Scedosporium*. In this study, we tested APX001A and comparators against 1,706 contemporary clinical fungal isolates collected in 2017 from 68 medical centers in North America (37.3%), Europe (43.4%), the Asia-Pacific region (12.7%), or Latin America (6.6%). Among the isolates tested, 78.5% were *Candida* spp., 3.9% were non-*Candida* yeasts, including 30 (1.8%) *Cryptococcus neoformans* var. *grubii* isolates, 14.7% were *Aspergillus* spp., and 2.9% were other molds. All isolates were tested by CLSI reference broth microdilution. APX001A (MIC_{50}, 0.008 μg/ml; MIC_{90}, 0.06 μg/ml) was the most active agent tested against *Candida* sp. isolates; corresponding anidulafungin, micafungin, and fluconazole MIC_{90} values were 16- to 64-fold higher. Similarly, APX001A (MIC_{50}, 0.25 μg/ml; MIC_{90}, 0.5 μg/ml) was $\geq$8-fold more active than anidulafungin, micafungin, and fluconazole against *C. neoformans* var. *grubii*. Against *Aspergillus* spp., AXP001A (50% minimal effective concentration [MEC_{50}], 0.015 μg/ml; MEC_{90}, 0.03 μg/ml) was comparable in activity to anidulafungin and micafungin. *Aspergillus* isolates (>98%) exhibited a wild-type phenotype for the mold-active triazoles (itraconazole, posaconazole, and voriconazole). APX001A was highly active against uncommon species of *Candida*, non-*Candida* yeasts, and rare molds, including 11 isolates of *Scedosporium* spp. (MEC values, 0.015 to 0.06 μg/ml). APX001A demonstrated potent *in vitro* activity against recent fungal isolates, including echinocandin- and fluconazole-resistant strains. The extended spectrum of APX001A was also notable for its potency against many less common but antifungal-resistant strains. Further studies are in progress to evaluate the clinical utility of the methyl phosphate prodrug, APX001, in difficult-to-treat resistant fungal infections.

KEYWORDS APX001A, Gwt1, antifungal, manogepix

The systemically active antifungal agents currently include the polyenes (amphotericin B), triazoles (fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole), and echinocandins (anidulafungin, caspofungin, and micafungin) (1–3). Although these agents cover the vast majority of opportunistic fungal pathogens and are increasingly utilized in prophylactic or preemptive treatment strategies, breakthrough

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invasive fungal infections continue to occur and increasingly involve yeast and mold isolates that are relatively uncommon and tend to exhibit decreased susceptibility to current antifungal agents (4–6). Data from multiple sources demonstrate that mortality rates and resource utilization increase significantly when therapy is delayed or inadequate (e.g., incorrect dose or resistant isolate), further highlighting the importance of detailed epidemiological data, including the evaluation of new classes of antifungal agents (1, 7–12).

APX001 (fosmanogepix [formerly E1211]) is a first-in-class (small-molecule) antifungal agent (13–15). It is a water-soluble prodrug (intravenous and oral formulations are available) that is rapidly metabolized by systemic phosphatases to the active moiety, APX001A (manogepix [formerly E1210]). APX001A possesses a novel mechanism of action distinct from those of other classes of antifungal agents. APX001A targets the highly conserved fungal enzyme Gwt1 (15). Inhibition of Gwt1 blocks the inositol acylation step during synthesis of glycosylphosphatidylinositol-anchored proteins of the fungal cell wall. This compromises cell wall integrity, biofilm formation, and germ tube formation and results in severe fungal growth defects (14, 15). In many nonclinical studies, APX001A has shown broad-spectrum activity against common species of *Candida*, *Cryptococcus neoformans*, *Cryptococcus gattii*, *Aspergillus* spp., multidrug-resistant strains such as *Candida auris*, and rare hard-to-treat molds, including *Fusarium* spp., *Scedosporium* spp., and *Lomentospora* (*Scedosporium*) *prolificans* (13, 14, 16–22).

Concurrent with the increasing number of invasive fungal infections, antifungal surveillance programs have become important in defining the species distribution and resistance patterns of the responsible pathogens, providing needed information for appropriate empirical antifungal treatment (23–28). The SENTRY Antimicrobial Surveillance Program is a global program (https://www.jmilabs.com/sentry-surveillance-program) that has been ongoing for more than 20 years (from 1997 to 2019) and collects, in each calendar year, consecutive invasive isolates of *Candida*, *Aspergillus*, and other opportunistic fungi from medical centers located in North America, Europe, Latin America, and the Asia-Pacific region (1, 29–31). Applying modern methods for species identification (e.g., sequence-based identification and matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI–TOF MS]), testing of antifungal susceptibility, and characterization of antifungal resistance mechanisms provides a level of standardization and clarity that makes these observations useful in the ongoing fight against resistance (1, 4, 9, 25, 29, 32–34).

In this study, we have utilized the SENTRY Antimicrobial Surveillance Program to examine the activities of APX001A, anidulafungin, micafungin, fluconazole, itraconazole, posaconazole, voriconazole, and amphotericin B against 1,706 contemporary clinical fungal isolates from bloodstream infections (BSIs), respiratory tract infections (RTIs), skin and skin structure infections (SSSIs), urinary tract infections (UTIs), intra-abdominal infections (IAIs), and other infections. The isolates were collected in 2017 from 68 medical centers in 24 countries in North America (637 isolates from 29 medical centers located in Canada or the United States), Europe (740 isolates from 26 medical centers located in Belgium, Czech Republic, France, Germany, Greece, Hungary, Ireland, Italy, Portugal, Romania, Slovenia, Spain, Sweden, or Turkey), the Asia-Pacific region (217 isolates from 9 medical centers located in Australia, Korea, New Zealand, or Thailand), or Latin America (112 isolates from 4 medical centers located in Argentina, Brazil, Chile, or Mexico).

RESULTS AND DISCUSSION

Fungal isolates tested. The frequency distributions for APX001A and the species/organism groups tested are shown in Table 1. All fungal species possessing $\geq$10 isolates in the surveillance program were analyzed separately. APX001A results for species with $<$10 isolates are listed in Tables S1 to S4 in the supplemental material.

Among the 1,706 fungal clinical isolates tested, 1,340 (78.5%) were *Candida* spp., 66 (3.9%) were non-*Candida* yeasts, including 30 (1.8%) *Cryptococcus neoformans* var. *grubii* isolates, 251 (14.7%) were *Aspergillus* spp., and 49 (2.9%) were other

TABLE 1 Antimicrobial activity of APX001A against the main organisms and organism groups tested

Organism/organism group	No. (cumulative %) of isolates with MIC/MEC of:[a,b]															
	≤0.002 μg/ml	0.004 μg/ml	0.008 μg/ml	0.015 μg/ml	0.03 μg/ml	0.06 μg/ml	0.12 μg/ml	0.25 μg/ml	0.5 μg/ml	1 μg/ml	2 μg/ml	4 μg/ml	8 μg/ml	>[c]	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Candida spp. (1,340 isolates)	70 (5.2)	213 (21.1)	**400 (51.0)**	215 (67.0)	141 (77.5)	190 (91.7)	58 (96.0)	7 (96.6)	3 (96.8)	0 (96.8)	0 (96.8)			43 (100.0)	0.008	0.06
Candida albicans (414 isolates)	63 (15.2)	141 (49.3)	**183 (93.5)**	26 (99.8)	1 (100.0)										0.008	0.008
Candida glabrata (321 isolates)	0 (0.0)	2 (0.6)	10 (3.7)	11 (7.2)	79 (31.8)	**167 (83.8)**	48 (98.8)	4 (100.0)							0.06	0.12
Candida parapsilosis (270 isolates)	1 (0.4)	30 (11.5)	**147 (65.9)**	70 (91.9)	19 (98.9)	3 (100.0)									0.008	0.015
Candida tropicalis (151 isolates)	0 (0.0)	3 (2.0)	31 (22.5)	**85 (78.8)**	31 (99.3)	1 (100.0)									0.015	0.03
Candida krusei (43 isolates)											0 (0.0)			**43 (100.0)**	>2	>2
Candida dubliniensis (49 isolates)	3 (6.1)	**26 (59.2)**	19 (98.0)	1 (100.0)											0.004	0.008
Candida lusitaniae (39 isolates)			0 (0.0)	**11 (28.2)**	9 (51.3)	**11 (79.5)**	6 (94.9)	1 (97.4)	1 (100.0)						0.03	0.12
Candida kefyr (13 isolates)					0 (0.0)	**5 (38.5)**	4 (69.2)	2 (84.6)	2 (100.0)						0.12	0.5
Other *Candida* spp. (40 isolates)	3 (7.5)	**11 (35.0)**	10 (60.0)	**11 (87.5)**	2 (92.5)	3 (100.0)									0.008	0.03
Cryptococcus neoformans var. *grubii* (30 isolates)				0 (0.0)	2 (6.7)	3 (16.7)	7 (40.0)	7 (63.3)	**8 (90.0)**	1 (93.3)	2 (100.0)				0.25	0.5
Other yeasts (36 isolates)	0 (0.0)	1 (2.8)	1 (5.6)	4 (16.7)	**9 (41.7)**	2 (47.2)	1 (50.0)	2 (55.6)	4 (66.7)	3 (75.0)	1 (77.8)			8 (100.0)	0.12	>2
Aspergillus spp. (251 isolates)			24 (9.6)	**170 (77.3)**	54 (98.8)	2 (99.6)	0 (99.6)	1 (100.0)							0.015	0.03
Aspergillus fumigatus (182 isolates)			2 (1.1)	**144 (80.2)**	35 (99.5)	1 (100.0)									0.015	0.03
Aspergillus section *Flavi* (18 isolates)			3 (16.7)	6 (50.0)	**8 (94.4)**	1 (100.0)									0.015	0.03
Aspergillus section *Nigri* (23 isolates)			**17 (73.9)**	5 (95.7)	1 (100.0)										≤0.008	0.015
Aspergillus section *Terrei* (10 isolates)			0 (0.0)	**7 (70.0)**	3 (100.0)										0.015	0.03
Other *Aspergillus* spp. (18 isolates)			2 (11.1)	**8 (55.6)**	7 (94.4)	0 (94.4)	0 (94.4)	1 (100.0)							0.015	0.03
Other molds (49 isolates)			7 (14.3)	6 (26.5)	8 (42.9)	**9 (61.2)**	1 (63.3)	0 (63.3)	0 (63.3)	2 (67.3)	3 (73.5)	2 (77.6)	6 (89.8)	5 (100.0)	0.06	>8

[a]Twenty-four-hour MICs were recorded for *Candida* spp., 48-h MICs were recorded for other yeasts and *C. neoformans* var. *grubii*, 72-h MECs were recorded for *Scedosporium* spp., and 48-h MECs were recorded for *Aspergillus* spp. and other molds.

[b]Numbers in bold are modal MIC values.

[c]Greater than the highest dilution tested.

molds (Table 1; also see Tables S1 to S4). The geographic distribution was as follows: 37.3% of the isolates were from North America, 43.4% from Europe, 12.7% from the Asia-Pacific region, and 6.6% from Latin America (data not shown).

Overall activity of APX001A against *Candida* sp. and *Cryptococcus neoformans* var. *grubii* isolates. Among the 8 species of *Candida* shown in Table 1, APX001A was most active against *Candida albicans* (MIC_{90}, 0.008 μg/ml) and *Candida dubliniensis* (MIC_{90}, 0.008 μg/ml) and least active against *Candida kefyr* (MIC_{90}, 0.5 μg/ml) and *Candida krusei* (MIC_{90}, >2 μg/ml). The upper limit (UL) of the APX001A wild-type (WT) MIC distribution (WT-UL; two 2-fold dilutions higher than the modal MIC value) was 0.015 μg/ml for *C. dubliniensis* (100.0% WT), 0.03 μg/ml for *C. albicans* (100.0% WT), 0.03 μg/ml for *Candida parapsilosis* (98.9% WT), 0.06 μg/ml for *Candida tropicalis* (100.0% WT), and 0.25 μg/ml for *Candida glabrata* (100.0% WT) (Table 1). The WT-UL MIC values for *C. kefyr* and *Candida lusitaniae* could not be determined due to the lack of a clear mode among the isolates tested. *C. krusei* (MIC_{50}, >2 μg/ml; MIC_{90}, >2 μg/ml) is considered intrinsically resistant to APX001A (14). Overall, 91.7% of the *Candida* sp. isolates tested were inhibited by ≤0.06 μg/ml of APX001A and 96.6% were inhibited by ≤0.25 μg/ml of APX001A (Table 1). APX001A showed a broad MIC distribution (seven 2-fold dilution steps; range, 0.03 to 2 μg/ml), with no clear mode against 30 *C. neoformans* var. *grubii* isolates; 90.0% were inhibited at a concentration of ≤0.5 μg/ml (MIC_{50}, 0.25 μg/ml; MIC_{90}, 0.5 μg/ml) (Table 1).

***In vitro* activity of APX001A and comparators against *Candida* sp. and *Cryptococcus neoformans* var. *grubii* isolates.** All 414 *C. albicans* isolates tested were inhibited by ≤0.03 μg/ml of APX001A (100.0% WT; MIC_{50}, 0.008 μg/ml; MIC_{90}, 0.008 μg/ml), and 99.8% of the isolates were susceptible to the echinocandins (anidulafungin and micafungin), using current CLSI breakpoint interpretive criteria (35) (Tables 1 and 2). All except 2 *C. albicans* isolates were susceptible to fluconazole (99.5%), all were susceptible to voriconazole (100.0%), and 93.2% had WT susceptibility (MIC, ≤0.06 μg/ml) to posaconazole (Table 2). Among 3 isolates displaying echinocandin MIC values greater than the epidemiological cutoff value (ECV) that were screened for the presence of *fks* hot spot (HS) mutations, all 3 displayed amino acid substitutions (*fks1* HS1 R647G, *fks1* HS1 S645P, and *fks1* HS2 R1361H) (Table 3). The corresponding APX001A MIC values were 0.008 μg/ml for all 3 strains (Table 3).

Among 321 *C. glabrata* isolates tested, 100.0% were inhibited by APX001A (MIC_{50}, 0.06 μg/ml; MIC_{90}, 0.12 μg/ml) at the WT-UL MIC cutoff value of ≤0.25 μg/ml (Tables 1 and 2). Micafungin (MIC_{50}, 0.015 μg/ml; MIC_{90}, 0.03 μg/ml) and anidulafungin (MIC_{50}, 0.06 μg/ml; MIC_{90}, 0.12 μg/ml) inhibited 96.9% and 94.4% of these isolates, respectively, at the current CLSI breakpoints for these compounds (35). Among 15 *C. glabrata* isolates displaying echinocandin MIC values greater than the ECV that were screened for the presence of *fks* HS mutations, 10 harbored amino acid substitutions (Table 3). The most common substitutions were *fks2* HS1 S663P (5 isolates) and *fks2* HS1 Y657_Y657 del F658Y (3 isolates). Among the echinocandin-nonsusceptible isolates with *fks* mutations, 3 were from the United States (2.1% of North American *C. glabrata* isolates), 6 were from Europe (4.4% of European *C. glabrata* isolates), and 1 was from Mexico (Table 3). The corresponding APX001A MIC values ranged from 0.008 to 0.12 μg/ml (all below the WT-UL cutoff value) for all 10 isolates (Table 3). A total of 8.4% of the *C. glabrata* isolates from 2017 were categorized as resistant to fluconazole; 7.2% and 11.2% had non-wild-type (NWT) susceptibility to posaconazole and voriconazole, respectively, using the ECVs published by the CLSI (36) (Table 2).

Among 270 *C. parapsilosis* isolates, 98.9% were inhibited by APX001A (MIC_{50}, 0.008 μg/ml; MIC_{90}, 0.015 μg/ml) at the WT-UL cutoff value of ≤0.03 μg/ml (Tables 1 and 2). Micafungin (MIC_{50}, 1 μg/ml; MIC_{90}, 1 μg/ml) and anidulafungin (MIC_{50}, 2 μg/ml; MIC_{90}, 2 μg/ml) inhibited 100.0% and 93.7% of these isolates, respectively, at the current CLSI breakpoint for these compounds; 6.3% had intermediate susceptibility to anidulafungin (MIC, 4 μg/ml) (Table 2). None of the *C. parapsilosis* isolates displayed MIC values above the ECV for the echinocandins. Fluconazole and voriconazole were

TABLE 2 Activity of APX001A and comparator agents tested against *Candida* spp. and *Cryptococcus neoformans* using the CLSI broth microdilution method

Organism and antifungal agent	MIC (μg/ml)			CLSI category (%)[a]			ECV category (%)[b]	
	MIC_{50}	MIC_{90}	Range	S	I/SDD	R	WT	NWT
C. albicans (414 isolates tested)								
APX001A	0.008	0.008	≤0.002–0.03				100.0	0.0
Anidulafungin	0.015	0.03	≤0.008–1	99.8	0.0	0.2	99.8	0.2
Micafungin	0.015	0.015	≤0.008–2	99.8	0.0	0.2	99.3	0.7
Fluconazole	≤0.12	0.25	≤0.12–8	99.5	0.2	0.2	98.8	1.2
Posaconazole	0.03	0.06	≤0.008–0.25				93.2	6.8
Voriconazole	≤0.008	0.015	≤0.008–0.12	100.0	0.0	0.0	99.8	0.2
Amphotericin B	0.5	1	0.25–1				100.0	0.0
C. glabrata (321 isolates tested)								
APX001A	0.06	0.12	0.004–0.25				100.0	0.0
Anidulafungin	0.06	0.12	0.015–4	94.4	2.2	3.4	96.6	3.4
Micafungin	0.015	0.03	≤0.008–2	96.9	0.3	2.8	95.0	5.0
Fluconazole	4	32	≤0.12–>128	91.6		8.4	88.5	11.5
Posaconazole	0.5	1	0.03 –>4				92.8	7.2
Voriconazole	0.06	1	≤0.008–>4				88.8	11.2
Amphotericin B	1	1	0.25–2				100.0	0.0
C. parapsilosis (270 isolates tested)								
APX001A	0.008	0.015	≤0.002–0.06				98.9	1.1
Anidulafungin	2	2	0.015–4	93.7	6.3	0.0	100.0	0.0
Micafungin	1	1	0.015–2	100.0	0.0	0.0	100.0	0.0
Fluconazole	0.25	2	≤0.12–32	90.7	2.2	7.0	88.9	11.1
Posaconazole	0.06	0.12	0.015–0.5				99.6	0.4
Voriconazole	≤0.008	0.06	≤0.008–0.5	93.3	6.7	0.0	89.3	10.7
Amphotericin B	0.5	1	0.5–1				100.0	0.0
C. tropicalis (151 isolates tested)								
APX001A	0.015	0.03	0.004–0.06				100.0	0.0
Anidulafungin	0.03	0.06	≤0.008–0.25	100.0	0.0	0.0	98.7	1.3
Micafungin	0.03	0.06	≤0.008–0.12	100.0	0.0	0.0	97.4	2.6
Fluconazole	0.25	0.5	≤0.12–32	98.0	0.0	2.0	96.7	3.3
Posaconazole	0.06	0.12	0.015–0.25				90.7	9.3
Voriconazole	0.015	0.06	≤0.008–1	98.0	1.3	0.7	98.0	2.0
Amphotericin B	0.5	1	0.5–1				100.0	0.0
C. krusei (43 isolates tested)								
APX001A	>2	>2	>2–>2					
Anidulafungin	0.06	0.12	0.015–0.12	100.0	0.0	0.0	100.0	0.0
Micafungin	0.06	0.12	≤0.008–0.12	100.0	0.0	0.0	100.0	0.0
Fluconazole	32	32	16–64					
Posaconazole	0.5	0.5	0.06–0.5				100.0	0.0
Voriconazole	0.25	0.5	0.12–1	97.7	2.3	0.0	97.7	2.3
Amphotericin B	1	1	1–2				100.0	0.0
C. dubliniensis (49 isolates tested)								
APX001A	0.004	0.008	≤0.002–0.015				100.0	0.0
Anidulafungin	0.03	0.12	0.015–0.12				100.0	0.0
Micafungin	0.015	0.03	≤0.008–0.03				100.0	0.0
Fluconazole	≤0.12	0.25	≤0.12–2				98.0	2.0
Posaconazole	0.03	0.06	0.015–0.12					
Voriconazole	≤0.008	0.015	≤0.008–0.03					
Amphotericin B	0.25	0.5	≤0.12–0.5					
C. lusitaniae (39 isolates tested)								
APX001A	0.03	0.12	0.015–0.5					
Anidulafungin	0.25	0.5	0.12–0.5				100.0	0.0
Micafungin	0.12	0.25	0.06–0.5				100.0	0.0
Fluconazole	0.25	2	≤0.12–64				89.7	10.3
Posaconazole	0.06	0.12	0.03–0.25				64.1	35.9
Voriconazole	≤0.008	0.015	≤0.008–0.5					
Amphotericin B	0.5	1	0.25–1					

(Continued on next page)

TABLE 2 (Continued)

Organism and antifungal agent	MIC (μg/ml)			CLSI category (%)[a]			ECV category (%)[b]	
	MIC_{50}	MIC_{90}	Range	S	I/SDD	R	WT	NWT
C. kefyr (13 isolates tested)								
APX001A	0.12	0.5	0.06–0.5					
Anidulafungin	0.06	0.12	0.03–0.12					
Micafungin	0.03	0.06	0.03–0.06					
Fluconazole	0.25	0.25	≤0.12–0.5					
Posaconazole	0.12	0.12	0.06–0.25					
Voriconazole	≤0.008	≤0.008	≤0.008–0.015					
Amphotericin B	1	1	0.5–1					
Cryptococcus neoformans var. grubii (30 isolates tested)								
APX001A	0.25	0.5	0.03–2					
Anidulafungin	>4	>4	2–>4					
Micafungin	>4	>4	4–>4					
Fluconazole	2	4	0.5–4				100.0	0.0
Posaconazole	0.12	0.25	0.03–0.25				100.0	0.0
Voriconazole	0.03	0.06	0.015–0.12				100.0	0.0
Amphotericin B	0.5	1	0.25–1				61.5	38.5

[a]CLSI breakpoint criteria for susceptible (S), intermediate/susceptible dose-dependent (I/SDD), and resistant (R).
[b]The WT-UL MIC value was used in place of the ECV to designate WT and NWT strains for APX001A.

active against 90.7% and 93.3%, respectively, of the *C. parapsilosis* isolates using the current CLSI breakpoint criteria; 99.6% had WT susceptibility to posaconazole (Table 2).

Against 151 *C. tropicalis* isolates, APX001A (MIC_{50}, 0.015 μg/ml; MIC_{90}, 0.03 μg/ml) (Tables 1 and 2), anidulafungin (MIC_{50}, 0.03 μg/ml; MIC_{90}, 0.06 μg/ml), and micafungin (MIC_{50}, 0.03 μg/ml; MIC_{90}, 0.06 μg/ml) displayed comparable activities. All *C. tropicalis* isolates had WT susceptibility to APX001A (WT-UL cutoff value, 0.06 μg/ml), and both echinocandins inhibited 100.0% of the tested isolates at the current CLSI breakpoints (Table 2). Fluconazole and voriconazole inhibited 98.0% of these isolates according to current CLSI breakpoint criteria. Among 5 *C. tropicalis* isolates displaying echinocandin MIC values greater than the ECV, none screened positive for the presence of *fks* HS mutations (36).

APX001A MIC values were >2 μg/ml for all 43 *C. krusei* isolates tested, and all were considered susceptible to the echinocandins (Tables 1 and 2). All isolates had WT susceptibility to posaconazole, and 97.7% were susceptible to voriconazole (Table 2).

Among other *Candida* species, APX001A was more active against *C. dubliniensis* (MIC_{50}, 0.004 μg/ml; MIC_{90}, 0.008 μg/ml; 100.0% WT), compared to *C. lusitaniae* (MIC_{50}, 0.03 μg/ml; MIC_{90}, 0.12 μg/ml) and *C. kefyr* (MIC_{50}, 0.12 μg/ml; MIC_{90}, 0.5 μg/ml)

TABLE 3 *In vitro* activity of APX001A and echinocandin comparators against *Candida* sp. isolates with *fks* alterations

Isolate	Country	Organism	MIC (μg/ml)[a]			*fks* alteration(s)[b]			
			APX001A	ANF	MCF	*fks1* HS1	*fks1* HS2	*fks2* HS1	*fks2* HS2
997524	Mexico	*C. glabrata*	0.06	0.5	0.06	S625S	WT	WT	WT
1003214	USA	*C. glabrata*	0.12	2	1	WT	WT	S663P	WT
1015009	Spain	*C. glabrata*	0.06	1	0.25	WT	WT	Y657_Y657del F658Y	WT
1020417	USA	*C. glabrata*	0.06	1	0.25	WT	WT	Y657_Y657del F658Y	WT
1025460	USA	*C. glabrata*	0.06	1	1	S629P	WT	R665G	WT
1025998	Hungary	*C. albicans*	0.008	0.016	0.06	R647G	WT	NT	NT
1026179	Spain	*C. glabrata*	0.03	1	0.25	WT	WT	Y657_Y657del, F658Y	WT
1027877	Italy	*C. albicans*	0.008	1	2	S645P	WT	NT	NT
1034513	Ireland	*C. glabrata*	0.06	4	0.5	WT	WT	S663P	WT
1034514	Ireland	*C. glabrata*	0.016	0.5	0.12	WT	WT	S663P	WT
1034515	Ireland	*C. glabrata*	0.008	0.12	0.06	WT	WT	S663P	WT
1039585	Turkey	*C. glabrata*	0.06	2	1	WT	WT	S663P	WT
1040838	USA	*C. albicans*	0.008	0.03	0.06	WT	R1361H	NT	NT

[a]ANF, anidulafungin; MCF, micafungin.
[b]NT, not tested.

(Tables 1 and 2). All isolates of *C. dubliniensis* and *C. lusitaniae* were classified as having WT susceptibility to anidulafungin (ECVs of 0.12 and 1 μg/ml, respectively) and micafungin (ECVs of 0.12 and 0.5 μg/ml, respectively) (Table 2). Four (10.3%) *C. lusitaniae* isolates and 1 (2.0%) *C. dubliniensis* isolate had NWT susceptibility to fluconazole (Table 2).

Among 30 *C. neoformans* var. *grubii* isolates, 90.0% were inhibited by APX001A (MIC_{50}, 0.25 μg/ml; MIC_{90}, 0.5 μg/ml) at a concentration of ≤0.5 μg/ml (Tables 1 and 2). All *C. neoformans* var. *grubii* isolates displayed WT MIC values for fluconazole, voriconazole, and posaconazole (Table 2). Given that echinocandins are commonly employed for empirical therapy, it is important to note that echinocandin activity was limited (MIC_{50}, >4 μg/ml; MIC_{90}, >4 μg/ml) against this species (Table 2).

Overall activity of APX001A against *Aspergillus* sp. isolates. The most common *Aspergillus* species (with ≥10 isolates overall) in the 2017 survey against which APX001A was tested included the following 4 *Aspergillus* species complexes, in order of frequency: *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus terreus*. The cumulative frequencies of minimal effective concentration (MEC) distributions for APX001A for these *Aspergillus* species are presented in Table 1.

APX001A exhibited comparable activities (MEC_{90}, 0.015 to 0.03 μg/ml) against all 4 species shown in Table 1. The UL of the APX001A WT MEC distribution (WT-UL; two 2-fold dilutions higher than the modal MEC value) was 0.03 μg/ml for *A. niger* (100.0% WT), 0.06 μg/ml for *A. fumigatus* and *A. terreus* (100.0% WT), and 0.12 μg/ml for *A. flavus* (100.0% WT) (Table 1). Overall, 99.6% of the *Aspergillus* sp. isolates tested exhibited a WT phenotype (WT-UL, ≤0.06 μg/ml) for APX001A (Table 1).

***In vitro* activity of APX001A and comparators against *Aspergillus* sp. isolates.** All 182 *A. fumigatus* isolates were inhibited by APX001A (MEC_{50}, 0.015 μg/ml; MEC_{90}, 0.03 μg/ml) and the echinocandins at ≤0.06 μg/ml (Table 4). These isolates displayed WT MEC/MIC results for APX001A (100.0%), itraconazole (98.4%), and voriconazole (98.4%); 90% were inhibited by ≤0.5 μg/ml of posaconazole (MIC_{50}, 0.25 μg/ml; MIC_{90}, 0.5 μg/ml) (Table 4). Three isolates (1.6%) had NWT susceptibility to itraconazole and voriconazole (MIC, ≥2 μg/ml).

A. flavus isolates ($n = 18$) were inhibited by APX001A (MEC_{50}, 0.015 μg/ml; MEC_{90}, 0.03 μg/ml) at ≤0.06 μg/ml (100.0% WT) (Tables 1 and 4), and this compound displayed activity similar to that of micafungin (MEC_{50}, 0.015 μg/ml; MEC_{90}, 0.03 μg/ml) and anidulafungin (MEC_{50}, 0.015 μg/ml; MEC_{90}, 0.03 μg/ml). All isolates of *A. flavus* had WT susceptibility to the mold-active azoles (Table 4).

All *A. niger* isolates ($n = 23$) were inhibited by APX001A (MEC_{50}, ≤0.008 μg/ml; MEC_{90}, 0.015 μg/ml) at ≤0.03 μg/ml (100.0% WT) (Tables 1 and 4), and this compound displayed activity similar to that of micafungin (MEC_{50}, 0.015 μg/ml; MEC_{90}, 0.03 μg/ml) and anidulafungin (MEC_{50}, ≤0.008 μg/ml; MEC_{90}, 0.015 μg/ml). All isolates of *A. niger* had WT susceptibility to the mold-active azoles (Table 4).

Ten *A. terreus* isolates were inhibited by APX001A (MEC_{50}, 0.015 μg/ml; MEC_{90}, 0.03 μg/ml) at ≤0.03 μg/ml (100.0% WT) (Tables 1 and 4), and this compound displayed activity similar to that of micafungin (MEC_{50}, ≤0.008 μg/ml; MEC_{90}, 0.015 μg/ml) and anidulafungin (MEC_{50}, 0.03 μg/ml; MEC_{90}, 0.03 μg/ml). All *A. terreus* isolates had WT susceptibility to the mold-active azoles (Table 4).

Activity of APX001A against rare species of *Candida*, non-*Candida* yeasts, and rare molds. The APX001A MIC/MEC results obtained for isolates grouped as other *Candida* spp. ($n = 40$), other yeasts ($n = 36$), other *Aspergillus* spp. ($n = 18$), and other molds ($n = 49$) are listed in Tables S1, S2, S3, and S4, respectively. Notably, APX001A was active against many of these less common and frequently antifungal (azole and/or echinocandin)-resistant fungi, including azole-resistant species of *Aspergillus* such as *Aspergillus lentulus* (MEC, 0.015 μg/ml) and *Aspergillus ustus* (MEC, ≤0.008 to 0.015 μg/ml) (Table S3), *Fusarium solani* species complex (MEC, 0.015 to 0.03 μg/ml) (Table S4), multidrug-resistant *Candida auris* (MIC, 0.06 μg/ml) (Table S1), and *Scedosporium* spp. (MEC, 0.015 to 0.06 μg/ml) (Table S4).

TABLE 4 Activity of APX001A and comparator antifungal agents tested against *Aspergillus* spp. using the CLSI broth microdilution method

Organism and antifungal agent	MIC/MEC (μg/ml)			CLSI category (%)[a]			ECV category (%)[b]	
	MIC_{50}/MEC_{50}	MIC_{90}/MEC_{90}	Range	S	I/SDD	R	WT	NWT
A. fumigatus (182 isolates tested)								
APX001A	0.015	0.03	≤0.008–0.06				100.0	0.0
Anidulafungin	0.015	0.03	≤0.008–0.03					
Micafungin	≤0.008	0.015	≤0.008–0.03					
Itraconazole	0.5	1	0.25–8				98.4	1.6
Posaconazole	0.25	0.5	0.06–1					
Voriconazole	0.25	0.5	0.06–4				98.4	1.6
Amphotericin B	1	2	0.25–2				100.0	0.0
A. flavus (18 isolates tested)								
APX001A	0.015	0.03	≤0.008–0.06				100.0	0.0
Anidulafungin	0.015	0.03	≤0.008–0.03					
Micafungin	0.015	0.03	≤0.008–0.03					
Itraconazole	0.5	1	0.25–1				100.0	0.0
Posaconazole	0.25	0.5	0.12 – 0.5				100.0	0.0
Voriconazole	0.5	1	0.25–1				100.0	0.0
Amphotericin B	2	2	1–2				100.0	0.0
A. niger (23 isolates tested)								
APX001A	≤0.008	0.015	≤0.008–0.03				100.0	0.0
Anidulafungin	≤0.008	0.015	≤0.008–0.03					
Micafungin	0.015	0.03	≤0.008–0.06					
Itraconazole	2	4	0.5–4				100.0	0.0
Posaconazole	0.5	1	0.25–1				100.0	0.0
Voriconazole	1	2	0.12–2				100.0	0.0
Amphotericin B	0.5	1	0.25–1				100.0	0.0
A. terreus (10 isolates tested)								
APX001A	0.015	0.03	0.015–0.03				100.0	0.0
Anidulafungin	0.03	0.03	0.015–0.06					
Micafungin	≤0.008	0.015	≤0.008–0.015					
Itraconazole	0.5	0.5	0.5–1				100.0	0.0
Posaconazole	0.25	0.25	0.25–0.5				100.0	0.0
Voriconazole	0.25	0.5	0.25–0.5				100.0	0.0
Amphotericin B	2	2	1–2				100.0	0.0

[a]CLSI breakpoint criteria for susceptible (S), intermediate/susceptible dose-dependent (I/SDD), and resistant (R).
[b]The WT-UL MEC was used in place of the ECV to designate WT and NWT strains for APX001A.

Overall activity of APX001A against 1,706 fungal isolates. APX001A demonstrated potent *in vitro* activity against 1,706 fungal isolates, similar to that of azoles and echinocandins when read under the same test/endpoint conditions, and showed excellent coverage for most common contemporary and geographically diverse isolates of *Candida* spp., including echinocandin-resistant isolates, and *Aspergillus* spp. tested as part of this study. Although azole and echinocandin resistance among *Candida* species other than *C. glabrata* remains uncommon, it is notable that both elevated MIC values (resistance or NWT susceptibility) and *fks* mutations were seen for isolates of *C. albicans* and *C. glabrata*. APX001A showed good activity against both echinocandin-susceptible and echinocandin-resistant *Candida* isolates. The extended spectrum of APX001A was also notable for its potency against many of the less common but antifungal-resistant fungi, such as *C. auris*, *A. lentulus*, *A. ustus*, *F. solani* species complex, and *Scedosporium* spp.

MATERIALS AND METHODS

Organisms. A total of 1,706 nonduplicate fungal isolates were collected from 68 medical centers in 24 countries in North America (637 isolates from 29 medical centers [9 U.S. census divisions]), Europe (740 isolates from 26 medical centers), the Asia-Pacific region (217 isolates from 9 medical centers), or Latin America (112 isolates from 4 medical centers). These isolates were recovered from patients with BSIs (968 isolates), RTIs (275 isolates), SSSIs (88 isolates), UTIs (39 isolates), IAIs (16 isolates), or infections at other sites (320 isolates).

Fungal identification methods. Yeast isolates either were subcultured on CHROMagar *Candida* medium (Becton, Dickinson, Sparks, MD, USA) upon arrival, to differentiate *C. albicans*, *C. tropicalis*, and *C. krusei*, or were subjected to MALDI–TOF MS using a MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA). Yeasts that were not identified by these methods were identified using sequencing-based methods involving the internal transcribed spacer (ITS) region, 28S ribosomal subunit, or intergenic spacer 1 (IGS1) (for *Trichosporon* spp.) (1, 34, 37, 38).

Molds were cultured and identified by MALDI–TOF MS or by DNA sequencing analysis when an acceptable identification was not achieved by MALDI–TOF MS. Sequencing was performed for 28S (all isolates) and one of the following genes: β-tubulin (for *Aspergillus* spp.), translation elongation factor (TEF) (for *Fusarium* spp.), or the ITS (for all other species of filamentous fungi) (1, 34, 37, 38).

Nucleotide sequences were analyzed using Lasergene software (DNAStar, Madison, WI, USA) and compared to available sequences using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). TEF sequences were analyzed using the *Fusarium* multilocus sequence typing database (http://www.westerdijkinstitute.nl/fusarium).

Susceptibility testing. All isolates were tested by the broth microdilution method, as described in CLSI documents M27 (39) and M38 (40). Frozen-form panels used RPMI 1640 broth supplemented with MOPS (morpholinepropane sulfonic acid) and 0.2% glucose and were inoculated with suspensions of 0.5×10^3 to 2.5×10^3 cells/ml. APX001A MIC/MEC values were determined visually, after incubation at 35°C for 24 h (*Candida* spp. [MIC]) or 48 to 72 h (*Aspergillus* spp., 48 h [MEC]; other molds [*Scedosporium* spp.], 72 h [MEC]; other yeasts, 48 h [MIC]; *C. neoformans*, 48 h [MIC]).

MIC endpoints for yeasts were read as the lowest concentration of drug that caused a significant decrease (≥50% inhibition) in growth below control levels (for APX001A [18, 41], fluconazole, posaconazole, voriconazole, and the echinocandins) or as the concentration that prevented any discernible growth (for amphotericin B) (35, 39). MIC endpoints for molds were read as the lowest concentration of drug that prevented any discernible growth (for amphotericin B, posaconazole, voriconazole, and itraconazole) (40, 42). MEC endpoints (morphology change from flocculent growth to small matted colonies) were read for APX001A and the echinocandins (16, 19, 40, 42).

Interpretive criteria (clinical breakpoints and ECVs, where available) were those published in CLSI documents M27 (39), M38 (40), M59 (36), M60 (35), and M61 (42). Neither clinical breakpoints nor ECVs have been determined for APX001A or any fungal species. For comparison purposes, we used a WT-UL value (two 2-fold dilutions higher than the modal MIC value of each MIC distribution) as the cutoff value to define the WT (MICs at or below the WT-UL value) and NWT (MICs above the WT-UL value) populations for APX001A and each species (43–45).

Quality control (QC) was performed as recommended in CLSI documents M27 and M38 (39, 40), using *C. parapsilosis* ATCC 22019, *Aspergillus flavus* ATCC 204304, and *Aspergillus fumigatus* ATCC MYA-3626. All MIC/MEC values for APX001A against *C. parapsilosis* ATCC 22019, *A. flavus* ATCC 204304, and *A. fumigatus* ATCC MYA-3626 were within QC ranges approved at the January 2018 CLSI meeting.

Echinocandin resistance mechanisms. *Candida* sp. isolates showing echinocandin MIC values above ECVs were subjected to whole-genome sequencing (29). Specifically, total genomic DNA was used as the input material for library construction using the Nextera XT library construction protocol and index kit (Illumina, San Diego, CA, USA), following the manufacturer's instructions. Sequencing was performed on a MiSeq Sequencer (Illumina). Reads were error corrected using BayesHammer. Each sample was assembled using a reference-guided assembly in DNAStar SeqMan NGen v.14.0. DNA regions encoding *fks* were compared to sequences available in the literature.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00840-19.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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