Activities of E1210 and Comparator Agents Tested by CLSI and EUCAST Broth Microdilution Methods against *Fusarium* and *Scedosporium* Species Identified Using Molecular Methods

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Fusarium ($n = 67$) and *Scedosporium* ($n = 63$) clinical isolates were tested by two reference broth microdilution (BMD) methods against a novel broad-spectrum (active against both yeasts and molds) antifungal, E1210, and comparator agents. E1210 inhibits the inositol acylation step in glycosylphosphatidylinositol (GPI) biosynthesis, resulting in defects in fungal cell wall biosynthesis. Five species complex organisms/species of *Fusarium* (4 isolates unspciated) and 28 *Scedosporium apiospermum*, 7 *Scedosporium aurantiacum*, and 28 *Scedosporium prolificans* species were identified by molecular techniques. Comparator antifungal agents included anidulafungin, caspofungin, itraconazole, posaconazole, voriconazole, and amphotericin B. E1210 was highly active against all of the tested isolates, with minimum effective concentration (MEC)/MIC₉₀ values ($\mu\text{g/ml}$) for E1210, anidulafungin, caspofungin, itraconazole, posaconazole, voriconazole, and amphotericin B, respectively, for *Fusarium* of 0.12, >16, >16, >8, >8, 8, and 4 $\mu\text{g/ml}$. E1210 was very potent against the *Scedosporium* spp. tested. The E1210 MEC₉₀ was 0.12 $\mu\text{g/ml}$ for *S. apiospermum*, but 1 to >8 $\mu\text{g/ml}$ for other tested agents. Against *S. aurantiacum*, the MEC₅₀ for E1210 was 0.06 $\mu\text{g/ml}$ versus 0.5 to >8 $\mu\text{g/ml}$ for the comparators. Against *S. prolificans*, the MEC₉₀ for E1210 was only 0.12 $\mu\text{g/ml}$, compared to >4 $\mu\text{g/ml}$ for amphotericin B and >8 $\mu\text{g/ml}$ for itraconazole, posaconazole, and voriconazole. Both CLSI and EUCAST methods were highly concordant for E1210 and all comparator agents. The essential agreement (EA; ± 2 doubling dilutions) was >93% for all comparisons, with the exception of posaconazole and *F. oxysporum* species complex (SC) (60%), posaconazole and *S. aurantiacum* (85.7%), and voriconazole and *S. aurantiacum* (85.7%). In conclusion, E1210 exhibited very potent and broad-spectrum antifungal activity against azole- and amphotericin B-resistant strains of *Fusarium* spp. and *Scedosporium* spp. Furthermore, *in vitro* susceptibility testing of E1210 against isolates of *Fusarium* and *Scedosporium* may be accomplished using either of the CLSI or EUCAST BMD methods, each producing very similar results.

The *Fusarium* spp. and *Scedosporium* spp. are among the most antifungal drug-resistant organisms encountered in clinical practice (1, 2, 7, 21, 25, 32, 38). Optimal treatment against infection with these fungal pathogens has not been established, and anecdotal successes have been reported with various agents alone or in combination (4, 12, 14, 18, 20, 23, 26, 33, 34, 40).

Among more than 60 species of *Fusarium* associated with human infections (24, 35), *Fusarium solani* is the most frequent (~50% of cases), followed by *F. oxysporum* (~20%) and *F. verticilloides* (~10%) (21, 22). *F. solani* is also the most common species in fusarial keratitis (5, 9, 24, 25). Molecular phylogenetic studies have shown that the more commonly reported fusarial pathogens represent species complexes and cannot be identified to the species level by traditional morphological methods alone (21, 24, 25, 38). DNA sequence-based molecular identification tools are increasingly used to enable accurate species determination (3, 25, 36).

The typical *in vitro* antifungal susceptibility profile of *Fusarium* spp. demonstrates relative resistance to most antifungal agents (1, 21, 25, 32). Notably, some species may exhibit different patterns of susceptibility: *F. solani* and *F. verticilloides* are usually resistant to azoles and exhibit higher amphotericin B MIC values than other species, whereas *F. oxysporum* may be susceptible to voriconazole and posaconazole (1, 21, 32). The echinocandins are not active against *Fusarium* spp. (25).

Fungi of the genus *Scedosporium* (teleomorph *Pseudallescheria*) include members of the *Pseudallescheria boydii* species com-

plex (SC) (*Scedosporium aurantiacum*, *S. apiospermum*, and *S. boydii*) as well as *S. prolificans* (7, 11, 17). *S. apiospermum* is generally considered to be resistant to amphotericin B, the MIC values of which are elevated, and the clinical response is very poor despite the use of high-dose regimens (7). The extended-spectrum azoles are active *in vitro* against *S. apiospermum*, and both posaconazole and voriconazole have been used in the treatment of central nervous system (CNS) abscesses (18, 20). *S. prolificans* is considered to be resistant to virtually all of the systemically active antifungal agents, including the extended-spectrum azoles and the echinocandins (7, 32). Synergistic activity between azoles and terbinafine against *S. prolificans* has been demonstrated *in vitro* (7), and patients with invasive fungal infection (IFI) due to *S. prolificans* have been successfully managed with a combination of terbinafine and voriconazole in addition to aggressive surgical debridement and immune reconstitution (4, 12, 14, 34, 40). As with *Fusarium* spp., the identification of the various species of *Scedosporium* is best

Received 29 July 2011 Returned for modification 27 September 2011

Accepted 30 October 2011

Published ahead of print 14 November 2011

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doi:10.1128/AAC.05414-11

accomplished using molecular identification methods (3, 7, 11, 17, 35).

E1210 (Eisai, Ltd., Japan) is a novel first-in-class broad-spectrum antifungal agent (with activity against yeasts and molds) that was designed to specifically inhibit the function of the protein GWT1, which plays an important role in fungal cell wall assembly (19). E1210 inhibits the inositol acylation step in fungal glycosphosphatidylinositol (GPI) biosynthesis, resulting in defects in various steps in cell wall biosynthesis with accompanying inhibition of cell wall growth, hyphal elongation, and attachment of fungal cells to biological substrates (19, 36, 37, 39). Preliminary data using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution (BMD) method (6) have demonstrated excellent potency and spectrum against various species of *Fusarium* and *Scedosporium* (19). These studies were limited by the small number of molecularly identified isolates tested, lack of antifungal comparators, and a lack of comparison of E1210 activity as determined by the reference BMD methods of the CLSI and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (6, 10).

In the present study, we have used a panel of *Fusarium* spp. and *Scedosporium* spp., all of which have been identified by molecular methods, to examine the activities of E1210 and comparator agents as determined by both CLSI and EUCAST BMD methods.

MATERIALS AND METHODS

Organisms. A total of 130 fungal isolates initially identified as *Fusarium* spp. (67 strains) and *Scedosporium* spp. (63 strains) were included in the study. This collection comprises all viable *Fusarium* isolates from two global survey collections: the ARTEMIS (27) and SENTRY (30) surveillance program collections. The *Scedosporium* isolates were also obtained from the ARTEMIS and SENTRY collections, as well as strains kindly offered by colleagues (see Acknowledgments). All experiments were carried out in the Mycology and Molecular Research Sections of JMI Laboratories (North Liberty, IA).

Species identification. All isolates were subcultured from storage and identified to the species or species complex (SC) level by molecular methods. DNA extractions were performed using the QIAquick extraction kit (Qiagen, Hilden, Germany), and amplification followed by sequencing of the translation elongation factor (TEF) (EF-1 α ; for *Fusarium* spp. only) and/or internal transcribed spacer (ITS) was carried out as described previously (3, 11, 16, 25). Nucleotide and amino acid sequences were analyzed using Lasergene software (DNASTar, Madison, WI) and compared to available sequences through the internet using BLAST (<http://www.ncbi.nlm.nih.gov/blast/>; for *Scedosporium* spp.), the FUSARIUM-ID database (<http://isolate.fusariumdb.org/index.php>), and/or the *Fusarium* multilocus sequence typing (MLST) database (<http://www.cbs.knaw.nl/fusarium/BiolomicsInfo.aspx>).

Antifungal susceptibility testing. All isolates were tested for *in vitro* susceptibility to E1210 and comparators using the CLSI and EUCAST BMD methods (6, 10). E1210 reference powder was obtained from the manufacturer (Eisai, Ltd.). Stock solutions were prepared in dimethyl sulfoxide, and the final range of E1210 concentrations tested was 0.008 to 16 μ g/ml. CLSI BMD testing was performed as outlined in document M38-A2 (6) by using RPMI 1640 medium with 0.2% glucose in round-bottom microdilution trays, an inoculum of 0.4×10^4 to 5×10^4 CFU/ml, and incubation at 35°C for 48 h (*Fusarium* spp.) or 72 h (*Scedosporium* spp.). MIC values for the triazoles and amphotericin B were determined visually as the lowest concentration of drug that caused complete inhibition of growth (first clear well) relative to that of the growth control. Minimum effective concentration (MEC) values for E1210, anidulafungin, and caspofungin were determined as described previously (19, 28, 29) and by the CLSI (6). The MEC endpoint was chosen for E1210 due to the

fact that, similar to the echinocandins, E1210 inhibits cell wall synthesis and hyphal extension of filamentous fungi resulting in aberrant hyphal growth (short, stubby, highly branched hyphae), but not complete growth inhibition (19). The use of the MEC endpoint was demonstrated by Miyazaki et al. (19), who found in testing E1210 and micafungin against *Aspergillus* and other molds (*Fusarium* and *Scedosporium*) that as the drug concentration increased, both E1210 and micafungin induced white and small colonies attached to the bottom of the microdilution plate that persisted throughout the dilution range. Using a complete inhibition MIC endpoint, such as that for the azoles, would make E1210 (and micafungin) appear less potent, when in fact it is quite active *in vivo* against these molds (13).

EUCAST BMD testing was performed as detailed by EUCAST (10) by using RPMI 1640 medium with 2.0% glucose, flat-bottom microdilution trays, an inoculum of 2×10^5 to 5×10^5 CFU/ml, and incubation at 35°C. MIC (triazoles and amphotericin B) and MEC (E1210 and echinocandins) results were determined visually after 48 h (*Fusarium* spp.) or 72 h (*Scedosporium* spp.) of incubation, as described above and by both CLSI (6) and EUCAST (10).

Quality control (QC) was ensured by testing the following strains recommended by CLSI (6) and by EUCAST (8): *C. parapsilosis* ATCC 22019, *A. flavus* ATCC 204304, and *A. fumigatus* ATCC MYA-3626. The E1210 MIC/MEC values for the QC strains obtained by the CLSI and EUCAST methods, respectively, were 0.015 to 0.06 and 0.03 to 0.12 μ g/ml for *A. fumigatus* ATCC 3626, 0.03 to 0.06 and 0.03 to 0.06 μ g/ml for *A. flavus* ATCC 204304, and ≤ 0.008 to 0.015 and ≤ 0.008 to 0.015 μ g/ml for *C. parapsilosis* ATCC 22019.

Analysis. The MIC/MEC results for each antifungal agent obtained by the EUCAST method were compared to those obtained by the CLSI BMD method. High off-scale MIC/MEC results were converted to the next highest concentration, and low off-scale MIC/MEC results were left unchanged. Discrepancies of more than $\pm 2 \log_2$ dilutions among MIC/MEC results were used to calculate the essential agreement (EA) between the two methods.

RESULTS AND DISCUSSION

The molecular identification of 67 *Fusarium* isolates was performed by TEF sequencing, and analysis was performed using the FUSARIUM-ID and Fusarium MLST databases. ITS and 28S sequencing was performed in some instances to confirm TEF results. Isolates were identified to the SC level, as follows: *Gibberella* (*Fusarium*) *fujikuroi* SC (30 strains), *Fusarium solani* SC (15 strains), *Fusarium oxysporum* SC (15 strains), *Fusarium dimerum* SC (two strains), and *Fusarium incarnatum-equisetii* SC (one strain). Four isolates could not be identified to the SC level and remained as *Fusarium* spp. "NOS" (not otherwise specified). TEF, ITS and 28S sequences for these four isolates had similarity below 98% to sequences deposited in the international databases.

Scedosporium isolates (63 strains) were identified using ITS and/or 28S sequencing, and among 63 isolates, 28 were *Scedosporium apiospermum*, 28 were *S. prolificans*, and 7 were *S. aurantiacum*. Table 1 summarizes the *in vitro* susceptibilities of the 130 isolates of *Fusarium* spp. and *Scedosporium* spp. to E1210 and six comparators as determined by CLSI and EUCAST BMD methods. Overall, E1210 was highly active against all *Fusarium* spp. tested (67 strains): MEC_{50/90} of 0.06/0.12 μ g/ml for the CLSI method and 0.06/0.25 for the EUCAST method (Table 1). The activity of this antifungal agent was higher than the activities noted for anidulafungin, caspofungin, itraconazole, posaconazole, voriconazole, and amphotericin B (MEC/MIC₉₀ values [CLSI and EUCAST] of >8 , >8 , >8 , >8 , >8 , and 4 μ g/ml, respectively).

F. solani SC strains (MEC_{50/90} [CLSI], 0.03/0.06 μ g/ml) were slightly more susceptible to E1210 than isolates identified as *Gibberella fujikuroi* SC and *F. oxysporum* SC (MEC_{50/90} [CLSI], 0.03/

TABLE 1 *In vitro* susceptibilities of *Fusarium* and *Scedosporium* spp. to E1210 and comparators as determined by the CLSI and EUCAST broth microdilution methods^a

Species (no. tested)	Antifungal agent	Test method	No. of isolates at MIC/MEC ($\mu\text{g/ml}$) of:												%EA ^b
			0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8	
<i>G. fujikuroi</i> SC (30)	E1210	EUCAST	1	1	11	7	3	7							96.7
		CLSI		7	8	11	2	2							
	Caspofungin	EUCAST												30	100.0
		CLSI												30	
	Anidulafungin	EUCAST												30	100.0
		CLSI												30	
	Itraconazole	EUCAST											3	27	93.3
		CLSI									2	4	2	22	
<i>F. solani</i> SC (15)	Posaconazole	EUCAST							1	4	8	5	1	11	96.7
		CLSI						4	9	5	2			10	
	Voriconazole	EUCAST									4	9	15	2	93.3
		CLSI								1	12	10	7		
	Amphotericin B	EUCAST							1	7	21	1	— ^c	—	100.0
		CLSI								2	15	13	—	—	
	E1210	EUCAST			3	4	8								93.3
		CLSI		2	8	5									
<i>F. oxysporum</i> SC (15)	Caspofungin	EUCAST												15	100.0
		CLSI												15	
	Anidulafungin	EUCAST												15	100.0
		CLSI												15	
	Itraconazole	EUCAST												15	100.0
		CLSI											1	14	
	Posaconazole	EUCAST								1				14	100.0
		CLSI								1				14	
<i>F. oxysporum</i> SC (15)	Voriconazole	EUCAST									1	2	7	5	100.0
		CLSI									1	5	5	4	
	Amphotericin B	EUCAST							3	5	6	1	—	—	100.0
		CLSI								4	8	3	—	—	
	E1210	EUCAST			2	8	4	1							93.3
		CLSI		2	3	5	1	3	1						
	Caspofungin	EUCAST												15	100.0
		CLSI												15	
<i>F. oxysporum</i> SC (15)	Anidulafungin	EUCAST												15	100.0
		CLSI												15	
	Itraconazole	EUCAST												15	100.0
		CLSI												15	
	Posaconazole	EUCAST									4	4		7	60.0
		CLSI								1	13			1	
	Voriconazole	EUCAST										8	5	2	100.0
		CLSI									3	12			
<i>F. oxysporum</i> SC (15)	Amphotericin B	EUCAST								2	12	1	—	—	100.0
		CLSI									8	7	—	—	
	E1210	EUCAST	2	1	16	21	17	10							94.0
		CLSI		11	19	26	5	5	1						
	Caspofungin	EUCAST												67	100.0
		CLSI												67	
	Anidulafungin	EUCAST												67	100.0
		CLSI												67	
<i>Fusarium</i> spp. (67) ^c	Itraconazole	EUCAST											3	64	95.5
		CLSI									3	5	4	55	
	Posaconazole	EUCAST							2	6	13	9	1	36	88.1
		CLSI							4	13	19	2		29	
	Voriconazole	EUCAST									6	24	27	10	98.5
		CLSI								2	18	29	13	5	
	Amphotericin B	EUCAST						1	6	15	42	3	—	—	100.0
		CLSI								9	34	24	—	—	

Continued on following page

TABLE 1 (Continued)

Species (no. tested)	Antifungal agent	Test method	No. of isolates at MIC/MEC ($\mu\text{g/ml}$) of:												%EA ^b
			0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8	
<i>S. apiospermum</i> (28)	E1210	EUCAST			1	21	6								100.0
		CLSI			1	15	11	1							
	Caspofungin	EUCAST								13	3	5	3	4	96.4
		CLSI						3	3	6	6	4	6		
	Anidulafungin	EUCAST								6	17	5			100.0
		CLSI								1	6	21			
	Itraconazole	EUCAST							7	5	13		1	2	96.3
		CLSI							1	9	8	7	1	2	
	Posaconazole	EUCAST							12	14				2	96.3
		CLSI							2	19	4	1		2	
<i>S. prolificans</i> (28)	E1210	EUCAST			1	13	12	2							96.4
		CLSI			3	20	5								
	Caspofungin	EUCAST								1		1	9	17	96.4
		CLSI										1	10	17	
	Anidulafungin	EUCAST								1	4	14	5	4	100.0
		CLSI									2	26			
	Itraconazole	EUCAST											2	26	100.0
		CLSI												28	
	Posaconazole	EUCAST											3	25	100.0
		CLSI												28	
<i>Scedosporium</i> spp. (63) ^d	E1210	EUCAST			3	38	19	3							98.4
		CLSI			5	40	17	1							
	Caspofungin	EUCAST								14	3	6	15	25	96.8
		CLSI						3	3	6	7	17	27		
	Anidulafungin	EUCAST								7	21	24	6	5	100.0
		CLSI								1	8	53		1	
	Itraconazole	EUCAST							7	7	17		3	29	98.9
		CLSI							1	10	8	10	3	31	
	Posaconazole	EUCAST							14	19			3	27	96.8
		CLSI							2	23	6	1	1	30	
<i>Scedosporium</i> spp. (63) ^d	Voriconazole	EUCAST					4	20	9		2	14	14		98.8
		CLSI					7	16	9		1	1	29		
	Amphotericin B	EUCAST					3	2	5	9	44 ^f				100.0
		CLSI						1	5	7	50 ^f				

^a Only species with at least 10 samples were included. Abbreviations: CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimum inhibitory concentration; MEC, minimum effective concentration; SC, species complex.

^b %EA, percent essential agreement ($\pm 2 \log_2$ dilutions).

^c Includes *G. fujikuroi* SC (30 strains), *F. oxysporum* SC (15 strains), *F. solani* SC (15 strains), *Fusarium* spp. NOS (4 strains), *F. dimerum* SC (2 strains), and *F. incarnatum-equiseti* SC (1 strain).

^d Includes *S. apiospermum* (28 strains), *S. prolificans* (28 strains), and *S. aurantiacum* (7 strains).

^e —, dilution not tested.

^f Values correspond to $\geq 4 \mu\text{g/ml}$.

0.12 and 0.06/0.25 $\mu\text{g/ml}$, respectively) (Table 1). The highest CLSI E1210 MEC value for *F. solani* SC was only 0.06 $\mu\text{g/ml}$, whereas for *G. fujikuroi* SC and *F. oxysporum* SC, the values were 0.25 and 0.5 $\mu\text{g/ml}$, respectively (Table 1). *F. dimerum* SC and *F. incarnatum-equiseti* SC had E1210 MEC results of 0.06 $\mu\text{g/ml}$ by the CLSI method and from ≤ 0.008 to 0.12 $\mu\text{g/ml}$ by the EUCAST method (data not shown).

E1210 was very potent against the *Scedosporium* species tested (Table 1). E1210 CLSI MEC results ranged from 0.03 to 0.25

$\mu\text{g/ml}$ (MEC₉₀, 0.12 $\mu\text{g/ml}$) for *S. apiospermum* (Table 1). With the exception of voriconazole (MIC₉₀, 1 $\mu\text{g/ml}$), the comparator agents showed limited activity against this species: MIC/MEC₉₀ values were >8 (caspofungin), 4 (anidulafungin), 8 (itraconazole), 4 (posaconazole), and $>4 \mu\text{g/ml}$ (amphotericin B). *S. prolificans* strains had E1210 CLSI MEC results of 0.03 to 0.12 $\mu\text{g/ml}$ (MEC₉₀, 0.12 $\mu\text{g/ml}$) (Table 1). Comparator agents displayed limited activity against *S. prolificans* strains, and MIC/MEC₉₀ values were $\geq 4 \mu\text{g/ml}$ for amphotericin B and anidulafungin and >8

$\mu\text{g/ml}$ for caspofungin, itraconazole, posaconazole, and voriconazole (Table 1). The MEC_{50} for E1210 against *S. aurantiacum* was $0.06 \mu\text{g/ml}$ versus 0.5 to $>8 \mu\text{g/ml}$ for the comparators (data not shown). The $\text{MIC}/\text{MEC}_{90}$ was not calculated for this species due to the low number of strains tested.

The comparison of CLSI and EUCAST testing conditions demonstrates for the first time that these methods were highly concordant when used to test new and established antifungal agents against *Fusarium* and *Scedosporium* spp. (Table 1). The %EA ($\pm 2 \log_2$ dilutions) was $>93\%$ for all comparisons, with the exception of posaconazole and *F. oxysporum* SC (60%) and posaconazole and voriconazole against *S. aurantiacum* (85.7%; data not shown). Overall, when testing *Fusarium* spp. (67 isolates), the %EA between CLSI and EUCAST MIC/MEC results were 88.1% for posaconazole, 94.0% for E1210, 95.5% for itraconazole, 98.5% for voriconazole, and 100.0% for caspofungin, anidulafungin, and amphotericin B. Likewise, when testing *Scedosporium* spp. (63 isolates), the %EA were 96.8% for posaconazole and caspofungin, 98.4% for E1210, itraconazole, and voriconazole, and 100.0% for anidulafungin and amphotericin B (Table 1).

There are several important findings that can be derived from this study. First, we have documented the excellent spectrum and potency of E1210 against molecularly identified strains of *Fusarium* and *Scedosporium* species, including polyene-, azole-, and echinocandin-resistant strains. E1210 was the most active of the seven antifungal agents tested against these uncommon, but clinically important opportunistic fungal pathogens. These findings are especially notable for *S. prolificans*, a species that appears completely resistant to the antifungal agents currently available for the treatment of invasive fungal infections (Table 1). Second, we document for the first time the high level of intermethod agreement between the CLSI and EUCAST reference tests for testing E1210, the echinocandins, the triazoles, and amphotericin B against species of *Fusarium* and *Scedosporium*.

E1210 is a potent, novel antifungal agent with impressive *in vitro* activity against *Fusarium* and *Scedosporium* spp. as well as against *Aspergillus* (19, 29) and *Candida* spp. (19, 31, 39). *In vitro* susceptibility testing of E1210 and other mold-active antifungal agents may be accomplished by either of the CLSI or EUCAST broth microdilution methods with comparable results. Further development of this new antifungal agent (E1210) appears warranted.

ACKNOWLEDGMENTS

This work was supported in part by research/educational grants from Eisai, Ltd.

The assistance of A. Small and P. Clark with the preparation of the manuscript is greatly appreciated, as is the technical support of S. A. Messer and L. N. Woosley. We would also like to acknowledge the generosity of our colleagues A. Espinel-Ingroff and A. Fothergill for sharing strains of *Scedosporium* spp. from their collections.

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