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In vitro Activity of SCY-078 in Combination with Isavuconazole or Amphotericin B against Medically Important Moulds

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Abstract

Background. Invasive mould infections constitute important causes of morbidity and mortality in immunocompromised patients. Preclinical studies and preliminary clinical data demonstrate synergistic interaction and improved outcome when an antifungal triazole or polyene is combined with an echinocandin. SCY-078 (SCY) is a novel tri-terpene orally bioavailable inhibitor of (1->3)-beta-D-glucan synthesis. In order to better understand the potential of SCY in the treatment of invasive mould infections, we investigated its *in vitro* interaction with isavuconazole (ISA) or with amphotericin B (AMB) against medically important filamentous fungi.
Methods. Bliss independence drug interaction (BSDI) and Lowe additivity analysis were used to examine the *in vitro* interactions between SCY and ISA or AMB against the following pathogens: *Aspergillus* spp., *Scedosporium apiospermum*, and Mucorales. CLSI (M38-A2) broth microdilution methodology was used to determine MICs for SCY, ISA, and AMB against 4 isolates each of *A. fumigatus*, *A. flavus*, *A. terreus*, *S. apiospermum, Rhizopus oryzae, Rhizopus microsporus, and Cunninghamella bertholettiae* in triplicate. Two-drug combinations were studied in 96-well plates in checkerboard dilutions. After 48 h incubation at 37° C, OD measurements were recorded at 550 nm. Bliss surface interaction and fractional inhibitory concentration indices (FICIs) were then calculated. The effect of antifungal agents on hyphal structure was evaluated by light microscopy.
Results. The combination of SCY and ISA resulted in synergistic interaction against all tested *Aspergillus* spp., *including A. fumigatus, A. flavus, A. terreus,* and *A. niger*. The median *in vitro* FICIs for SCY+ISA against *A. fumigatus* were 0.68 (0.38–1.06), while that for SCY+AMB were 0.95 (0.53–1.5). The greatest synergistic interaction was observed for *A. fumigatus*. There was less synergistic *in vitro* interaction between SCY and ISA against other mould pathogens and no significant interaction between SCY and AMB against *Aspergillus* spp. or other filamentous fungi studied.
Conclusion. These results indicate that the combination of SCY plus ISA may be more effective than either agent alone in treatment of invasive aspergillosis and warrants further investigation.

Introduction

Invasive mould infections caused by *Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp. and Mucorales spp. are important causes of morbidity and mortality in immunocompromised hosts. Response of these infections to single agent therapy is often inadequate. Combination antifungal therapy provides a potential strategy by which to improve antimicrobial activity and improve clinical outcome.

The combination of antifungal triazoles and echinocandins provides the most rational mechanistic basis for this approach. Antifungal triazoles inhibit ergosterol biosynthesis through lanosterol C14-demethylase, while echinocandins inhibit cell wall synthesis through inhibition of the (1→3)-β-D-glucan synthase complex. This triazole-echinocandin combination has been found to range in activity from synergistic to additive against *Aspergillus* spp. *in vitro* and *in vivo*.

The discovery and development of SCY-078 (SCY) with its unique structure-activity relationship (SAR) for inhibition of (1→3)-β-D-glucan synthase has the potential to enhance antifungal therapy, prevent emergence of resistance, and achieve synergy in combination with triazole antifungal agents and with AMB. Whether in fact SCY possesses these properties for synergistic activity with triazoles and AMB in combination therapy against moulds is unknown. Isavuconazole (ISA) is a recently developed antifungal agent with *in vitro* activity against *Aspergillus* spp., as well as non-*Aspergillus* moulds, including *Fusarium* spp., *Scedosporium* spp. and Mucorales spp.. ISA was used in these studies as the companion anti-mould triazole. Understanding the potential role of combination therapy with SCY will be important in advancing therapeutic strategies with this novel cell wall inhibitor against the devastating infections caused by these pathogens.

The *in vitro* combination studies conducted in our laboratory were analyzed by the Lowe Additivity (Fractional Inhibitory Concentration Index (FICI) determinations) and by Bliss Surface Analysis. The equations through Bliss Surface Analysis provide a robust and unbiased interpretation, compared to conventional Lowe Additivity (FICI determinationss). This analysis has correlated strongly with *in vivo* outcomes. The interaction was measured at 24 and 48 hours in order to understand the time-related dynamics of the combination.

We therefore studied the combinations of SCY plus ISA and SCY plus AMB against the following pathogens: *Aspergillus* spp., *Fusarium* spp., *Scedosporium apiospermum*, and Mucorales spp. These *in vitro* studies will provide critical data for the potential of combination therapy with SCY against invasive fungal infections caused by these medically important moulds.

Materials and Methods

- **Isolates.** Eleven clinical isolates of *Aspergillus* spp., *Fusarium* spp. *Scedosporium* spp., and *Mucorales* (Weill Cornell Transplantation-Oncology Infectious Disease Program collection) were used (see Tables).
- **Antifungal agents**. SCY-078 (SCY), isavuconazole (ISA), and amphotericin B (AMB) were used. The antifungal agents were obtained in lyophilized powder form and prepared according to the manufacturer's instructions.
- **Inoculum preparation.** Inocula were prepared spectrophotometrically (530 nm) and further diluted in RPMI 1640 to obtain an initial inoculum in a range of approximately 0.4 to 5 x 10^4 CFU/ml. In each well 100 µl of the mould suspension was dispensed.
- **Antifungal susceptibility testing.** The minimum inhibitory concentrations (MICs) were determined according to the reference procedure of the antifungal susceptibility testing of filamentous fungi of CLSI (M38-A2) after 48 h incubation. The range of concentrations tested were: for SCY from 0.0625-32 µg/ml, for ISA from 0.015 to 8 µg/ml, and for AMB from 0.125 to 8 µg/ml.
- ***In vitro* combination testing.** The *in vitro* interactions between SCY and ISA or SCY and AMB were studied using a two-dimensional checkerboard method in 96-well microtitration plates. Each isolate was tested three times on different days. Antifungal agents were prepared in serial twofold dilutions and ranged from 0.0625-32 µg/ml for SCY, 0.25-16 µg/ml for ISA, and 0.03-2 µg/ml for AMB.
- **Incubation and reading method.** The combined effects of antifungal agents were quantified after 24 and 48 h incubation, spectrophotometrically (550/620 nm) using the metabolic reduction assay 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]2H-tetrazolim-5-carboxanilide (XTT, 0.25 mg/ml) plus menadione (25 µM). The % growth inhibition was calculated according to color absorbance (A) as

$$100\% \times (A_{well} - A_{background}) / (A_{drug\ free\ well} - A_{background})$$

- **Drug interaction modeling and analysis.** Interactions between antifungal agents were analyzed using:
  1. The Fractional Inhibitory Concentration (FIC) index expressed with the following equation:

$$\sum FIC = FICA + FICB = C_A^{comb} / MIC_A^{alone} + C_B^{comb} / MIC_B^{alone}$$

where MIC_A^{alone} and MIC_B^{alone} the MICs of drugs A and B when acting alone and C_A^{comb} and C_B^{comb} are the concentrations of drugs A and B in combination, respectively, corresponding to a MIC (isoeffective combinations). When the FIC indices in all three replicates were smaller than 1, significant synergy was claimed and in all other cases additivity or indifference was concluded.

  2. Bliss independence model, where the theoretical percentage of growth (E_{ind}) (compared to an antifungal-agent free control) describing the effect of the combination of two antifungal agents was calculated with the following equation:

$$E_{ind} = E_A \times E_B$$

where E_A and E_B are the experimental percentages of growth when each antifungal agent acts alone.

For each combination of x mg/l of antifungal agent A with y mg/l of antifungal agent B in each of the independent replicate experiments, the experimental observed percentage of growth, E_{obs}, was subtracted from E_{ind}.

- o When the **ΔE** ($\Delta E = E_{ind} - E_{obs}$) was **positive** and its 95% confidence interval (CI) did not include 0, significant **synergy** was claimed for the specific combination of x µg/ml of antifungal agent A with y µg/ml of antifungal agent B. The higher the positive number for ΔE, the stronger is the synergistic interaction.
- o When the **ΔE** was **negative** without its CI overlapping 0, statistically significant **antagonism** was claimed. The higher the negative number for ΔE, the stronger is the antagonistic interaction.
- o In any other case, when **ΔE=0**, **indifference** was concluded.

Results

Table 1. Minimal inhibitory concentrations (MIC's) of SCY-078 (SCY) of isavuconazole (ISA) and amphotericin B (AMB)

Isolate	MIC (µg/ml)		
	SCY[a]	ISA[a]	AMB[a]
Aspergillus fumigatus	> 32	0.5-1	0.5-1
Aspergillus flavus	> 32	2-4	0.5-1
Aspergillus terreus	> 32	0.25-0.5	0.5-1
Scedosporium apiospermum	> 32	4-8	0.5-1
Cunninghamella bertholettiae	> 32	16-32	1-2
Rhizopus oryzae	> 32	0.5-1	0.25-0.5
Rhizopus microsporus	> 32	2-4	0.5-1
Mucor circinelloides	> 32	4-8	0.06-0.12
Lichtheimia corymbifera	> 32	1-2	0.125
Fusarium oxysporum species complex	> 32	16	0.5
Fusarium solani species complex	> 32	>16	1-2

[a] The lowest drug concentration that prevents any discernible growth (100% inhibition)

Table 2. *In vitro* interactions of SCY-078 (SCY) with isavuconazole (ISA) or amphotericin B (AMB) against different mould isolates (Results according to FIC index)

Isolate	SCY + ISA	SCY +AMB
	FIC Index, Median (range)	FIC Index, Median (range)
Aspergillus fumigatus	0.65 (0.31 – 1.1)	0.96 (0.76 – 1.2)
Aspergillus flavus	0.78 (0.38 – 1.06)	0.95 (0.53 – 1.5)
Aspergillus terreus	0.52 (0.57 – 1.07)	1.01 (0.62 – 1.4)
Scedosporium apiospermum	0.72 (0.43 – 1)	0.96 (0.5 – 1.4)
Cunninghamella bertholettiae	0.65 (0.42 – 1.9)	0.95 (0.6 – 2.4)
Rhizopus oryzae	1.09 (0.76 – 2.1)	1.5 (0.6 – 2.4)
Rhizopus microsporus	0.74 (0.65 – 1.6)	1.3 (0.56 – 2.2)
Mucor circinelloides	1.06 (1 – 1.5)	1.01 (0.5 – 2.1)
Lichtheimia corymbifera	1.07 (0.65 – 1.8)	1.01 (0.72 – 2.1)
Fusarium oxysporum species complex	0.64 (0.52 – 1.5)	0.76 (0.5 – 1.4)
Fusarium solani species complex	0.74 (0.59 – 1.3)	0.92 (0.84 – 1.8)

Results

Table 3A. *In vitro* interactions between SCY-078 (SCY) and isavuconazole (ISA) against different mould isolates (after 24 hrs incubation)

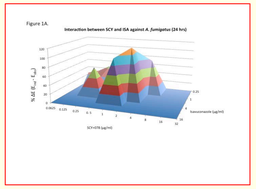
Isolate	Type of interaction	Mean %ΔE value (range)	Mean %SE (range)
Aspergillus fumigatus	SYN	34.7 (25.6 – 39.8)	8.6 (6.5 – 9.4)
Aspergillus flavus	SYN	16.5 (12.8 – 22.5)	3.5 (2.8 – 5.2)
	ANT	-11.6 (-10.3– -12.9)	1.4 (1.3 – 1.5)
Aspergillus terreus	SYN	77.9 (41.5 – 91.5)	14.5 (8.9 – 19.4)
	ANT	-4 (-2 – -6.4)	0.7 (0.3 -1.3)
Scedosporium apiospermum	SYN	26.8 (12.6 – 34.6)	4.1 (1.7 – 7.3)
Cunninghamella bertholettiae	INT	-	-
Rhizopus oryzae	INT	-	-
Rhizopus microsporus	SYN	39.2 (23.9 – 69.1)	6.6 (2.5 – 13.4)
Mucor circinelloides	INT	-	-
Lichtheimia corymbifera	INT	-	-
Fusarium oxysporum	SYN	22.9 (12.6 – 30.8)	3.7 (1.9 – 6.2)
Fusarium solani	INT	-	-

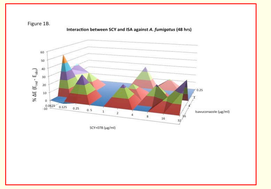
Table 3B. *In vitro* interactions between SCY-078 (SCY) and isavuconazole (ISA) against different mould isolates (after 48 hrs incubation)

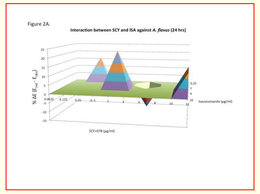
Isolate	Type of interaction	Mean %ΔE value (range)	Mean %SE (range)
Aspergillus fumigatus *	SYN	25.2 (11.5 – 50.7)	3.8 (0.4 – 7.1)
Aspergillus flavus	SYN	22.5 (15.2 – 30)	3.9 (0.8 – 6.2)
Aspergillus terreus	SYN	79.2 (49.6 – 89.4)	11.5 (9.8 – 17.6)
	ANT	-2.1 (-1 – -5.3)	0.5 (0.3 – 1.1)
Scedosporium apiospermum *	SYN	25.9 (7.5 – 49.8)	3 (0.3 – 8.5)
Cunninghamella bertholettiae	SYN	56.7 (41.9 – 83.5)	7 (1.4 – 12.3)
Rhizopus oryzae	ANT	-29.7 (-13.6 – - 45.5)	4.1 (2.4 – 7.2)
Rhizopus microsporus *	ANT	-112 (-103 – - 127.6)	19 (15 - 27.5)
Mucor circinelloides	INT	-	-
Lichtheimia corymbifera	ANT	-30.5 (-25.2 - - 38.5)	5.2 (3.9 – 7.9)
Fusarium oxysporum	SYN	23 (12.9 – 29.9)	3.8 (1.3 – 5.6)
Fusarium solani	SYN	19.2 (13.1 - -29.2)	3.2 (1.3 – 5.3)

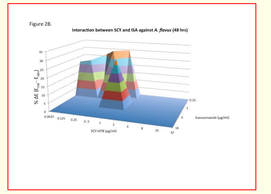
* , Minor one cell interactions were noted

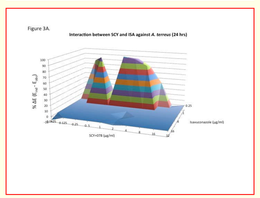
SE, standard error; ANT, antagonistic interaction; INT, indifferent interaction; SYN, synergistic interaction;

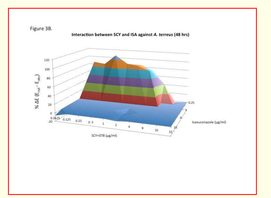




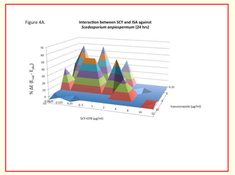


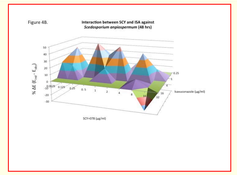


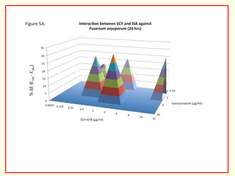


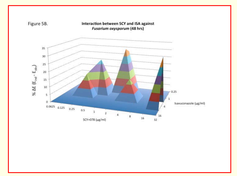


Results









Figures.
Interaction surface plots obtained from analysis with the Bliss independence model of SCY-078 (SCY) and isavuconazole (ISA) interactions against *A. fumigatus* (Fig1), *A. flavus* (Fig2), *A. terreus* (Fig3), *Scedosporium apiospermum* (Fig4), and *Fusarium oxysporum* (Fig5). The zero plane (ΔE=0) represents indifferent interactions and volumes above (ΔE>0) zero plane synergistic interactions.

Table 4A. *In vitro* interactions between SCY-078 (SCY) and amphotericin B (AMB) against different mould isolates (after 24 hrs incubation)

Isolate	Type of interaction	Mean %ΔE value (range)	Mean %SE (range)
Aspergillus fumigatus *	INT	-	-
Aspergillus flavus	SYN	25.6 (9 - 36.8)	4.4 (1.9 - 7.7)
Aspergillus terreus	INT	-	-
Scedosporium apiospermum	INT	-	-
Cunninghamella bertholettiae	INT	-	-
Rhizopus oryzae *	INT	-	-
Rhizopus microspores *	INT	-	-
Mucor circinelloides	INT	-	-
Lichtheimia corymbifera	INT	-	-
Fusarium oxysporum *	INT	-	-
Fusarium solani *	SYN	23.4 (18.6 – 29.8)	3.9 (2.7 – 4.6)

Table 4B. *In vitro* interactions between SCY-078 (SCY) and amphotericin B (AMB) against different mould isolates (after 48 hrs incubation)

Isolate	Type of interaction	Mean %ΔE value (range)	Mean %SE (range)
Aspergillus fumigatus	SYN	17.5 (4.1 – 33.6)	2.5 (0.4 -3.4)
	ANT	-8.8 (-2.8 – -11.7)	0.9 (0.2 – 1.8)
Aspergillus flavus	SYN	25.3 (15.3 – 31.9)	3.7 (1.9 – 6.8)
Aspergillus terreus	INT	-	-
Scedosporium apiospermum *	SYN	37 (3 -63.3)	3.9 (0.4 – 13.3)
Cunninghamella bertholettiae	SYN	41.2 (24 - 60.8)	6.5 (1.5 – 10.4)
Rhizopus oryzae	ANT	-78.6 (-76 – -81)	15 (13 -16.9)
Rhizopus microsporus *	INT	-	-
Mucor circinelloides	INT	-	-
Lichtheimia corymbifera *	INT	-	-
Fusarium oxysporum	SYN	38.9 (19.3 – 64.6)	5.8 (0.9 – 8.1)
Fusarium solani	INT	-	-

* , Minor one cell interactions were noted

SE, standard error; ANT, antagonistic interaction; INT, indifferent interaction; SYN, synergistic interaction;

* , Minor one cell interactions were noted

Conclusions/Summary

- *In vitro* SCY-078 and isavuconazole are synergistically active against *A. fumigatus*, *A. flavus*, *A. terreus*, *C. bertholletiae*, *S. apiospermum, F. oxysporum* and *F. solani* complexes.
- These results indicate that the combination of SCY-078 plus isavuconazole may be more effective than either agent alone in treatment of invasive aspergillosis, as well as other invasive mould infections, and warrant further investigation.