Lopinavir and ritonavir act synergistically with azoles against *Candida auris in vitro* and in a mouse model of disseminated candidiasis

Ehab A. Salama[a,b*], Hassan E. Eldesouky[a,b*], Yehia Elgammal[a,b], Nader S. Abutaleb[a,b], Mohamed N. Seleem[a,b#]

[a]*Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061, USA.*

[b]*Center for One Health Research, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061, USA.*

#Corresponding Author:

Mohamed N. Seleem
Department of Biomedical Sciences and Pathobiology
Virginia-Maryland College of Veterinary Medicine
Virginia Polytechnic Institute and State University
1410 Prices Fork Rd, Blacksburg, Virginia, 24061
Phone: 540-231-7173
Email: seleem@vt.edu

***** Ehab A. Salama and Hassan E. Eldesouky contributed equally to this work. Authors order was determined based on manuscript writing.

ABSTRACT

The emergence of *Candida auris* has created a global health challenge. Azole antifungals are the most affected antifungal class due to the extraordinary capability of *C. auris* to develop resistance against these drugs. Here, we used a combinatorial therapeutic approach to sensitize *C. auris* to azole antifungals. We have demonstrated the capability of the HIV protease inhibitors lopinavir and ritonavir, at clinically relevant concentrations, to be used with azole antifungals to treat *C. auris* infections both *in vitro* and *in vivo*. Both lopinavir and ritonavir exhibited potent synergistic interactions with the azole antifungals, particularly with itraconazole against 24/24 (100%) and 31/34 (91%) of tested *C. auris* isolates, respectively. Furthermore, ritonavir significantly interfered with the fungal efflux pump, resulting in significant increase in Nile red fluorescence by 44%. In a mouse model of *C. auris* systemic infection, ritonavir boosted the activity of lopinavir to work synergistically with fluconazole and itraconazole and significantly reduced the kidney fungal burden by a 1.2 log (~94%), and a 1.6 log (~97%) CFU, respectively. Our results urge further comprehensive assessment of azoles and HIV protease inhibitors as a novel drug regimen for the treatment of serious invasive *C. auris* infections.

KEYWORDS

HIV protease inhibitors, *Candida auris*, checkerboard assay, azole resistance, *in vivo* infection model.

INTRODUCTION

Candida auris is an emerging fungal pathogen responsible for serious invasive outbreaks worldwide [1, 2]. The United States Centers for Disease Control and Prevention (CDC) has categorized *C. auris* infections as an urgent threat for which new drugs are urgently needed [3]. *C. auris* isolates have already developed resistance to two (azoles and polyenes) out of the three available classes of antifungal agents and strains resistant to the third class (echinocandins) are on the rise [4]. Azole antifungals remained the first-line treatment options for *Candida* infections for decades [5, 6]. Currently, fluconazole (the most widely used azole antifungal) is no longer considered a reliable therapeutic option because ~ 90% of the circulating *C. auris* isolates are reported to be fluconazole-resistant [7]. Nevertheless, the susceptibility of *C. auris* to other azole antifungals, such as itraconazole and voriconazole, varies [8]. Most worrisome, pan-resistant *C. auris* isolates have also been reported in the United States and other countries which highlight the global threat posed by *C. auris* and the urgent need for alternative approaches to manage the challenging *C. auris* infection [9].

In a previous work, we screened 1,547 FDA-approved drugs and identified the HIV protease inhibitor lopinavir as a potent *in vitro* enhancer to azole antifungals [10]. Herein, we sought to examine the potential of lopinavir to enhance the *in vivo* efficacy of azole antifungals in a mouse model of disseminated candidiasis. However, lopinavir exhibits poor bioavailability when administered alone [11], which may prevent it from enhancing the activity of azole antifungals *in vivo*. Thus, we hypothesized that ritonavir, a dual action drug with pharmacokinetic (PK)-boosting activity and efflux inhibition, can synergize with lopinavir to enhance the antifungal activity of azole antifungals. Therefore, we investigated the synergistic activity of ritonavir and lopinavir with azoles against *C. auris*, the efflux inhibitory activity of ritonavir, and finally, the *in vivo* efficacy of lopinavir/ritonavir/azoles combination in a mouse model of *C. auris* disseminated infection.

MATERIALS AND METHODS

Fungal isolates, media and reagents

A total of 34 *C. auris* isolates were provided from the CDC (Atlanta, GA, USA), BEI Resources (Manassas, VA, USA), and Westerdijk Fungal Biodiversity Institute (Utrecht, the Netherlands). Isolate ID and clade distribution are listed in **Table S1**. The media and tested drugs were obtained

as follows: RPMI 1640 (Gibco, Grand Island, NY, USA); 3-(N-Morpholino) propane sulfonic acid (MOPS) (Fisher Bioreagents, Fairlawn, NJ, USA); yeast extract peptone dextrose agar and broth (YPD, Becton, Dickinson, and Company, Sparks, MD, USA); HEPES buffer (DOT Scientific Inc, Burton, MI, USA); phosphate-buffered saline (PBS) (Corning, Manassas, VA, USA); cyclophosphamide (Cayman Chemical, Ann Arbor, MI, USA); chloramphenicol (Sigma-Aldrich, St. Louis, MO, USA); fluconazole (FLC), itraconazole (ITC), and lopinavir (LPV) (Acros Organics, Morris Plains, NJ, USA); voriconazole (VRC), ritonavir (RTV), and Nile red (TCI America, Portland, OR, USA).

Minimum inhibitory concentration (MIC) and checkerboard assay

MICs identification and checkerboard assays were performed according to the Clinical and Laboratory Standards Institute M27-A3 guidelines [12]. The fractional inhibitory concentration index (ΣFICI) was calculated and interpreted as follows: ΣFICI ≤0.5 was classified as synergism (SYN), ΣFICI from >0.5 to ≤4 was classified as indifference (IND), and ΣFICI >4 was classified as antagonism (ANT) [13, 14]. Every assay was performed in triplicate.

Efflux pump assay

Azole-resistant *C. auris* AR0390 was used to evaluate the effect of RTV on the efflux pump using the established glucose-induced Nile red efflux assay [15, 16]. Briefly, *C. auris* cells were cultivated in YPD broth overnight in a shaker incubator (150 rpm at 35 °C). Next, cells were collected and washed three times with PBS. *Candida* cells were then resuspended in PBS, allowed to starve for 4 h in the shaker, and then incubated overnight at 4 °C. The next day, cells were suspended in HEPES buffer and 7.5 mM Nile red was added to the suspension for 30 min at 35 °C. Extra Nile red was washed out twice with PBS; cells were treated with either DMSO or RTV (8 µg/ml) and transferred to an opaque 96-well plate. Efflux was initiated by the addition of glucose (10 mM). The fluorescence signal of Nile red at 485/528 nm was measured before and 10 min after adding glucose using a Biotek Synergy H1 microplate reader.

***In vivo* kidney fungal burden mouse model**

We evaluated the *in vivo* efficacy of the tested combinations in a previously described *C. auris* disseminated infection mouse model with modifications [17]. Briefly, female CD-1 mice (5-6 weeks old) were randomly distributed into groups and were injected intraperitoneally (i.p.) with

cyclophosphamide 4 days (150 mg/kg) and 1 day (100 mg/kg) before infection. On the challenge day, mice were injected i.p. with ~ 4×10^7/mouse cells of *C. auris* AR0390 suspended in PBS in the FLC sensitization experiment. For the ITC sensitization experiment, mice were infected with 2×10^7 CFU/mouse of *C. auris* AR0390. Treatments started two hours after infection. All treatments were given twice daily via oral gavage for two consecutive days. On the third day, mice were humanely euthanized, and kidneys were aseptically extracted and homogenized in PBS. The kidney homogenate was serially diluted and then plated on YPD agar supplemented with chloramphenicol (100 µg/ml). Animal model results were analyzed via a one-way ANOVA with post hoc Dunnett's test for multiple comparison using GraphPad Prism version 8 for Windows (GraphPad Software, La Jolla, CA, USA).

RESULTS

In vitro interactions between lopinavir/ritonavir and azole drugs against *C. auris* isolates

First, we identified the susceptibility of all isolates to the three azole antifungal drugs (FLC, ITC, and VRC) and HIV protease inhibitors (LPV and RTV) **(Table S1)**. Approximately 74% (25/34) of the tested isolates were resistant to FLC (MIC values ranged from 32 to >128 µg/ml). The MIC values for ITC and VRC against *C. auris* ranged between 0.125 to 2 µg/ml and 0.015 to 4 µg/ml respectively. LPV and RTV alone did not inhibit growth of any of the tested *C. auris* isolates (MICs >128 µg/ml).

Afterwards, we investigated the interactions between LPV and the three azole antifungals (FLC, VRC, and ITC) against 24 *C. auris* isolates (**Table 1**). LPV interacted synergistically with FLC and with VRC against 62.5% (15/24) of the isolates. The ΣFICI for the synergistic interactions ranged from 0.14 to 0.38 and 0.27 to 0.38 for FLC/LPV and VRC/LPV, respectively. LPV exhibited the most potent synergistic interaction with ITC against 100% (24/24) of the tested isolates with ΣFICI ranged from 0.14 to 0.31.

Similarly, RTV interacted synergistically with FLC and with VRC against 21% (7/34) of the *C. auris* isolates. The ΣFICI ranged from 0.25 to 0.38 for FLC/RTV and from 0.31 to 0.5 for VRC/RTV. Furthermore, RTV exhibited a potent synergistic interaction with ITC against 91% (31/34) of the tested *C. auris* isolates, with ΣFICI ranging from 0.07 to 0.31 (**Table 2**).

Ritonavir's effect on efflux pump activity in *C. auris*

We used the glucose-induced Nile red efflux assay to evaluate the ability of RTV to inhibit the efflux pump activity of *C. auris* AR0390. RTV was able to interfere with the efflux of Nile red, which led to a significant increase in the Nile red fluorescence signal in yeast cells (by 44% ± 9.3%) (**Fig. 1A**).

In vivo activity of lopinavir/ritonavir and azole drugs against *C. auris* infection in a mouse model of disseminated candidiasis

We evaluated the ability of LPV to re-sensitize/enhance the susceptibility of drug-resistant *C. auris* to the antifungal activity of FLC and ITC in two independent experiments of a disseminated candidiasis mouse model.

For FLC sensitization, groups of immunocompromised CD-1 mice (n=8) were challenged with 4 × 10^7 CFU/mouse of *C. auris* AR0390, and the treatment was initiated orally 2 h after fungal challenge. Mice were treated twice daily with FLC (75 mg/kg), LPV (160 mg/kg), LPV-R (160-40 mg/kg), FLC/LPV (75/160 mg/kg), FLC/LPV-R (75/160-40 mg/kg) and one group was kept as an untreated control. The treatment continued for 48 h then all mice were euthanized to determine the fungal burden in the kidneys. As shown in **Fig. 1B**, FLC/LPV treatments did not significantly reduce the *C. auris* burden compared to the untreated or FLC-treated controls. On the other hand, the FLC/LPV-R combination significantly reduced the burden of *C. auris* in the kidneys relative to all other treatments and produced a 1.2 log CFU reduction (~94%) compared to the vehicle control and a 1 log CFU reduction (~90%) compared to FLC alone.

For ITC sensitization experiment, groups of immunocompromised CD-1 mice (n=6) were infected with 2 × 10^7 CFU/mouse *C. auris* AR0390 and then treated orally with ITC (5 mg), LPV-R (160-40 mg/kg), a combination of ITC/LPV-R (5/160-40 mg/kg), and one group was kept as an untreated control. As shown in **Fig. 1C**, ITC-LPV-R combination significantly reduced the burden of *C. auris* in murine kidneys producing a 1.6 log CFU reduction (~97%) compared to the vehicle control and a 1.3 log CFU reduction (~95%) compared to ITC alone.

DISCUSSION

Candida auris is an emerging fungal pathogen characterized by its alarming multidrug-resistance profile. Combination therapy is considered as an alternative approach for the management of hard-to-treat pathogens. We previously reported the potent azole re-sensitization activities and many

advantageous qualities of the HIV protease inhibitor, LPV against 10 isolates of multidrug-resistant *C. auris* [10]. Here, we confirmed this synergistic interaction against 24 additional isolates of *C. auris*. Additionally, we evaluated another HIV protease inhibitor, RTV, which is clinically used with LPV to enhance its pharmacokinetic profile [11].

Our results indicate that LPV and RTV exhibit a synergistic interaction when combined with the azole antifungals FLC, VRC, or ITC. Checkerboard assays revealed that LPV exhibited better synergistic interactions with FLC and VRC against 62.5% of *C. auris* isolates tested compared to RTV (only 21%). Interestingly, the most potent synergistic interactions were observed with the combination of HIV protease inhibitors (LPV or RTV) and the azole drug ITC against almost all the tested isolates (100% and 91% respectively).

Overexpression of efflux pumps is a major mechanism by which *C. auris* exhibits resistance to azole drugs. Here, we were curious if RTV (like LPV) can interfere with the efflux pump of *C. auris*. As expected, our findings revealed that RTV significantly interfered with the efflux pump of *C. auris* and increased the Nile red florescence intensity in the fungal cells by 44 %.

The favorable *in vitro* results encouraged us to explore the *in vivo* efficacy of these combinations in a *C. auris* disseminated infection mouse model. We first evaluated the *in vivo* efficacy of FLC/LPV (75/160 mg/kg) in a neutropenic mouse model in the presence and absence of the bioavailability enhancer RTV. The FLC/LPV (75/160) combination did not significantly reduce *C. auris* kidney burden in mice. We speculated that the low *in vivo* efficacy of the FLC/LPV combination could be due to the poor oral bioavailability of LPV (~25%). Since RTV possesses PK boosting and efflux inhibition activities, we hypothesized that RTV could synergize with LPV to enhance the antifungal activity of FLC. It is worth mentioning that the pharmacokinetic of protease inhibitors (LPV and RTV) were previously studied in a mouse experiment at the doses of 200 and 50 mg/kg, respectively. These doses resulted in plasma levels that are equivalent to those of therapeutic doses in humans [18]. In our study, we used lopinavir and ritonavir at the dose of 160-40 mg/kg which is lower than the previously reported dose. This indicates that lopinavir-ritonavir, at clinically achievable concentrations, could induce in vivo efficacy against *C. auris*. As expected, the FLC/LPV-R combination (75/160-40 mg/kg) significantly reduced the burden of *C. auris* in the kidneys of mice. FLC/LPV-R generated a 1.2 log (~94%) CFU reduction compared to the vehicle control and a 1 log (90%) CFU reduction compared to FLC alone.

Then, we moved to test the *in vivo* synergy with ITC. ITC/LPV-R (5/160-40 mg/kg) combination exhibited the most potent *in vivo* efficacy leading to a significant decline in the fungal burden in the kidneys compared to all other treatments. ITC/LPV-R generated a 1.6 log (~97%) CFU reduction compared to the vehicle control and a 1.3 log (~95%) CFU reduction compared to ITC alone. Interestingly, the *in vivo* activity of ITC/LPV-R against *C. auris* is comparable to that observed with a newly developed antifungal drug (APX001A) currently undergoing clinical trials [19].

Moreover, there have been reports of the clinical resolution of azole-resistant oropharyngeal candidiasis following the administration of HIV protease inhibitor therapy [20, 21] that further validates our approach. Taken together, the characteristics of protease inhibitors as re-sensitizing antifungal agents may offer a novel strategy to current approaches for treating serious *C. auris* infections.

ACKNOWLEDGMENTS

We acknowledge the CDC and BEI Resources for providing the clinical isolates utilized in this work. This work was supported by National Institute of Health Grant R01AI141439.

CONFLICT OF INTEREST

No conflict of interest.

ETHICAL APPROVAL FOR ANIMAL EXPERIMENT

Animal experiments were approved and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee at Virginia Tech (protocol #: 20-199).

Table 1: Interactions between lopinavir and azole antifungals (fluconazole, itraconazole, and voriconazole) against 24 *C. auris* isolates

C. auris Isolate ID	FLC/LPV combination			ITC/LPV combination			VRC/LPV combination		
	MIC (µg/ml)	ΣFICI	Mode	MIC (µg/ml)	ΣFICI	Mode	MIC (µg/ml)	ΣFICI	Mode
CBS 12766	32/16	0.31	SYN	0.25/8	0.28	SYN	0.125/8	0.28	SYN
CBS 12768	64/16	0.31	SYN	0.25/8	0.28	SYN	0.125/16	0.31	SYN
CBS 12770	64/8	0.28	SYN	0.25/8	0.16	SYN	0.25/4	0.27	SYN
CBS 12771	64/8	0.28	SYN	0.5/4	0.27	SYN	0.25/4	0.27	SYN
CBS 12772	64/16	0.31	SYN	0.25/8	0.28	SYN	0.125/16	0.31	SYN
CBS 12773	64/8	0.28	SYN	0.25/16	0.31	SYN	0.125/32	0.38	SYN
CBS 12774	32/4	0.14	SYN	0.5/8	0.28	SYN	0.25/4	0.27	SYN
NR-52713	32/8	0.28	SYN	0.06/8	0.15	SYN	0.125/8	0.53	IND
NR-52714	16/8	0.53	IND	0.125/4	0.27	SYN	0.125/4	0.52	IND
NR-52715	0.5/8	0.53	IND	0.06/8	0.15	SYN	0.015/1	1	IND
NR-52716	4/16	0.56	IND	0.125/8	0.16	SYN	0.06/1	1	IND
NR-52717	32/8	0.16	SYN	0.25/8	0.16	SYN	0.25/8	0.28	SYN
AR 1101	16/16	0.31	SYN	0.25/8	0.28	SYN	0.125/16	0.31	SYN
CBS 12372	32/8	0.28	SYN	0.5/8	0.28	SYN	0.125/16	0.31	SYN
CBS 10913	0.5/1	1	IND	0.03/4	0.14	SYN	0.015/1	1	IND
CBS 12373	32/8	0.28	SYN	0.5/8	0.28	SYN	0.25/8	0.28	SYN
AR 1102	>128/>128	2	IND	0.25/4	0.27	SYN	2/1	1	IND
AR 1103	128/8	0.53	IND	0.25/8	0.28	SYN	0.5/8	0.28	SYN
AR 0931	64/32	0.38	SYN	0.25/16	0.31	SYN	1/16	0.31	SYN
AR 1104	16/8	0.28	SYN	0.5/4	0.27	SYN	0.25/8	0.53	IND
AR 1097	8/16	0.31	SYN	0.25/8	0.28	SYN	0.06/16	0.30	SYN
AR 1099	1/4	0.52	IND	0.125/4	0.27	SYN	0.015/1	1	IND
AR 1100	1/16	0.56	IND	0.06/4	0.26	SYN	0.015/1	1	IND
AR 1105	0.5/8	0.53	IND	0.125/4	0.27	SYN	0.015/4	0.35	SYN

FLC: fluconazole, **ITC**: itraconazole, **VRC**: voriconazole, **LPV**: lopinavir, **SYN**: synergy, **IND**: indifference.

Table 2: Interactions between ritonavir and azole antifungals (fluconazole, itraconazole, and voriconazole) against 34 *C. auris* isolates

C. auris Isolate ID	FLC/RTV combination			ITC/RTV combination			VRC/RTV combination		
	MIC (µg/ml)	ΣFICI	Mode	MIC (µg/ml)	ΣFICI	Mode	MIC (µg/ml)	ΣFICI	Mode
AR0382	1/1	1	IND	0.25/16	0.56	IND	0.015/1	1	IND
AR0387	1/1	1	IND	0.06/8	0.27	SYN	0.015/1	1	IND
AR0388	64/16	0.31	SYN	0.125/16	0.19	SYN	0.25/32	0.38	SYN
AR0389	>128/>128	2	IND	0.25/2	0.26	SYN	2/1	1	IND
AR0390	64/16	0.31	SYN	0.25/8	0.28	SYN	0.25/8	0.53	IND
CBS 12766	128/1	1	IND	0.25/8	0.28	SYN	0.5/1	1	IND
CBS 12768	128/1	1	IND	0.25/16	0.31	SYN	0.5/1	1	IND
CBS 12770	128/16	0.56	IND	0.25/8	0.28	SYN	0.25/16	0.31	SYN
CBS 12771	64/32	0.38	SYN	0.25/8	0.28	SYN	0.25/16	0.31	SYN
CBS 12772	128/16	0.56	IND	0.25/16	0.31	SYN	0.5/1	1	IND
CBS 12773	64/16	0.31	SYN	0.25/16	0.31	SYN	0.5/1	1	IND
CBS 12774	128/32	0.63	IND	0.5/2	0.26	SYN	0.25/16	0.31	SYN
NR-52713	32/8	0.28	SYN	0.06/8	0.15	SYN	0.125/8	0.53	IND
NR-52714	32/1	1	IND	0.125/4	0.27	SYN	0.125/8	0.53	IND
NR-52715	1/1	1	IND	0.06/8	0.15	SYN	0.015/1	1	IND
NR-52716	4/16	0.56	IND	0.25/8	0.28	SYN	0.06/1	1	IND
NR-52717	32/32	0.25	SYN	0.25/8	0.16	SYN	0.25/16	0.31	SYN
AR0381	1/1	1	IND	0.01/8	0.07	SYN	0.007/1	1	IND
AR 1101	32/16	0.56	IND	0.5/2	0.51	IND	0.25/1	0.50	SYN
CBS 12372	64/16	0.56	IND	0.5/4	0.27	SYN	0.5/1	1	IND
CBS 10913	0.5/1	1	IND	0.06/8	0.27	SYN	0.015/1	1	IND
CBS 12373	64/16	0.56	IND	0.5/4	0.27	SYN	1/1	1	IND
AR0383	>128/>128	2	IND	0.25/4	0.27	SYN	0.5/1	1	IND
AR0384	>128/>128	2	IND	0.25/4	0.27	SYN	1/1	1	IND
AR 1102	>128/>128	2	IND	0.25/4	0.27	SYN	1/1	1	IND
AR 1103	>128/>128	2	IND	0.25/8	0.28	SYN	2/1	1	IND
AR0385	>128/>128	2	IND	0.25/8	0.28	SYN	2/32	0.63	IND
AR0386	>128/>128	2	IND	0.25/8	0.28	SYN	2/1	1	IND

AR 0931	>128/>128	2	IND	0.5/4	0.52	IND	2/16	0.56	IND
AR 1104	64/1	1	IND	0.5/4	0.27	SYN	0.5/1	1	IND
AR 1097	8/16	0.31	SYN	0.125/8	0.28	SYN	0.125/8	0.53	IND
AR 1099	1/8	0.53	IND	0.125/4	0.27	SYN	0.015/1	1	IND
AR 1100	1/16	0.56	IND	0.125/4	0.27	SYN	0.015/1	1	IND
AR 1105	0.25/16	0.56	IND	0.125/8	0.28	SYN	0.015/8	0.36	SYN

FLC: fluconazole, **ITC**: itraconazole, **VRC**: voriconazole, **RTV**: ritonavir, **SYN**: synergy, **IND**: indifference.

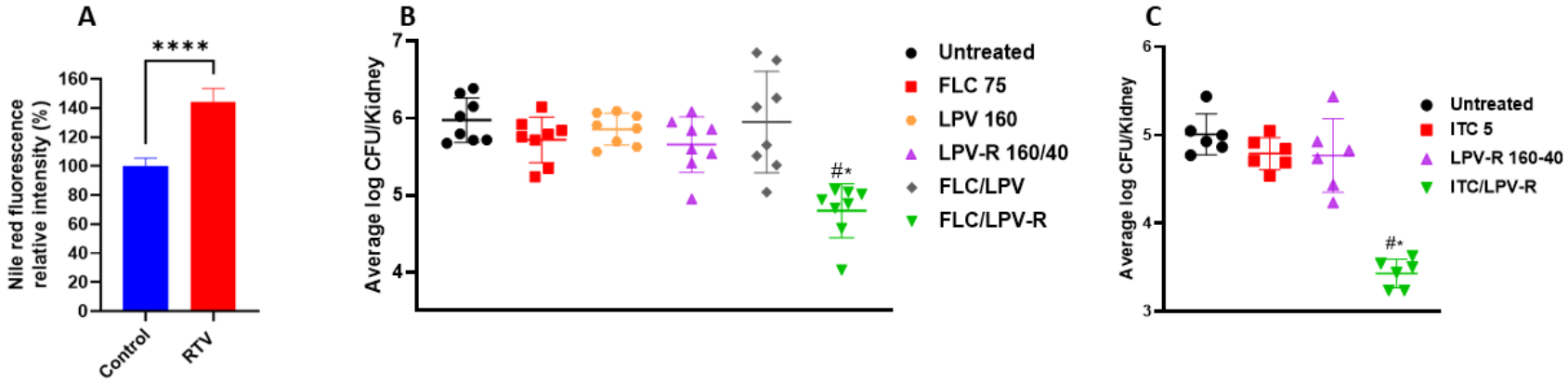

Figure 1: Efflux assay and *in vivo* efficacy of HIV protease inhibitors in combination with azole antifungals against *C. auris*. A) Inhibitory effect of ritonavir (RTV) on the efflux of Nile red from *C. auris*. RTV, at 8 µg/ml, interferes with the efflux of Nile red from *C. auris* AR0390. The data, which represent the average of 3 independent experiments, were analyzed via a Student's t-test. Asterisks denote a statistical difference in the intensity of Nile red fluorescence between the untreated and RTV-treated *C. auris* cells (P <0.0001). **B) *In vivo* efficacy of FLC/LPV-R combination.** Female CD-1 mice (n = 8), infected with azole-resistant *C. auris* AR0390 (4×10^7 CFU/mouse) and treated with vehicle control (untreated), fluconazole (FLC) 75 mg/kg, lopinavir (LPV) 160 mg/kg, lopinavir-ritonavir (LPV-R) 160-40 mg/kg, fluconazole/lopinavir (FLC/LPV) 75/160 mg/kg, and fluconazole/lopinavir-ritonavir (FLC/LPV-R) 75/160-40 mg/kg. **C) *In vivo* efficacy of ITC/LPV-R combination.** Female CD-1 mice (n = 6), infected with azole-resistant *C. auris* AR0390 (2×10^7 CFU/mouse) and treated with vehicle control, itraconazole (ITC) 5 mg/kg, lopinavir-ritonavir (LPV-R) 160-40 mg/kg, or the itraconazole/lopinavir-ritonavir combination (ITC/LPV-R) 5/160-40 mg/kg. The data were analyzed via a one-way analysis of variance (ANOVA) using post-hoc Dunnett's test for multiple comparisons. The asterisk (*) indicates a statistically significant difference ($P < 0.05$) compared to the untreated control. The pound sign (#) indicates a statistically significant difference for the combination treatment compared to the treatment with the azole drug alone ($P < 0.05$).

REFERENCES

[1] Mohammad H, Eldesouky HE, Hazbun T, Mayhoub AS, Seleem MN. Identification of a Phenylthiazole Small Molecule with Dual Antifungal and Antibiofilm Activity Against Candida albicans and Candida auris. Sci Rep. 2019;9:18941.

[2] Chen J, Tian S, Han X, Chu Y, Wang Q, Zhou B, et al. Is the superbug fungus really so scary? A systematic review and meta-analysis of global epidemiology and mortality of Candida auris. BMC Infect Dis. 2020;20:827.

[3] CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC. . 2019.

[4] Carolus H, Pierson S, Munoz JF, Subotic A, Cruz RB, Cuomo CA, et al. Genome-Wide Analysis of Experimentally Evolved Candida auris Reveals Multiple Novel Mechanisms of Multidrug Resistance. Mbio. 2021;12.

[5] Eldesouky HE, Li X, Abutaleb NS, Mohammad H, Seleem MN. Synergistic interactions of sulfamethoxazole and azole antifungal drugs against emerging multidrug-resistant Candida auris. Int J Antimicrob Agents. 2018;52:754-61.

[6] Allen D, Wilson D, Drew R, Perfect J. Azole antifungals: 35 years of invasive fungal infection management. Expert Rev Anti Infect Ther. 2015;13:787-98.

[7] CDC. Antifungal susceptibility testing and interpretation in Candida auris, https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html. Accessed on February, 2022.

[8] Chowdhary A, Prakash A, Sharma C, Kordalewska M, Kumar A, Sarma S, et al. A multicentre study of antifungal susceptibility patterns among 350 Candida auris isolates (2009-17) in India: role of the ERG11 and FKS1 genes in azole and echinocandin resistance. J Antimicrob Chemoth. 2018;73:891-9.

[9] Jacobs SE, Jacobs JL, Dennis EK, Taimur S, Rana M, Patel D, et al. Candida auris Pan-Drug-Resistant to Four Classes of Antifungal Agents. Antimicrob Agents Chemother. 2022;66:e0005322.

[10] Eldesouky HE, Salama EA, Lanman NA, Hazbun TR, Seleem MN. Potent Synergistic Interactions between Lopinavir and Azole Antifungal Drugs against Emerging Multidrug-Resistant Candida auris. Antimicrob Agents Ch. 2021;65.

[11] Chandwani A, Shuter J. Lopinavir/ritonavir in the treatment of HIV-1 infection: a review. Ther Clin Risk Manag. 2008;4:1023-33.

[12] CLSI. 2017. Reference method for broth dilution antifungal susceptibility testing of yeasts, 4th ed. CLSI standard M27. Clinical and Laboratory Standards Institute, Wayne, PA.

[13] Eldesouky HE, Mayhoub A, Hazbun TR, Seleem MN. Reversal of Azole Resistance in Candida albicans by Sulfa Antibacterial Drugs. Antimicrob Agents Chemother. 2018;62.

[14] Eldesouky HE, Lanman NA, Hazbun TR, Seleem MN. Aprepitant, an antiemetic agent, interferes with metal ion homeostasis of Candida auris and displays potent synergistic interactions with azole drugs. Virulence. 2020;11:1466-81.

[15] Eldesouky HE, Salama EA, Li X, Hazbun TR, Mayhoub AS, Seleem MN. Repurposing approach identifies pitavastatin as a potent azole chemosensitizing agent effective against azole-resistant Candida species. Sci Rep. 2020;10:7525.

[16] Eldesouky HE, Salama EA, Hazbun TR, Mayhoub AS, Seleem MN. Ospemifene displays broad-spectrum synergistic interactions with itraconazole through potent interference with fungal efflux activities. Sci Rep. 2020;10:6089.

66 [17] Xin H, Mohiuddin F, Tran J, Adams A, Eberle K. Experimental Mouse Models of Disseminated
67 Candida auris Infection. mSphere. 2019;4.
68 [18] Prot M, Heripret L, Cardot-Leccia N, Perrin C, Aouadi M, Lavrut T, et al. Long-term treatment with
69 lopinavir-ritonavir induces a reduction in peripheral adipose depots in mice. Antimicrob Agents
70 Chemother. 2006;50:3998-4004.
71 [19] Hager CL, Larkin EL, Long L, Zohra Abidi F, Shaw KJ, Ghannoum MA. In Vitro and In Vivo Evaluation
72 of the Antifungal Activity of APX001A/APX001 against Candida auris. Antimicrob Agents Chemother.
73 2018;62.
74 [20] Valdez H, Gripshover BM, Salata RA, Lederman MM. Resolution of azole-resistant oropharyngeal
75 candidiasis after initiation of potent combination antiretroviral therapy. AIDS. 1998;12:538.
76 [21] Hood S, Bonington A, Evans J, Denning D. Reduction in oropharyngeal candidiasis following
77 introduction of protease inhibitors. AIDS. 1998;12:447-8.

78