ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Potent Antifungal Synergy of Phthalazinone and Isoquinolones with Azoles Against *Candida albicans*

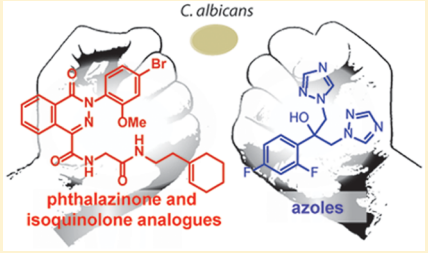
Aaron D. Mood,[†,∥] Ilandari Dewage Udara Anulal Premachandra,[†,∥] Stanley Hiew,[†] Fuqiang Wang,[‡] Kevin A. Scott,[†] Nathan J. Oldenhuis,[†] Haoping Liu,[‡] and David L. Van Vranken*[,†]

[†]Department of Chemistry, University of California, 1102 Natural Sciences 2, Irvine, California 92697-2025, United States

[‡]Department of Biological Chemistry, University of California, Irvine, California 92697-1700, United States

Supporting Information

ABSTRACT: Four phthalazinones (CIDs 22334057, 22333974, 22334032, 22334012) and one isoquinolone (CID 5224943) were previously shown to be potent enhancers of antifungal activity of fluconazole against *Candida albicans*. Several even more potent analogues of these compounds were identified, some with EC_{50} as low as 1 nM, against *C. albicans*. The compounds exhibited pharmacological synergy (FIC < 0.5) with fluconazole. The compounds were also shown to enhance the antifungal activity of isavuconazole, a recently FDA approved azole antifungal. Isoquinolone **15** and phthalazinone **24** were shown to be active against several resistant clinical isolates of *C. albicans*.



KEYWORDS: *Candida albicans, antifungal agents, fluconazole, synergy, phthalazinone, isoquinolone*

Azoles continue to be the drug of choice for many types of invasive fungal infections, acting on a key enzyme, sterol 14α-demethylase, in the ergosterol biosynthesis pathway. The azole family of antifungals has evolved continuously since the initial introduction of ketoconazole in the 1980s[1] with the goal of achieving high affinity toward the fungal P450 14α-demethylase, low affinity toward human CYP enzymes,[2,3] and, more recently, evasion of fungal resistance mechanisms.[4] Fluconazole, introduced in 1990, proved well-tolerated in patients and has been used ubiquitously to treat invasive candidiasis.[1] The emergence of fluconazole resistance has led to the increasing use of echinocandins and the development of third-generation azoles (voriconazole, posoconazole, isavuconazole) with higher affinity.[5] Of the third-generation drugs, posoconazole and voriconazole work against a broader range of fungal pathogens but are more expensive and have other disadvantages: posoconazole has a less flexible dosing and absorption profile than fluconazole; voriconazole may be ineffective against strains that have already developed resistance toward fluconazole.[6] Newer azole drugs like albaconazole and fosravuconazole are still in development.

The development of improved azoles has been paralleled by the search for small molecules that enhance the antifungal effect of existing azoles,[7−10] but the efforts have been met with limited success.[11,12] A wide range of azole enhancers have been shown to exhibit antifungal synergy;[13] two approved drugs, flucytosine[14] and calcineurin inhibitors,[15−19] have been shown to synergize with fluconazole against at low concentrations (<10 μg/mL) against strains of *C. albicans* but have limitations for general use. Against other species,[20] flucytosine instead antagonizes the effect of fluconazole, so the benefits of flucytosine-azole combinations are not clear.[21] Calcineurin inhibitors such as sirolimus and tacrolimus depend on human CYP enzymes for metabolic clearance; azole drugs like fluconazole exert off-target effects on these human CYP enzymes. Buildup of these calcineurin inhibitors in plasma increases risk of nephrotoxicity and, as immunosuppressants, may increase rates of infection from other pathogenic fungal species.[22−24] Given the limitations of these existing azole synergizers, new potent alternatives are needed.

Lindquist, Schreiber, and co-workers carried out a screening campaign to identify small molecules that could enhance the antifungal effect of fluconazole against *C. albicans*.[25,26] After an initial screen of over 300,000 compounds and a subsequent rescreening, 296 compounds were found to enhance the antifungal effect of fluconazole against a partially resistant clinical strain CaCi-8 without cytotoxicity against mammalian cells. Three of those compounds[27,28,26] were selected for further optimization, but none of the resulting compounds (ML189, ML212, and ML229) were active below 0.7 μM against CaCi-8. Following up on another hit from the Lindquist−Schreiber screen, we recently reported an analogue called synazo-1 that could enhance the antifungal effect of fluconazole with an EC_{50} of 300 pM.[29] Encouraged by the discovery of potent fluconazole synergizers we set out to identify new lead compounds, optimize their structures, study their synergy with other azoles, and assess their selectivity for *C. albicans*.

Among the compounds initially screened by Lindquist and coworkers were 233 structurally related *N*-arylphthalazinones (X = N, Figure 1) and *N*-arylisoquinolones (X = CH). Four phthalazinones and one isoquinolone were active in the initial screen. The most active compound of the five was phthalazinone

Received: September 6, 2016
Accepted: January 11, 2017
Published: January 11, 2017

DOI: 10.1021/acsmedchemlett.6b00355
ACS Med. Chem. Lett. 2017, 8, 168−173

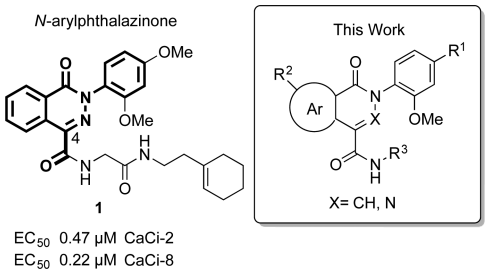


Figure 1. Structure of lead phthalazinone (CID 22334057) and representative structure of analogues synthesized in this study.

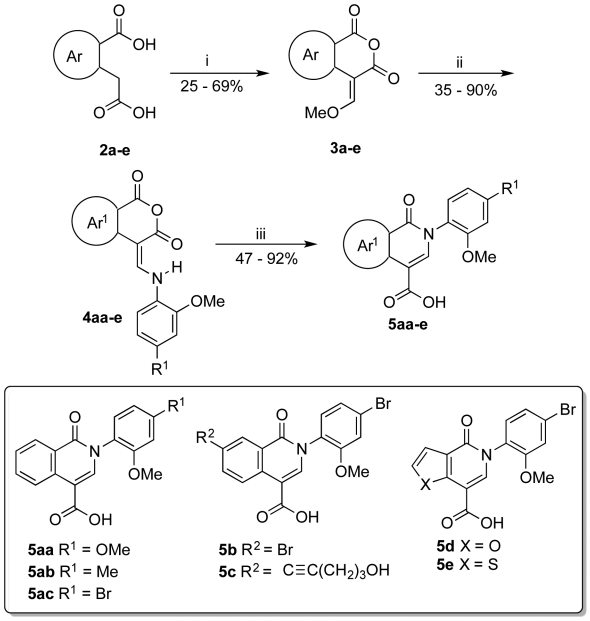
1 (CID 22334057), which showed an EC_{50} below 0.5 μM for *C. albicans* clincal isolates CaCi-8 and CaCi-2.

An initial analysis of the Lindquist data suggested that the biological activity of phthalazinones was sensitive to substituents on the *N*-phenyl ring and the C4 carboxamide side chain (Figure 1). In particular, a *para* substituent on the *N*-phenyl ring was favorable for activity, and an *ortho* substituent seemed essential. Additionally, the glycine linker was present in all of the active phthalazinones. The effect of modifications within the bicyclic core, at the atom X within the heterocycle or the fused benzo ring, were unclear. Analogues of the isoquinolone and phthalazinone compounds were therefore synthesized to explore chemical modification at three sites: (a) modification of the bicyclic core, including substitution of X (N versus CH), substitution of the fused benzo ring, or replacement of the benzo ring with a heterocycle; (b) substitution at the *para* position of the *N*-phenyl ring; (c) variation of the C4 carboxamide side chain.

All analogues were synthesized by coupling heterocyclic carboxylic acids with amines. Isoquinolone analogues were synthesized from the corresponding dicarboxylic acids **2a−e** following a route developed by Wolfbeis (Scheme 1).[30] A one-step anhydride formation and formylation generated enol ethers **3a−e**. Conjugate substitution of the methoxy group with various anilines generated the corresponding enamines **4aa−e**. Ethoxide catalyzed the Wolfbeis rearrangement of anhydrides **4aa−e** to isoquinolones **5aa−e**.[31]

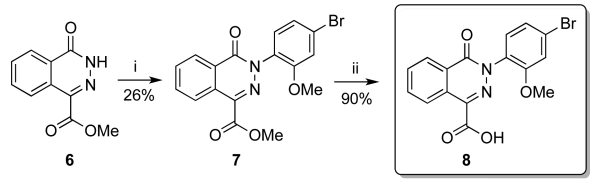
The original Dieckmann synthesis[32] of *N*-arylphthalazinones was problematic due to the capricious reactivity of electron-rich arylhydrazines. *N*-Arylphthalazinone **7** was instead synthesized using a Cu-promoted cross-coupling of phthalazinone **6** with an arylboronic acid (Scheme 2). Ester **7** was saponified to afford carboxylic acid **8**.

Scheme 1. Synthesis of Isoquinolone Carboxylic Acids[a]



[a]Reagents and conditions: (i) 1.0 mol-equiv of $CH(OCH_3)_3$, Ac_2O, 140 °C, 5 min-2.5 h; (ii) 1.0 mol-equiv of $ArNH_2$, 1,4-dioxane, 23 °C, 1−4 h; (iii) 5.0 mol-equiv of NaOH, EtOH, 80 °C, 1−4 h.

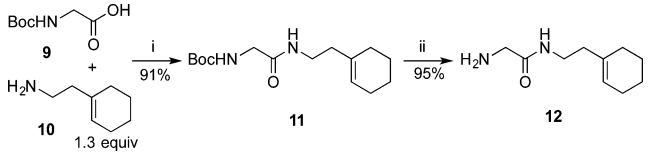
Scheme 2. Synthesis of Phthalazinone Carboxylic Acid[a]



[a]Reagents and conditions: (i) 2 mol-equiv of $Cu(OAc)_2$, 5 mol-equiv of pyridine, CH_2Cl_2, 23 °C, 24 h; (ii) 3.8 mol-equiv of LiOH, 3:1 THF/H_2O, 23 °C, 16 h.

Several 2-amino acetamide fragments were synthesized in order to couple with carboxylic acids **5aa−e** and **8** (Scheme 3).

Scheme 3. Representative Synthesis of 2-Amino Acetamide Analogues[a]



[a]Reagents and conditions: (i) 1.3 mol-equiv of EDC, 1.7 mol-equiv of HOBT, 5.5 mol-equiv of DIPEA, THF, 23 °C, 16 h; (ii) H_2O, 100 °C, 16 h.

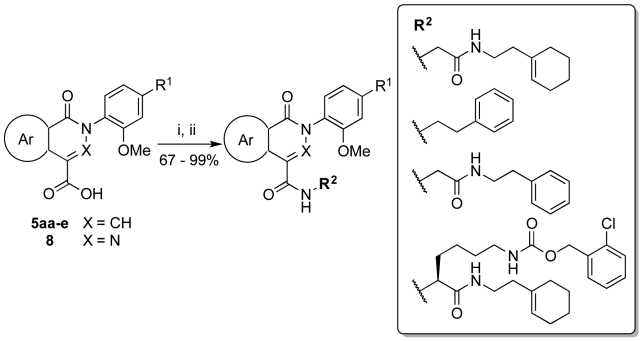
For illustrative purposes, the synthesis of amine **12** is described in Scheme 3. *N*-Boc-glycine **9** was coupled with 2-(1-cyclohexenyl)ethylamine **10** through a carbodiimide coupling reaction to afford amide **11** in good yield.[33] Deprotection of the Boc group in the presence of the cyclohexenyl group, however, was found to be challenging. Common methods such as treatment with trifluoroacetic acid resulted in complete degradation of the starting material due to protonation of the alkene. Consequently, a thermal deprotection was employed to generate amine **12**. Compound **11** was deproprotected by heating at reflux in water to remove the Boc group.[34] However, related compounds with limited solubility in water were instead heated under reflux in ethylene glycol.[35]

Carboxylic acids **5aa−e** and **8** were converted to the corresponding acid chlorides under Vilsmeier conditions and then directly coupled with amines in the presence of triethylamine (Scheme 4) to afford analogues **13−15** and **17−24** in good yields (Table 1).[36]

To test the steric demands around the *N*-phenyl group the bromine substituent of isoquinolone **15** was converted to the corresponding ω-hydroxyalkyne using a palladium-catalyzed Sonogashira coupling (Scheme 5).

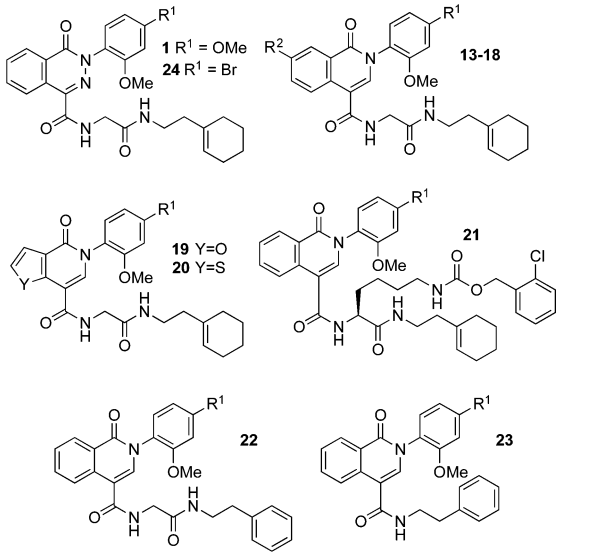
DOI: 10.1021/acsmedchemlett.6b00355
ACS Med. Chem. Lett. 2017, 8, 168−173

Scheme 4. Coupling of Carboxylic Acid and Amine Fragments[a]



[a]Reagents and conditions: (i) 1.5 mol-equiv of oxalyl chloride, 10 mol % DMF, CH_2Cl_2, 0 °C, 2 h; (ii) 1.2 mol-equiv of Et_3N, CH_2Cl_2, 0−23 °C, 30 min.

Table 1. Antifungal Activities for Phthalazinone and Isoquinolone Analogues Against *C. albicans* in the Presence of Fluconazole[a]

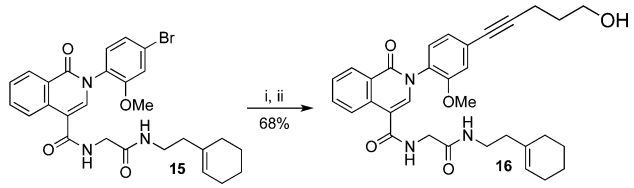


compd	R^1	R^2	EC_{50} (μM)
1	OMe		0.015 ± 0.002
13	OMe	H	0.033 ± 0.005
14	Me	H	0.065 ± 0.02
15	Br	H	0.003 ± 0.001
16	C≡C$(CH_2)_3$OH	H	>100
17	Br	Br	0.003 ± 0.001
18	Br	C≡C$(CH_2)_3$OH	>100
19	Br		>10
20	Br		0.230 ± 0.01
21	Br		0.023 ± 0.008
22	Br		0.020 ± 0.006
23	Br		0.220 ± 0.05
24	Br		0.001 ± 0.0003

[a]Cells incubated with 0.25 μg/mL fluconazole.

We determined the enhancement of antifungal activity for all of the new analogues against a susceptible strain of *C. albicans*, HLY4123, derived from the common laboratory strain CAI4.[29] In the presence of a constant, nonlethal concentration of fluconazole (0.25 μg/mL), the analogues inhibited fungal cell growth in a dose-dependent fashion, from which EC_{50} values were determined (Table 1).

Scheme 5. Derivatization of Analogue 15 via Sonogashira Coupling[a]



[a]Reagents and conditions: (i) 1.2 mol-equiv of 4-pentyn-1-ol, 5 mol % $Pd(Cl)_2(PPh_3)_2$, 5 mol % CuI, 3:2 DMF/Et_3N, 100 °C, 45 min.

Against HLY4123, phthalazinone **1** demonstrated promising activity (EC_{50} 0.015 μM) in the presence of 0.25 μg/mL fluconazole. The isostere, isoquinolone **13**, exhibited a similar EC_{50} of 0.033 μM suggesting that both the isoquinolone and phthalazinone cores are competent scaffolds for eliciting antifungal activity. Only one of five active compounds in the initial Lindquist−Schreiber screen possessed an isoquinolone core, so it was unclear which structural features most influenced antifungal activity. We set out to examine the isoquinolone core further by making modifications to the *N*-phenyl ring and benzo ring of the core, as well as the C4 carboxamide side chain.

First, we explored the effect of substitution at the *para* position of the *N*-phenyl ring (R^1). A decrease in activity was observed when methoxy (isoquinolone **13**) was replaced by a methyl group (isoquinolone **14**) at this position.

A bromine atom at the *para* position led to a surprising improvement in activity, with bromo-analogue **15** exhibiting 10-fold better activity than methoxy-analogue **13**, and also better activity than phthalazinone **1**. Substitution at R^1 with an alcohol-terminated alkyne abolishes activity, suggesting that larger or more rigid substituents are detrimental at this position.

After establishing the importance of the bromine substituent in isoquinolone **15**, we proceeded to evaluate the arene ring fused to the central heterocycle. Substitution with bromine at the C7 position of the benzo ring (R^2) resulted in activity of analogue **17** comparable to that of **15**. As with R^1, substitution at R^2 with an alcohol-terminated alkyne abolished activity. The sensitivity to substituents at R^1 and R^2 suggests that both positions play an important role in binding to the biological target.

Attempts to replace the benzo group with a smaller furano (analogue **19**) or thiopheno (analogue **20**) group resulted in a significant loss of potency. Even so, the thiophene analogue **20** showed significantly better activity than its electron-rich furan isostere **19**.

Neither contraction of the benzo ring nor expansion by substitution improved activity. Therefore, we directed our attention to the C4 carboxamide side chain. Using analogue **15** again as a reference point, we evaluated the effect of substitution at the glycine α-carbon. The activity of ClCbz-lysine analogue **21** was only slightly attenuated relative to glycine analogue **15**, revealing some tolerance for bulky substituents at this position of the side chain. We then prepared phenethyl amide **22** to test the importance of the cyclohexenylethyl group. The activity of cyclohexenylethyl analogue **15** was superior to that of the aromatic phenethyl analogue **22**. Shortened analogue **23**, missing the glycine linker, was 70-fold less active than full-length analogue **15**.

DOI: 10.1021/acsmedchemlett.6b00355
ACS Med. Chem. Lett. 2017, 8, 168−173

Table 2. Strains Used in This Study

species	strain	relevant characteristics or genotype					ref
Candida albicans	HLY4123	ERG3-GFP					Premachandra[29]
Candida glabrata	BG2	WT					Fidel[39,40]
Cryptococcus neoformans var. *grubii*	H99	WT					Broad Institute[41]
			expression (fold)[a]				
Candida albicans	clinical isolate	mutations	*ERG11*	*CDR1*	*CDR2*	*MDR1*	Flowers[37]
	17	ERG11A114S,Y257H	1.6	2.7	23.9	0.8	
	26	ERG11G307S,G448R	1.0	0.9	0.5	2.6	
	33	ERG11F145L,E266D	1.7	16.2	1465.6	4.1	
	36	UPC2Y642F	12.6	6.8	201.5	1.5	
	45	ERG11E266D,G464S, UPC2A646V	3.5	4.8	147.8	15.5	

[a]Fold increase relative to expression levels averaged across three unrelated fluconazole-susceptible strains.

Phthalazinone **24** was slightly more active than the isosteric bromophenyl isoquinolone **15**. With an EC_{50} of 1 nM, bromophenyl phthalazinone **24** was the most active of all compounds tested.

Lead compound **1**, isoquinolone **15**, and phthalazinone **24** showed potent activity even at fluconazole concentrations of 0.05 μg/mL, 5-fold lower than that used in Table 1. The EC_{50} values were measured as 49, 24, and 7 nM, respectively.

Next, highly active bromophenyl analogues **15** and **24** were tested against five fluconazole-resistant clinical isolates of *C. albicans* (Table 2). Resistance in these isolates has been linked to multiple factors, including overexpression and point mutations in *ERG11*,[37] the gene encoding sterol 14α-demethylase. Point mutations in *ERG11* may contribute to fluconazole resistance by reducing binding affinity to the enzyme active site.[38] In some cases, the isolates also exhibit gain-of-function mutations in *UPC2*, a transcription factor regulating *ERG11* expression. The isolates also show varying levels of *CDR1*, *CDR2*, and *MDR1* ression, implicating efflux pumps as an important source of fluconazole resistance.

In dose-dependent broth microdilution assays, analogues **15** and **24** showed potent activities (EC_{50} < 1 μM) against all resistant isolates. The EC_{50} values were determined by varying the analogue concentration while keeping the fluconazole concentration fixed. The fluconazole MIC_{50} was used and was determined for each isolate individually. Compound **24** exhibited comparable or better activity than compound **15** against the isolates tested (Table 3). At present, the origin of fluconazole synergy is uncertain. Many factors contribute to fluconazole resistance in these isolates, and we did not observe a correlation of compound activity with one specific resistance mechanism, such as levels of *CDR1* expression, among the isolates we tested here. Therefore, we are unable to attribute the synergistic effect of compounds **15** and **24** with fluconazole to any single variable. Investigation into the possible causes of fluconazole synergy is ongoing.

Table 3. Antifungal Activities of Analogues 15 and 24 against Resistant Isolates of *C. albicans* in the Presence of Fluconazole

		EC_{50}[a]	
isolate	[Flu][b]	**15**	**24**
17	12	74 ± 10	39 ± 3
26	32	11 ± 1	17 ± 2
33	64	371 ± 23	72 ± 6
36	64	32 ± 4	20 ± 2
45	96	170 ± 20	65 ± 15

[a]EC_{50} (nM) expressed as arithmetic mean ± SD of three independent experiments. [b]Measured MIC_{50} values for fluconazole alone; EC_{50} values were determined at the fluconazole concentrations listed.

Compounds **1**, **15**, and **24** were also tested against *Candida glabrata* BG2[39,40] and *Cryptococcus neoformans* var. *grubii* H99[41] and showed no activity up to 30 μM at their MIC_{50} values (128 and 4.0 μg/mL, respectively). It is not clear why analogues **15** and **24** are active against *C. albicans* but not *C. glabrata* or *Cryptococcus neoformans*. We are currently investigating this issue.

Isavuconazole is one of the newest azole drugs, approved by FDA in 2015. We tested the potency of compounds **15** and **24** against HLY4123 and the highly resistant CaCi-45 in the presence of isavuconazole (Table 4). Compounds **15** and **24** showed potent antifungal activity with isavuconazole at much lower concentrations than was required for fluconazole.

Table 4. Antifungal Activities[a] of Analogues 15 and 24 against *C. albicans* in the Presence of Isavuconazole

	isolate	
compd	HLY4123 ([isa] = 0.001 μg/mL)[b]	CaCi-45 ([isa] = 0.5 μg/mL)[b]
15	6 ± 0.2	110 ± 20
24	4 ± 0.2	13 ± 4

[a]EC_{50} (nM) expressed as arithmetic mean ± SD of three independent experiments. [b]MIC values

At 30 μM or below, the analogues do not significantly affect fungal growth in the absence of azoles. Checkerboard assays revealed that compounds **15** and **24** are true synergizers with fluconazole, exhibiting fractional inhibitory concentration indices of less than 0.17 and 0.12, respectively. These indices are well below the upper-limit of 0.5 for pharmacological synergy (Supporting Information, 29−30).

All analogues were also tested for cytotoxicity against 3T3 mammalian fibroblasts. No cytotoxicity was observed up to 10 μM, indicating high selectivity for inhibiting *C. albicans* cell growth over that of mammalian fibroblasts.

In order to assess the potential for compounds **15** and **24** as drug leads, the physicochemical properties were calculated using FAF-Drugs3 (Supporting Information, 32). The bromine atom is beneficial for activity but puts the molecular weight over 500. Both compounds exhibit favorable characteristics according to other criteria including tPSA, predicted bioavailability, and predicted induction of phospholipidosis but were slightly lipophilic based on logD.

In conclusion we have designed, synthesized, and studied phthalazinone and isoquinolone analogues of lead compound **1** (CID 22334057), which was previously shown to be active

DOI: 10.1021/acsmedchemlett.6b00355
ACS Med. Chem. Lett. 2017, 8, 168−173

against *C. albicans* in the presence of fluconazole. Most of the analogues synthesized showed highly potent activities against a susceptible strain of *C. albicans*, HLY4123. Two compounds, isoquinolone analogue **15** and the other a phthalazinone analogue **24**, were selected for further studies and shown to be active against clinical isolates with high resistance to fluconazole. Checkerboard assays confirmed compounds **15** and **24** as true synergizers of fluconazole. Also, compounds **15** and **24** were shown to increase the efficacy of the new azole drug, isavuconazole.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.6b00355.

Complete experimental details on synthetic and bioassay methods (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*(D.L.V.V.) E-mail: david.vv@uci.edu.

ORCID

David L. Van Vranken: 0000-0001-5964-7042

Author Contributions

‖A.D.M. and I.D.U.A.P. contributed equally to this work.

Funding

This work was supported by the NIH R01 AI099190.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Dr. David Rogers for providing us with resistant clinical isolates of *C. albicans* used for this study. We are also grateful to Dr. Zhibin Guan for assistance with the mammalian cytotoxicity assays.

■ REFERENCES

(1) Maertens, J. A. History of the development of azole derivatives. *Clin. Microbiol. Infect.* **2004**, *1*, 1−10.

(2) Mast, N.; Zheng, W.; Stout, C. D.; Pikuleva, I. A. Antifungal Azoles: Structural Insights into Undesired Tight Binding to Cholesterol-Metabolizing CYP46A1. *Mol. Pharmacol.* **2013**, *84*, 86−94.

(3) Zonios, I.; Bennett, J. Update on Azole Antifungals. *Semin. Respir. Crit. Care Med.* **2008**, *29*, 198−210.

(4) Lass-Flörl, C. Triazole antifungal agents in invasive fungal infections: a comparative review. *Drugs* **2011**, *71*, 2405−2419.

(5) Mast, N.; Zheng, W.; Stout, C. D.; Pikuleva, I. A. Antifungal Azoles: Structural Insights into Undesired Tight Binding to Cholesterol-Metabolizing CYP46A1. *Mol. Pharmacol.* **2013**, *84*, 86−94.

(6) Panackal, A.; Gribskov, J.; Staab, J.; Kirby, A.; Rinaldi, M.; Marr, K. Clinical significance of azole antifungal drug cross-resistance in *Candida glabrata*. *J. Clin. Microbiol.* **2006**, *44*, 1740−1743.

(7) LaFleur, M.; Lucumi, E.; Napper, A.; Diamond, S.; Lewis, K. Novel high-throughput screen against *Candida albicans* identifies antifungal potentiators and agents effective against biofilms. *J. Antimicrob. Chemother.* **2011**, *66*, 820−826.

(8) Spitzer, M.; Griffiths, E.; Blakely, K.; Wildenhain, J.; Ejim, L.; Rossi, L.; De Pascale, G.; Curak, J.; Brown, E.; Tyers, M.; Wright, G. D. Cross-species discovery of syncretic drug combinations that potentiate the antifungal fluconazole. *Mol. Syst. Biol.* **2011**, *21*, 1−14.

(9) Robbins, N.; Spitzer, M.; Yu, T.; Cerone, R.; Averette, A.; Bahn, Y.; Heitman, J.; Sheppard, D.; Tyers, M.; Wright, G. An Antifungal Combination Matrix Identifies a Rich Pool of Adjuvant Molecules that Enhance Drug Activity against Diverse Fungal Pathogens. *Cell Rep.* **2015**, *13*, 1481−1492.

(10) Liu, H.; Wang, L.; Li, Y.; Liu, J.; An, M.; Zhu, S.; Cao, Y.; Jiang, Z.; Zhao, M.; Cai, Z.; Dai, L.; Ni, T.; Liu, W.; Chen, S.; Wei, C.; Zang, C.; Tian, S.; Yang, J.; Wu, C.; Zhang, D.; Liu, H.; Jiang, Y. Structural optimization of berberine as a synergist to restore antifungal activity of fluconazole against drug-resistant *Candida albicans*. *ChemMedChem* **2014**, *9*, 207−216.

(11) Chaturvedi, V.; Ramani, R.; Andes, D.; Diekema, D.; Pfaller, M.; Ghannoum, M.; Knapp, C.; Lockhart, S.; Ostrosky-Zeichner, L.; Walsh, T.; Marchillo, K.; Messer, S.; Welshenbaugh, A.; Bastulli, C.; Iqbal, N.; Paetznick, V.; Rodriguez, J.; Sein, T. Multilaboratory testing of two-drug combinations of antifungals against *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis*. *Antimicrob. Agents Chemother.* **2011**, *55*, 1543−1548.

(12) Mukherjee, P.; Sheehan, D.; Hitchcock, C. Combination treatment of invasive fungal infections. *Clin. Microbiol. Rev.* **2005**, *18*, 163−194.

(13) Cui, J.; Ren, B.; Tong, Y.; Dai, H.; Zhang, L. Synergistic combinations of antifungals and anti-virulence agents to fight against *Candida albicans*. *Virulence* **2015**, *6*, 362−71.

(14) Girmenia, C.; Venditti, M.; Martino, P. Fluconazole in combination with flucytosine in the treatment of fluconazole-resistant Candida infections. *Diagn. Microbiol. Infect. Dis.* **2003**, *46*, 227−31.

(15) Onyewu, C.; Blankenship, J.; Del Poeta, M.; Heitman, J. Ergosterol biosynthesis inhibitors become fungicidal when combined with calcineurin inhibitors against Candida albicans, Candida glabrata, and Candida krusei. *Antimicrob. Agents Chemother.* **2003**, *47*, 956−964.

(16) Sun, S.; Li, Y.; Guo, Q.; Shi, C.; Yu, J.; Ma, L. In vitro inter- actions between tacrolimus and azoles against *Candida albicans* determined by different methods. *Antimicrob. Agents Chemother.* **2008**, *52*, 409−417.

(17) Uppuluri, P.; Nett, J.; Heitman, J.; Andes, D. Synergistic effect of calcineurin inhibitors and fluconazole against *Candida albicans* biofilms. *Antimicrob. Agents Chemother.* **2008**, *52*, 1127−1132.

(18) Denardi, L.; Mario, D.; Loreto, É.; Santurio, J.; Alves, S. Synergistic effects of tacrolimus and azole antifungal compounds in fluconazole-susceptible and fluconazole-resistant Candida glabrata isolates. *Braz. J. Microbiol.* **2015**, *46*, 125−129.

(19) Steinbach, W.; Schell, W.; Blankenship, J.; Onyewu, C.; Heitman, J.; Perfect, J. In vitro interactions between antifungals and immunosuppressants against *Aspergillus fumigatus*. *Antimicrob. Agents Chemother.* **2004**, *48*, 1664−1669.

(20) Te Dorsthorst, D.; Verweij, P.; Meletiadis, J.; Bergervoet, M.; Punt, N.; Meis, J.; Mouton, J. In vitro interaction of flucytosine combined with amphotericin B or fluconazole against thirty-five yeast isolates determined by both the fractional inhibitory concentration index and the response surface approach. *Antimicrob. Agents Chemother.* **2002**, *46*, 2982−2989.

(21) Johnson, M.; MacDougall, C.; Ostrosky-Zeichner, L.; Perfect, J.; Rex, J. Combination Antifungal Therapy. *Antimicrob. Agents Chemother.* **2004**, *48*, 693−715.

(22) Kuypers, D.; Claes, K.; Evenepoel, P.; Vanrenterghem, Y. Clinically Relevant Drug Interaction Between Voriconazole and Tacrolimus in a Primary Renal Allograft Recipient. *Transplantation* **2006**, *81*, 1750−1752.

(23) Imbert, S.; Bresler, P.; Boissonnas, A.; Gauthier, L.; Souchet, L.; Uzunov, M.; Leblond, V.; Mazier, D.; Nguyen, S.; Fekkar, A. Calcineurin inhibitors impair neutrophil activity against *Aspergillus fumigatus* in allogenic hematopoietic stem cell transplant recipients. *J. Allergy Clin. Immunol.* **2016**, *138*, 860.

(24) Dodds-Ashley, E. Management of drug and food interactions with azole antifungal agents in transplant recipients. *Pharmacotherapy* **2010**, *30*, 842−854.

(25) National Center for Biotechnology Information. Summary of Broad Institute MLPCN Reversing Antifungal Drug Resistance Project. https://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=2007 (accessed Mar 11, 2015).

DOI: 10.1021/acsmedchemlett.6b00355
ACS Med. Chem. Lett. 2017, 8, 168−173

(26) Youngsaye, W.; Hartland, C.; Morgan, B.; Ting, A.; Nag, P.; Vincent, B.; Mosher, C.; Bittker, J.; Dandapani, S.; Palmer, M.; Whitesell, L.; Lindquist, S.; Schreiber, S.; Munoz, B. ML212: A small-molecule probe for investigating fluconazole resistance mechanisms in *Candida albicans*. *Beilstein J. Org. Chem.* **2013**, *9*, 1501−1507.
(27) Youngsaye, W.; Vincent, B.; Hartland, C.; Morgan, B.; Buhrlage, S.; Johnston, S.; Bittker, J.; MacPherson, L.; Dandapani, S.; Palmer, M.; Whitesell, L.; Lindquist, S.; Schreiber, S.; Munoz, B. Piperazinyl quinolines as chemosensitizers to increase fluconazole susceptibility of *Candida albicans* clinical isolates. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5502−5505.
(28) Youngsaye, W.; Dockendorff, C.; Vincent, B.; Hartland, C.; Bittker, J.; Dandapani, S.; Palmer, M.; Whitesell, L.; Lindquist, S.; Schreiber, S. L.; Munoz, B. Overcoming fluconazole resistance in *Candida albicans* clinical isolates with tetracyclic indoles. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3362−3365.
(29) Premachandra, I. D. U. A.; Scott, K. A.; Shen, C.; Wang, F.; Lane, S.; Liu, H.; Van Vranken, D. L. Potent Synergy between Spirocyclic Pyrrolidinoindolinones and Fluconazole against Candida albicans. *ChemMedChem* **2015**, *10*, 1672−1686.
(30) Wolfbeis, O. Synthesen von Fluoreszenzfarbstoffen, 11 über die Umlagerung von Alkoxymethylen- und Aminomethylenhomophthalsäureanhydriden zu Isocumarinen bzw. Isochinolinonen. *Liebigs Ann. Chem.* **1981**, *5*, 819−827.
(31) Patil, C. D.; Sadana, A.; Deodhar, S. Syntheses of 2-substituted-1,2-dihydro-1-oxo-4-isoquinoline-carboxamides and 4-amidoximino-2-substituted-1,2-dihydro-1-oxoisoquinolines as antimicrobial agents. *J. Indian Chem. Soc.* **1990**, *67*, 654−656.
(32) Dieckmann, W.; Meiser, W. Über Homophthalsäureester, Oxymethylen-homophthalsäureester und die aus ihm entstehenden Isocumarin-und Isocarbostyril-Derivate. *Ber. Dtsch. Chem. Ges.* **1908**, *41*, 3253−3269.
(33) Liu, B.; Yi, W.; Zhang, J.; Liu, Q.; Liu, Y.; Fan, S.; Yu, X. Synthesis and gene transfection of cyclen-based cationic lipids with asymmetric acyl-cholesteryl hydrophobic tails. *Org. Biomol. Chem.* **2014**, *12*, 3484−3492.
(34) Zinelaabidine, C.; Souad, O.; Zoubir, J.; Malika, B.; Nour-Eddine, A. A Simple and Efficient Green Method for the Deprotection of N-Boc in Various Structurally Diverse Amines under Water-mediated Catalyst-free Conditions. *Int. J. Chem.* **2012**, *4*, 73−79.
(35) Martin, L.; Coello, M.; Reyes, B.; Rodriguez, V.; Garranzo, G.; Murcia, P.; Francesch, S.; Sanchez, S.; Fernandez, R. Antitumoral dihydropyran-2-one compounds. WO Patent 2007 144 423, 2007.
(36) Liu, Z.; Wang, R.; Guo, R.; Hu, J.; Li, R.; Zhao, Y.; Gong, P. Design, synthesis and biological evaluation of novel 6,7-disubstituted-4-phenoxyquinoline derivatives bearing 4-oxo-3,4-dihydrophthalazine-1-carboxamide moieties as c-Met kinase inhibitors. *Bioorg. Med. Chem.* **2014**, *22*, 3642−3653.
(37) Flowers, S.; Barker, K.; Berkow, E.; Toner, G.; Chadwick, S.; Gygax, S.; Morschhauser, J.; Rogers, D. Gain-of-Function Mutations in *UPC2* Are a Frequent Cause of *ERG11* Upregulation in Azole-Resistant Clinical Isolates of *Candida albicans*. *Eukaryotic Cell* **2012**, *11*, 1289−1299.
(38) Warrilow, A. G.; Martel, C. M.; Parker, J. E.; Melo, N.; Lamb, D. C.; Nes, W. D.; Kelly, D. E.; Kelly, S. L. Azole Binding Properties of *Candida albicans* Sterol 14-α Demethylase (CaCYP51). *Antimicrob. Agents Chemother.* **2010**, *54*, 4235−4245.
(39) Cormack, B. P.; Falkow, S. Efficient homologous and illegitimate recombination in the opportunistic yeast pathogen *Candida glabrata*. *Genetics.* **1999**, *151*, 979−87.
(40) Fidel, P. L.; Cutright, J. L.; Tait, L.; Sobel, J. D. A Murine Model of *Candida glabrata Vaginitis*. *J. Infect. Dis.* **1996**, *173*, 425−431.
(41) Janbon, G.; et al. Analysis of the Genome and Transcriptome of *Cryptococcus neoformans var. grubii* Reveals Complex RNA Expression and Microevolution Leading to Virulence Attenuation. *PLoS Genet.* **2014**, *10*, 1−26.

DOI: 10.1021/acsmedchemlett.6b00355
ACS Med. Chem. Lett. 2017, 8, 168−173