Supporting Information

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SI Methods

Strains and Growth Conditions. The *Candida albicans* strains used were the WT clinical isolate SC5314 (1), SC5314-derived *edt1*$^{-/-}$ cells that lack an adhesion protein (2), or their respective GFP-expressing derivatives (2). Other fungi analyzed included *C. tropicalis* (a gift from A. B. Onderdonk, Harvard Medical School, Boston), *C. dubliniensis* (3), and *C. parapsilosis* strain CDC317 (4). These cells were grown in 6.7 g/L yeast nitrogen broth without amino acids (YNB) supplemented with 30 μg/mL L-leucine, 20 μg/mL L-histidine, 20 μg/mL L-tryptophan, and 25 μg/mL uridine (Supplemented YNB), with 2% glucose added except where indicated otherwise. The SGH284 strain (a gift from Aaron Mitchell, Carnegie Mellon University, Pittsburgh) was used as a hyphal reporter strain; this strain contains the red fluorescent protein (RFP) gene under the control of the endogenous, hyphal-specific *HWP1* promoter (5). This strain was grown in Supplemented YNB + 2% glucose that was additionally supplemented with 20 μg/mL L-arginine.

C. albicans strains taken in the 1990s from HIV-infected patients enrolled in a prospective clinical study at the University of Texas Health Science Center at San Antonio were obtained from Ted White (Fig. S4). The strains that we obtained were originally isolated from anonymous patient 59 as described (6, 7).

The *C. albicans* strains used for the chemical genetic experiments were gifts from Douglas Johnson (University of Vermont, VT) (8):

AV55 (*ura3::λimm434/ura3::λimm 434; LEU2::pCK1-efg1-T206E::URA3*),

DH409 (*ura3::λimm434/ura3; ras1-G13V*),

HTC7 (*ura3:: λimm 434/ura3::λimm 434; TRP1/TRP1::ADH1pr-gpa2-Q355L::URA3*),

CDH72-1 (*ura3/ura3 cph1Δ::hisG/cph1Δ::hisG; ADH1pr-CPH1*), and

YTC014 (*ura3:: λimm 434/ura3::λimm 434; TRP1/TRP1:: ADH1pr-GPR1-URA3*).

The *Caenorhabditis elegans* WT strain N2 was grown at 20 °C on nematode growth agar medium (NGM) spotted with *Escherichia coli* OP50 and maintained as described previously (9). *E. coli* OP50 was grown overnight in Luria broth at 37 °C.

Chemical Screen Data Analysis. Because of plate-to-plate variability in the magnitude of the signal for the DMSO control wells, we normalized the data. For each plate, the mean A_{590} value from column 1 was set to 0.0, and the mean A_{590} value from column 12 was set to 1.0, with each experimental well value normalized to these controls. Normalized values from the entire screen were then ranked. Forty compounds yielded a normalized value <0.25 (that is, >75% inhibition of adhesion). Many of these compounds fell into two structural subclasses that we termed Scaffolds 1 and 2 (Table S1). We reordered 26 of these compounds (Chembridge) for additional characterization, omitting highly similar ones. Compounds 27, Q1, and Q2 were also obtained from Chembridge. Statistical analyses were carried out using Graphpad Prism5.

alamarBlue-Based Adhesion Assay. Overnight cultures of SC5314 and *edt1*$^{-/-}$cells were grown and plated onto Immulon 2HB 96-well microtiter plates and treated with small molecules as described above. After incubations, media were decanted, and plates were washed two times with 100 μL/well PBS. alamarBlue (Life Technologies) was resuspended to a final concentration of 5% in RPMI buffered with 0.165 M Mops (pH 7.0), and then, 100 μL were added to each well followed by additional incubation for 2 h at 37 °C. Fluorescence signals at 555_{Ex}/585_{Em} were read using a SpectraMax M5 Plate reader (Molecular Devices).

For *Candida* species other than *C. albicans*, the standard alamarBlue-based adhesion assay conditions were followed, except for *C. tropicalis*, which adhered less well and therefore, was subjected to a single wash after coincubation with drugs (Fig. 2*E*).

GFP-Based Polystyrene Adhesion Assay. SC5314-GFP and *edt1*$^{-/-}$-GFP strains were pregrown overnight in Supplemented YNB + 2% glucose media at 30 °C, diluted into fresh Supplemented YNB + 2% glucose media to a final concentration of 5×10^6 cells/mL (equivalent to 0.5 OD_{600}/mL), and plated onto Immulon 2HB 96-well plates, with each well receiving 0.1 OD of cells. DMSO or compounds resuspended in DMSO were added to the desired final concentration (1% vol/vol DMSO was maintained). Plates were then incubated for 4 h at 37 °C. Media were decanted, and plates were washed three times with PBS using a MicroFill microplate dispenser (BioTek), with each well receiving 100 μL PBS. Fluorescence was measured using a Synergy HT plate reader (BioTek) after the addition of 100 μL PBS/well. For each experiment, untagged SC5314 was analyzed simultaneously to determine background fluorescence that was subtracted from the signal from each well. DIC and fluorescence images were acquired using a Axiovert 200 microscope (Zeiss) equipped with a QICAM FAST cooled mono 12-bit digital camera (Qimaging). Image acquisition was carried out using Openlab imaging software (PerkinElmer).

Epithelial Cell Binding Assay. Human lung epithelial A549 cells (10) were grown to confluence on Nunc 48 well plates in RPMI + 10% FBS and Penicillin/Streptomycin. Media were decanted, and plates were carefully washed three times with PBS to remove unbound cells. Pregrown overnight cultures of SC5314-GFP and *edt1*$^{-/-}$-GFP *C. albicans* cells were resuspended in fresh Supplemented YNB + 2% glucose at 0.5 OD/mL, and 250 μL yeast suspension were plated onto to each well. After incubation at 37 °C for 1.5 h, media were decanted, and the monolayers were washed carefully three times with 250 μL PBS. Fluorescence from bound yeast was measured (485_{Ex}/535_{Em}) using the Synergy HT plate reader (BioTek) after the addition of 100 μL PBS/well. DIC and fluorescence images were acquired as described above for the GFP-based adhesion assay.

Adhesion Reversal Assay. SC5314 cells were pregrown overnight in Supplemented YNB + 2% glucose and resuspended in fresh Supplemented YNB + 2% glucose to a final concentration of 1 OD_{600}/mL. Cells were then seeded into Immulon 2HB 96-well plates at 0.1 OD (100 μL) per well. One-half of the samples remained untreated at this point (to test for subsequent reversion test), and one-half received either 50 μM filastatin or 1% DMSO (measuring the effect of continuous exposure to compound). Plates were then incubated for 4 h at 37 °C. After this incubation, an additional 100 μL/well Supplemented YNB + 2% glucose were added, bringing final concentrations to 50 μM filastatin or 1% DMSO. Plates were then returned to the 37 °C incubator for an additional 4 h. At the end of 8 h of incubation, all media were decanted, and the plate was washed two times

with 100 μL/well PBS. This washing was followed by the addition of 100 μL/well 5% alamarBlue in RPMI buffered with 0.165 M Mops (pH 7.0) and additional incubation for 2 h at 37 °C. Fluorescence signals at $555_{Ex}/585_{Em}$ were read using a SpectraMax M5 Plate reader (Molecular Devices).

Filamentation Assays. ***HWP1-RFP reporter.*** Overnight cultures of SGH584 were pregrown in Supplemented YNB + 2% glucose + arginine media at 30 °C to an OD_{600} > 13, diluted 1:10 into prewarmed Spider medium [1% Peptone (Difco), 1% Mannitol (Sigma), and 0.2% K_2HPO_4 (Sigma)] (11) in glass tubes, and grown for 16 h at 37 °C shaking at 200 rpm in the presence or absence of drugs as indicated. DIC and fluorescence images were acquired using a Zeiss Axioplan 2 microscope equipped with an Orca ERG 1394 digital camera (Hamamatsu). Image acquisition was carried out using AxioVision 3 imaging software (Zeiss).

Chemical genetics. Overnight cultures were grown at 30 °C in Supplemented YNB + 2% glucose. The next day, cells were resuspended to 0.1 OD_{600}/mL in fresh Supplemented YNB + 2% glucose and grown at 30 °C to a density of 0.4 OD_{600}/mL. Cells were pelleted and resuspended in Spider medium supplemented with either 1% DMSO alone or 1% DMSO plus 50 μM filastatin and incubated at 37 °C for 4 h. Cells were stained with 1 μg/mL Calcofluor white for 15 min and vortexed thoroughly to disperse cells. DIC and fluorescent images of cells were obtained using an EVOS fluorescent microscope (Life Technologies) using a 20× objective.

Spider and serum liquid media (Fig. 3B). SC5314 cells were grown overnight at 30 °C in Supplemented YNB + 2% glucose to 13 OD_{600}/mL. Cells were diluted 1:10 into either Spider media or Supplemented YNB + 2% glucose + 10% bovine serum as indicated and grown at 37 °C for the indicated period. Cells were photographed with DIC optics on an EVOS microscope using a 10× objective.

GlcNAc media. SC5314 cells were grown overnight in Supplemented YNB + 2% glucose to 13 OD_{600}/mL. Cells were diluted 1:10 into GlcNAc media (0.5% GlcNAc, 0.5% peptone, 0.3% KH_2PO_4) (8) and grown at 37 °C for 16 h. Calcofluor-stained cells were imaged as above.

Solid Spider media for colony morphology assays. Spider media agar plates (25 mL agar/plate) were overlaid with 250 μL 100% DMSO or 5 mM stock of filastatin in DMSO, yielding final concentrations of 1% DMSO with or without 50 μM filastatin. SC5314 or YTC104 (*ADHpr-GPR1*) cells pregrown overnight in Supplemented YNB media were diluted into Supplemented YNB to a final concentration of 100 cells/mL, and 200 μL were spread onto the plates. Plates were sealed with Saran wrap and incubated at 37 °C for the indicated times. Images of colony edges were obtained using the EVOS microscope (10× objective). Fields of colonies were imaged using a Nikon SMZ800 microscope fitted with a Nikon Coolpix digital camera, and single colonies were imaged using a Leica M165 FC stereo microscope at 12.5× magnification.

Hydroxyurea-induced filamentation. SC5314 cells were pregrown overnight in YPD at 30 °C, diluted into fresh YPD at a concentration of 5×10^6 cells/mL, and grown for 2 h at 30 °C. Hydroxyurea was then added to a final concentration of 50 mM, and growth was continued at 30 °C for 5 h. Calcofluor-stained cells were imaged as for the GlcNac experiment.

Measurement of Toxicity of Filastatin. A549 cells in RPMI + 10% FBS were seeded into 24-well plates at 1×10^5 cells/well and grown overnight at 37 °C. Media were removed and replaced with fresh media containing indicated concentrations of filastatin or 1% sodium azide followed by incubation for 24 h. Viability was determined with 5% alamarBlue as above.

Biofilm Formation on Silicone Elastomers. We adapted a published method (12). Autoclaved, preweighed silicone elastomer squares (PR72034-04N; Bentec) were incubated overnight at 37 °C with undiluted bovine serum. After washing with PBS, the squares were transferred to 12-well plates and incubated with 0.5 OD/mL SC5314-GFP or *edt1*$^{-/-}$-GFP cells in Spider medium (with or without drugs as indicated) for 90 min at 37 °C and shaking at 200 rpm. Squares were washed in PBS and transferred to new 12-well plates containing fresh Spider medium with fresh drugs, and then, they were incubated for 60 h in a 37 °C shaker at 200 rpm. The plates were then photographed, the liquid media were collected for A_{600} measurements, and the dry weights of the bound biofilms were measured after drying of the silicone squares in a chemical hood.

Growth Rate Measurements in Spider Media. ***OD.*** Overnight cultures of SC5314 cells grown in Supplemented YNB + 2% glucose media were diluted into Spider media at a concentration of 0.1 OD/mL and allowed to grow at 37 °C in the presence of either 50 μM filastatin or 1% DMSO as a vehicle control. The means and SDs of OD measurements at the indicated times from three biological replicates are shown. Additional 100-μL aliquots taken at indicated times were fixed with 4% paraformaldehyde for 30 min at room temperature. These aliquots were then stained with Calcofluor and analyzed by microscopy as described for the chemical genetics experiments.

alamarBlue. Cultures were grown as above. At the indicated times after transfer to Spider media, 100 μL culture were added to an equal volume of 5% alamarBlue in RPMI and incubated at 37 °C for 30 min. The resulting fluorescent signals were measured on a BioTek plate reader as described above.

C. *elegans* Egg Preparation. Three L3/L4 stage larvae of *C. elegans* strain N2 were transferred to NGM agar plates seeded with *E. coli* OP50 and grown at 20 °C for 4 d. The worms were washed off the plates with M9 buffer, and the collected liquid was centrifuged at 900 × *g* for 2 min. The supernatants were aspirated, and the pellets were resuspended in 15 mL 1:4 dilution commercial bleach solution (5.25%) containing 0.25 M NaOH to release the eggs. The suspensions were mixed by inversion for 3 min and then centrifuged for 2 min at 2,000 × *g*. The supernatants were aspirated, and the pellets containing the eggs was washed two times with M9 buffer and resuspended to a final concentration of 5–6 eggs/μL; 20 μL egg prep solution were added to NGM plates seeded with *E. coli* OP50. The plates were incubated at 20 °C for 2.5 d to obtain a large number of young adults.

C. *elegans* Deformed Anal Region Disease Assays. Deformed anal region disease was monitored as previously described (13, 14). Briefly, *E. coli* OP50 and *C. albicans* SC5314 cells were grown separately overnight at 37 °C. Cells were harvested, and *E. coli* growth was attenuated by addition of 10 μL 50 mg/mL streptomycin; the mixture was spotted onto NGM agar plates. When needed, compounds were added to the mixture to a final concentration of 12.5 μM. Uninfected control plates were spotted with *E. coli* alone. A suspension of *C. elegans* eggs was transferred to each plate. Plates were incubated at 20 °C, and deformed anal region disease was visually evaluated for the hatched larvae on day 4.

C. *elegans* Survival Assays. *E. coli* and *C. albicans* strains were grown from single colonies overnight at 37 °C in LB and 30 °C in YPD, respectively. Culture aliquots were centrifuged at full speed for 1 min. The pellets washed two times in sterile water and finally resuspended to a final concentration of 200 mg/mL for *E. coli* and OD_{600} = 3 for *C. albicans*; 10 μL 50 mg/mL streptomycin solution were mixed with 2.5 μL *E. coli*, 0.5 μL *C. albicans*, and 7 μL sterile water. When needed, drugs were

added to the mixture to a final concentration of 12.5 μM, and plates lacking *C. albicans* were used as negative controls (uninfected). The mixtures were spotted on NGM plates and maintained at room temperature overnight; 20 young adults worms were added to each of three plates ($n = 60$ for each experimental condition), and the plates were incubated at 20 °C. Worms were scored daily by gentle prodding with a platinum wire. Dead worms were discarded, whereas live ones were transferred to new seeded plates for growth overnight at room temperature. Worms accidentally killed while transferring or found dead on the edges of the plates were discarded and censored in our analysis. Statistical analysis was performed using SPSS and GraphPad Prism software: survival curves were obtained using the Kaplan–Meier method, and *P* values were calculated using the log-rank test that gives equal weight to all time points.

Mouse Mucosal Infection Studies. The protocol for the ex vivo infections was based on published studies (15). Mice were administered estrogen (1 μg/mL; 100 μL subQ), and vaginae were harvested after 72 h. Before inoculation, *C. albicans* SC5314 was pretreated with 50 μM filastatin for 1 h. Tissues were either left uninoculated or inoculated with treated or untreated SC5314 (10^6 blastoconidia) and incubated for 24 h. Tissues were bisected, with one-half used for cfu analysis and one-half processed for scanning EM or confocal microscopy (CM) after staining with Calcofluor to detect yeast (blue) and Concavalin A to visualize ECM (red). Scanning EM images were taken at 1,000× magnification, and CM images were taken at 200× magnification. We performed two independent repeats ($n = 6$ vaginal explants per experiment). Mouse tissues were acquired and analyzed under protocols approved by the institutional review board of Louisiana State University (Protocol 2835).

SI Results

Filastatin (Scaffold 1) and Compound 14 (Scaffold 2) Do Not Synergize for Inhibition of *C. albicans* Binding to Polystyrene. Many of our initial set of candidate compounds had one of two distinct chemical scaffolds (Fig. S2*A* and Table S1). We tested whether we could detect any synergistic effects on combining members of these two classes. Specifically, we combined doses of compounds that alone had partial effects on adhesion: filastatin (Scaffold 1) and compound 14 (Scaffold 2). Combining these compounds at these concentrations produced additive but not synergistic reductions in adhesion (Fig. S2*B*). Therefore, we focused subsequent experiments on single compounds alone.

Filastatin Is Functionally Distinct from Previously Described Compounds That Affect *Candida* Drug Resistance. During the course of our studies, results were published from a high-throughput screen for chemosensitizer compounds that do not impair growth of WT *C. albicans* but synergize with fluconazole to reduce viability of fluconazole-resistant strains (16). Notably, the best candidate compound identified (termed Q1 in Fig. S3*A* and Table S1) is a piperazinyl quinoline. Q1 has a backbone similar to our filastatin, with the exception of the quinoline group distal to the piperazinyl linker, which is in contrast to the single-ring nitrophenyl group present in filastatin (Fig. S3*A*). We, therefore, tested whether the presence of the quinoline group Q1 affected our adhesion assays or affected the activities of the candidate compounds in a variety of assays. We also tested whether different substitutions on the aryl group adjacent to the carbonyl at the other end of the molecules would correlate with activities, comparing compound 27 with filastatin and comparing a compound that we termed Q2 with Q1 (Fig. S3*A*).

We tested this set of compounds for effects on *C. albicans* adhesion to polystyrene. We observed that both filastatin and compound 27 were effective adhesion inhibitors but that neither compound Q1 nor compound Q2 was inhibitory (Fig. S3*B*). Therefore, the presence of the quinoline group on compounds Q1 and Q2 correlated with a lack of activity, and the substitutions on the other end of the compounds were less important. Furthermore, these data show that filastatin and Q1 are functionally distinct, despite their similar chemical backbones.

We also compared these compounds in the human cell binding assay. In this case, only filastatin strongly inhibited *C. albicans* to human A549 cells (Fig. S3*C*). Therefore, perturbation of either of the aryl side groups on both sides of the molecule can impair the human cell adhesion inhibition activity of filastatin, further illustrating the distinct properties of this compound.

Finally, we tested whether filastatin would inhibit growth of fluconazole-resistant *C. albicans* strains in the presence of low levels of fluconazole, because that activity is the activity described for compound Q1 (16). To measure cell viability rather than adhesion, we performed alamarBlue-based viability measurements without wash steps using fluconazole-resistant *C. albicans* strains obtained from an AIDS patient who had been treated with fluconazole for oral candidaisis (6, 7). We confirmed that compound Q1 does, indeed, synergize with fluconazole to inhibit growth of these strains (Fig. S4). In contrast, filastatin alone did not affect viability of these fluconazole-resistant clinical isolates, and it did not synergize with fluconazole. These data provide another example of how filastatin and Q1 are functionally distinct, despite some similarity in their structures.

Several Compounds Inhibit Hyphal Morphogenesis. Using the *HWP1-RFP* reporter strain, we initially tested each of the compounds that inhibit adhesion to polystyrene (Fig. 1*B*) as well as compounds 27, Q1, and Q2 (Fig. S3*A*) at a concentration of 12.5 μM. We observed that filastatin and compounds 14, 27, Q1, and Q2 most potently inhibited hyphal formation and induction of RFP (Fig. 3*B* and Fig. S6). Therefore, the compounds with a quinoline group can inhibit hyphae formation but not adhesion to polystyrene or human cells (Fig. S3). In contrast, other Scaffold 1 compounds (Fig. S2 and Table S1) (e.g., compounds 1, 6, 25, and 26) did not block hyphal formation as efficiently as did filastatin, 27, Q1, or Q2, suggesting that minor substitutions on this backbone can affect this assay. Additionally, multiple chemical backbones are able to inhibit hyphae formation, because compound 14 has a Scaffold 2 backbone (Table S1). To better discriminate the inhibitory compounds, we tested filastatin, 14, and Q1 at lower concentrations. At 10 μM, all three of these completely blocked the hyphae formation induced by Spider media (Fig. 3*A* and Fig. S6). A few hyphae were observed at 5 μM in the presence of compound 14, and hyphae were detectable in the presence of any of the three compounds at 2.5 μM. We conclude that filastatin, 14, and Q1 all potently inhibit hyphal formation, although these compounds behave differently in adhesion assays (Figs. 1 and 2).

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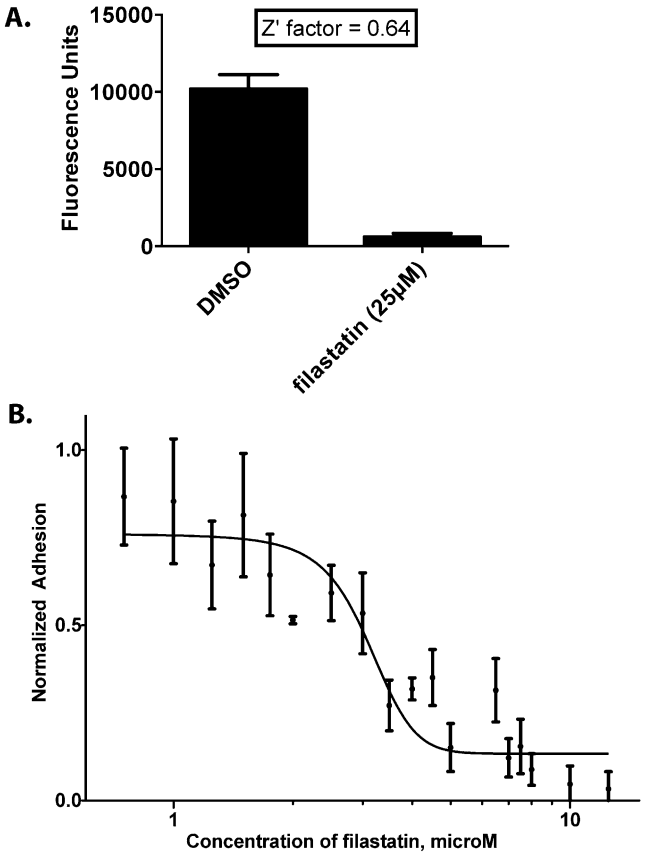

Fig. S1. Z′-factor measurement and titration of filastatin. (*A*) Overnight cultures of SC5314 cells were diluted to 0.5 OD/mL in fresh synthetic complete media (SCM) and plated onto an Immulon 2HB 96-well microtiter plate; 48 wells were treated with either 1% DMSO or 25 μM filastatin, and alamarBlue-based adhesion assays were performed as described in *Methods*. Z′ factor, a metric for the robustness of the assay, was calculated as described (1); this value should be >0.5 for high-throughput screens. Using alamarBlue, we found the Z′ factor routinely to be >0.6. Therefore, our modified protocol would be well-suited to additional high-throughput screens. Mean and SDs of fluorescence measurements of 48 wells are shown. (*B*) Assay performed like in Fig. 1*D* using the GFP-encoding SC5314 cells in the presence of indicated concentrations of filastatin. Mean and SDs from four replicate well measurements are shown.

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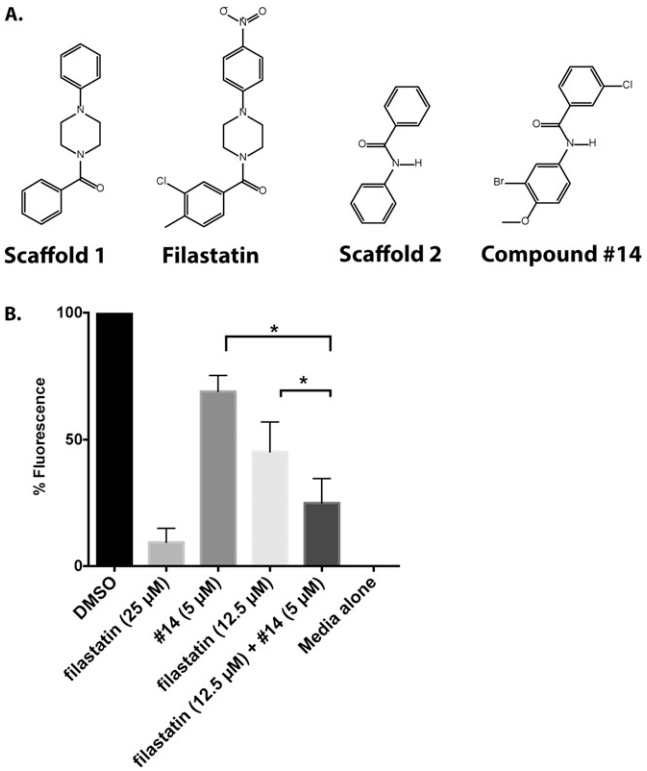


Fig. S2. Coincubation of compounds representing Scaffolds 1 and 2. (*A*) Chemical structures of the two scaffold groups identified alongside the structures of filastatin and compound 14. (*B*) Assay performed as in Fig. 1*B* with the indicated compound concentrations. For each biological replicate, alamarBlue-based fluorescence measurements were normalized to the mean value observed for DMSO-treated cells. Six biological replicate experiments were performed, and each experiment had 16 replicate technical measurements per condition. Mean and SDs are shown. Asterisks indicate that P was <0.05 (Wilcoxon matched pairs signed rank test; paired, nonparametric).

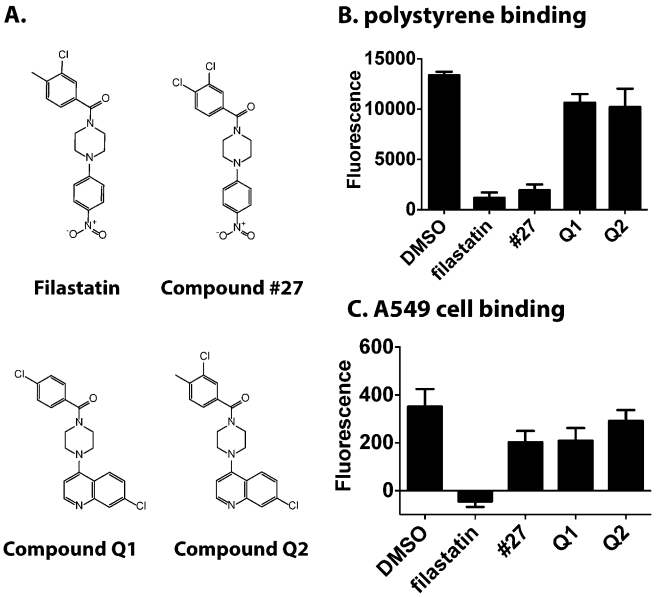


Fig. S3. Filastatin has distinct activities from structurally related piperazinyl quinolones. (*A*) Structures of compounds. Compound 27 was analyzed to determine if the aryl group substitution of filastatin is important. The piperzinyl quinolone Q1 was described (1) as a compound that reverses fluconazole resistance. Compound Q2 is a piperzinyl quinolone with the same aryl group substitutions as filastatin. (*B*) Compounds Q1 and Q2 do not inhibit *C. albicans* adhesion to polystyrene. Assay was performed like in Fig. 1*B* with 25 μM compounds. Mean and SD of measurements from eight wells are shown. (*C*) Compounds Q1 and Q2 do not inhibit *C. albicans* adhesion to human A549 cells. Assay was performed like in Fig. 2*A* with 25 μM compounds. Mean and SD of measurements from four wells are shown.

1. Youngsaye W, et al. (2011) Piperazinyl quinolines as chemosensitizers to increase fluconazole susceptibility of *Candida albicans* clinical isolates. *Bioorg Med Chem Lett* 21(18): 5502–5505.

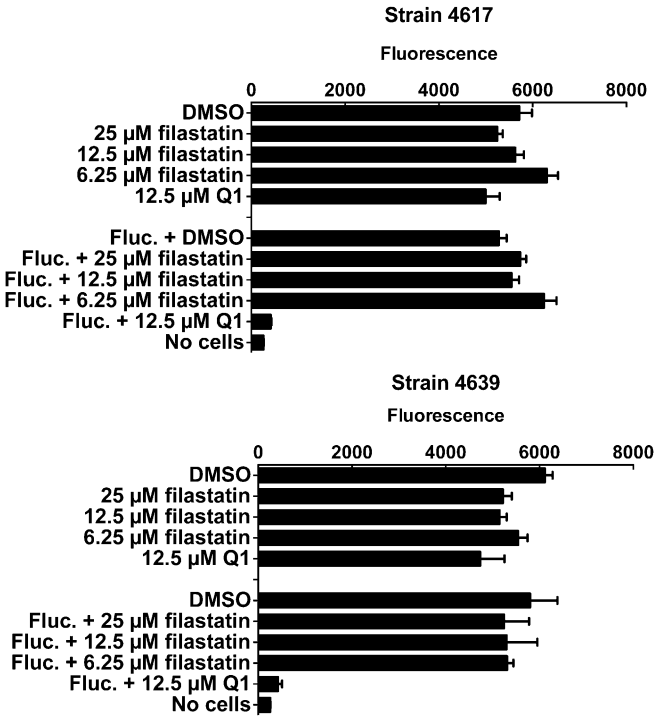


Fig. S4. Unlike compound Q1, filastatin does not synergize with low concentrations of fluconazole to inhibit growth of clinical *Candida* strains. Mean and SD of measurements from eight wells are shown.

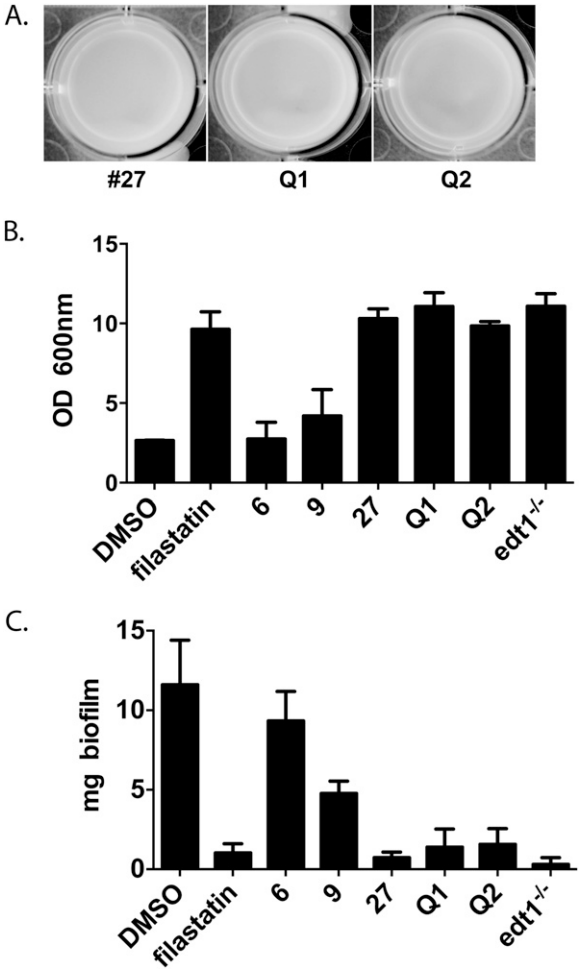


Fig. S5. Effects of small molecules on biofilm formation in vitro. (*A*) Biofilm formation on silicone elastomers. As in Fig. 3*A*, testing was done on compounds 27, Q1, and Q2. (*B*) Quantitation of turbidity of medium. Media were removed from the wells and vortexed vigorously, and the OD at 600 nm was measured in a spectrophotometer. Mean and SD of measurements from three replicates are shown. (*C*) Quantitation of dry weight of biofilm. Dry weight in milligrams of air-dried silicone elastomers. Mean and SD of measurements from three replicates are shown.

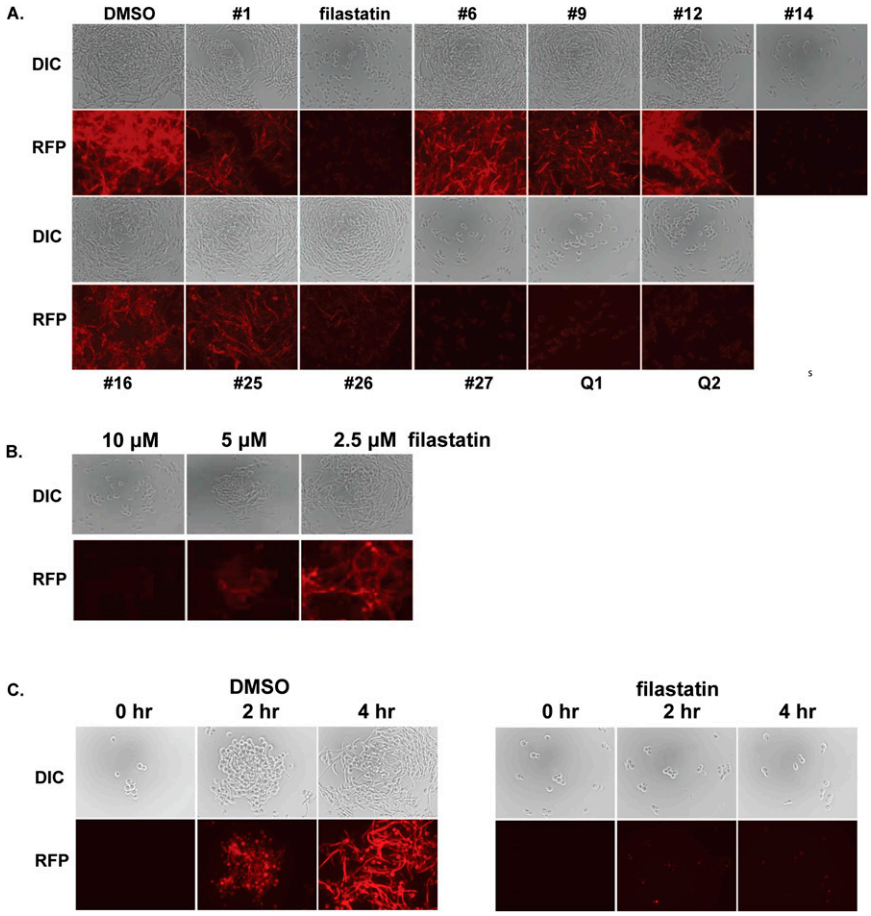


Fig. S6. Chemical modulation of *C. albicans* morphogenesis. (*A*) Microscopic analysis of hyphal development in the presence of the indicated compounds at 12.5 μM. Cells containing an *HWP1* promoter-driven RFP reporter were grown in Spider media for 16 h and then photographed using DIC or fluorescence microscopy (RFP) as indicated (64× objective). (*B*) Dose-dependent effects of filastatin on hyphal development. Filastatin at 10, 5, and 2.5 μM as indicated was tested as above. (*C*) Time course of hyphal development. Cells grown in Spider media for the indicated periods of time in the presence and absence of filastatin imaged as above.

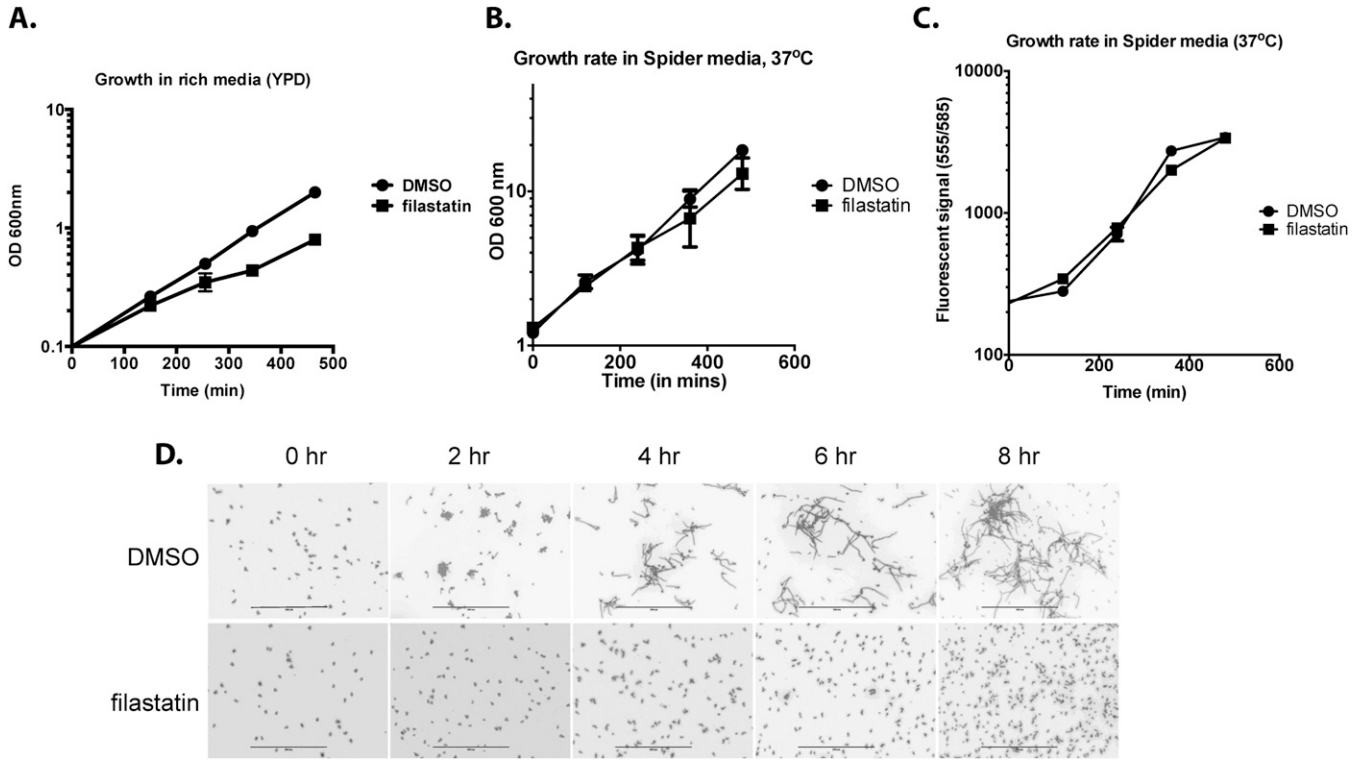


Fig. S7. Effects of filastatin on growth rates of *C. albicans*. (*A*) Growth rate of SC5314 in rich media. Overnight culture of SC5314 grown in YPD media was diluted into fresh YPD media to a concentration of 0.1 OD/mL and allowed to grow at 37 °C in the presence of 20 μM filastatin or 1% DMSO as a vehicle control. The mean and SD OD measurements of three replicates measured at indicated times are shown. (*B*) Growth rate of SC5314 in Spider media. Overnight culture of SC5314 grown in Supplemented YNB media was diluted into Spider media to a concentration of 0.1 OD/mL and allowed to grow at 37 °C in the presence of 50 μM filastatin or 1% DMSO as a vehicle control. The mean and SDs of OD measurements of three biological replicates are shown. (*C*) The same as in *B*, except samples were analyzed using alamarBlue. (*D*) The same as in *B*, except samples were analyzed by microscopy.

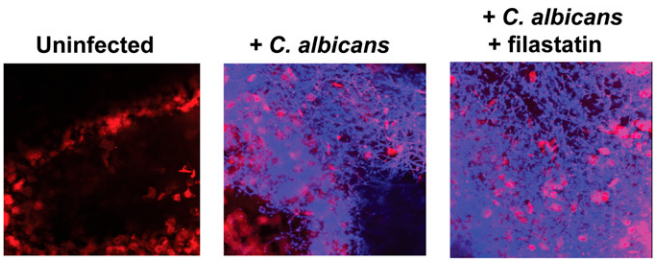


Fig. S8. Filastatin does not block ECM formation by *C. albicans* during growth on vaginal mucosae. Microscopic analysis of ex vivo vaginal mucosal biofilm formation. Tissues were processed for CM after staining with Calcofluor to detect yeast (blue) and Concavalin A to visualize ECM (red). (Magnification: 200×.) The representative images show areas of biofilm growth from two independent repeats (n = 6 vaginal explants per experiment).

Table S1. Compound structures and summary of activities

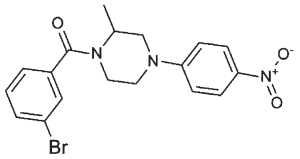
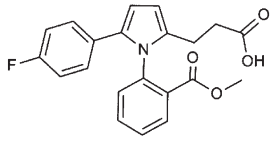
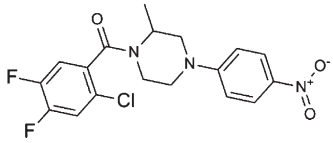
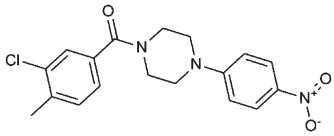
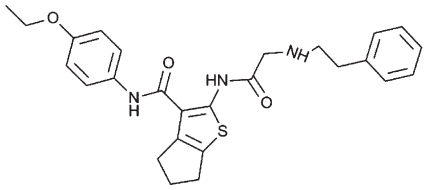
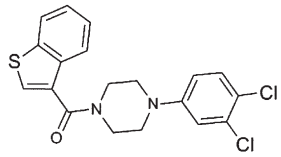
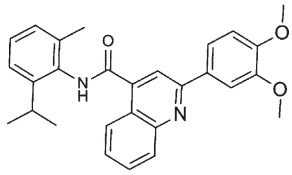
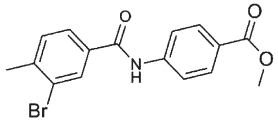
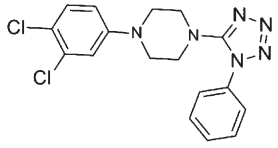
Candidate compound no.	Chembridge compound ID	Structure	Comments
1	6177408		Scaffold 1 variant; piperazine ring has an Me group
2	6870699		
3	6139415		Scaffold 1 variant
4 Filastatin	6714151		Scaffold 1
5	7021797		
6	7288338		Scaffold 1 variant
7	7291583		
8	7052098		Scaffold 2
9	7140676		

Table S1. Cont.

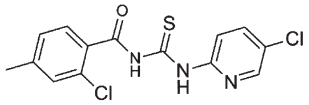
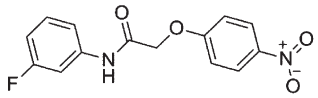
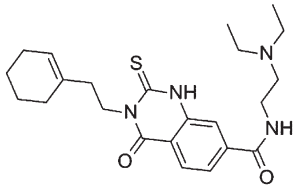
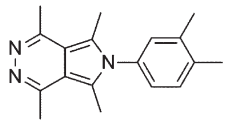
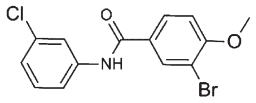
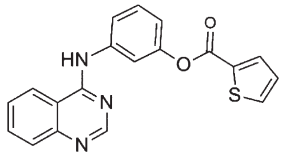
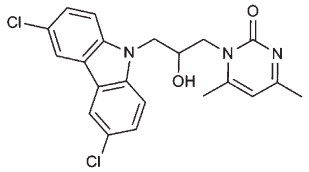
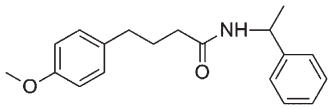
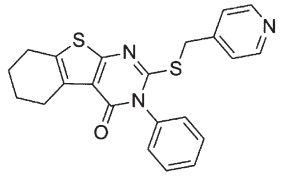
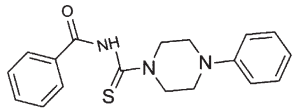
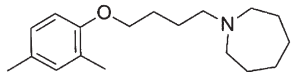
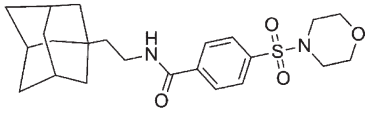
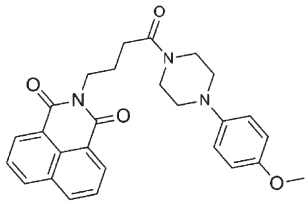
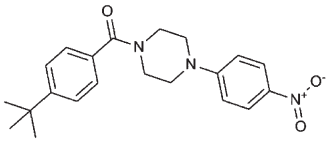
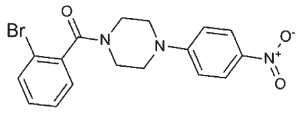
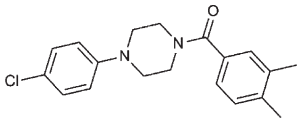
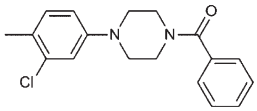
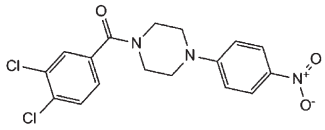
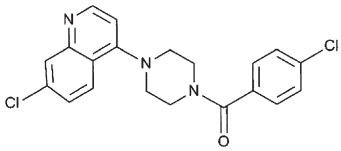
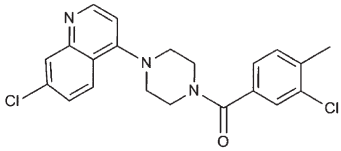
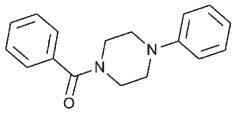
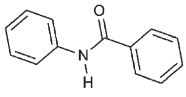
Candidate compound no.	Chembridge compound ID	Structure	Comments
10	6942569		
11	6988606		Scaffold 2 variant
12	7115759		
13	7024394		
14	6777751		Scaffold 2
15	6886186		
16	5605598		
17	6873245		
18	7111893		
19	7146132		
20	7319890		

Table S1. Cont.

Candidate compound no.	Chembridge compound ID	Structure	Comments
21	6937280		
22	7113600		
23	5742793		Scaffold 1
24	5221077		Scaffold 1
25	7239106		Scaffold 1
26	7276058		Scaffold 1
27	5653912		Methyl group of compound 4 (filastatin) substituted with Cl
Q1	7959790		Fluconazole sensitizer (1)
Q2	9009034		Derivative of Q1
Scaffold 1			
Scaffold 2			

Scaffold 1 compounds have a 1-benzoyl-4-phenylpiperazine backbone (that is, they are piperazines with a benzoyl group attached to one nitrogen and an aryl group attached to the other nitrogen). Scaffold 2 compounds are N-phenylbenzamides with two substituted aryl groups separated by an amide bond.

1. Youngsaye W, et al. (2011) Piperazinyl quinolines as chemosensitizers to increase fluconazole susceptibility of *Candida albicans* clinical isolates. *Bioorg Med Chem Lett* 21(18): 5502–5505.

Table S2. Summary of compound activities

Compound no.	Scaffold	Polystyrene adhesion	Human cell adhesion	Hyphae formation	Biofilm formation
1	Scaffold 1 variant; piperazine ring with methyl group	XX	Negative	Negative	ND
2		Negative	Negative	ND	ND
3	Scaffold 1 variant	Negative	Negative	ND	ND
4 (filastatin)	Scaffold 1	XX	X	X	X
5		X	Negative	ND	ND
6	Scaffold 1 variant	XX	Negative	Negative	Negative
7		Negative	Negative	ND	ND
8	Scaffold 2	Negative	Negative	ND	ND
9		XX	Negative	Negative	Negative
10		Negative	Negative	ND	ND
11	Scaffold 2 variant	Negative	Negative	ND	ND
12		XX	Negative	Negative	ND
13		Negative	Negative	ND	ND
14	Scaffold 2	X	Negative	X	ND
15		Negative	Negative	ND	ND
16		XX	Negative	Negative	ND
17		Negative	Negative	ND	ND
18		Negative	Negative	ND	ND
19		Negative	Negative	ND	ND
20		Negative	Negative	ND	ND
21		Negative	Negative	ND	ND
22		Negative	Negative	ND	ND
23	Scaffold 1	Negative	Negative	ND	ND
24	Scaffold 1	Negative	Negative	ND	ND
25	Scaffold 1	XX	Negative	Negative	ND
26	Scaffold 1	X	Negative	X	ND
27	Scaffold 1: methyl group of compound 4 substituted with Cl	XX	Negative	X	X
Q1	Piperazinyl quinoline (1)	Negative	Negative	X	X
Q2	Derivative of Q1	Negative	Negative	X	X

For polystyrene adhesion inhibition, XX indicates that <10% of *C. albicans* cells detected by alamarBlue remained at 25 μM compound; X indicates that <20% of *C. albicans* cells remained at 25 μM (Fig. 1*B*). For human cell adhesion, X indicates that <10% of *C. albicans* cells remained at 25 μM compound (Fig. 2*A*). For hyphae formation, X indicates that most cells were nonhyphal on treatment with 12.5 μM compound (Fig. S6). For biofilm formation, X indicates that most cells did not adhere to the silicone mesh in the presence of 50 μM compound (Fig. 3 and Fig. S5). ND, not determined.

1. Youngsaye W, et al. (2011) Piperazinyl quinolines as chemosensitizers to increase fluconazole susceptibility of *Candida albicans* clinical isolates. *Bioorg Med Chem Lett* 21(18): 5502–5505.