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***N,N'*-Diaryl-Bishydrazones in a Biphenyl Platform: Broad Spectrum Antifungal Agents**

Nishad Thamban Chandrika^a, Emily K. Dennis^a, Sanjib K. Shrestha^a, Huy X. Ngo^a, Keith D. Green^a, Stefan Kwiatkowski^{a,b}, Agripina Gabriela Deaciuc^a, Linda P. Dwoskin^a, David S. Watt^{b,c,d,*}, and Sylvie Garneau-Tsodikova^{a,*}

^aDepartment of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY, 40536-0596, USA.

^bCenter for Pharmaceutical Research and Innovation, College of Pharmacy, University of Kentucky, Lexington, KY, 40536-0596, USA.

^cDepartment of Molecular and Cellular Biochemistry, College of Medicine, University of Kentucky, Lexington, KY, 40536-0509, USA.

^dLucille Parker Markey Cancer Center, University of Kentucky, Lexington, KY, 40536-0093, USA.

Abstract

N,N'-Diaryl-bishydrazones of [1,1'-biphenyl]-3,4'-dicarboxaldehyde, [1,1'-biphenyl]-4,4'-dicarboxaldehyde, and 4,4'-bisacetyl-1,1-biphenyl exhibited excellent antifungal activity against a broad spectrum of filamentous and non-filamentous fungi. These *N,N'*-diaryl-bishydrazones displayed no antibacterial activity in contrast to previously reported *N,N'*-diamidinobishydrazones and *N*-amidino-*N'*-aryl-bishydrazones. The leading candidate, 4,4'-bis((*E*)-1-(2-(4-fluorophenyl)hydrazono)ethyl)-1,1'-biphenyl, displayed less hemolysis of murine red blood cells at concentrations at or below that of a control antifungal agent (voriconazole), was fungistatic in a time-kill study, and possessed no mammalian cytotoxicity and no toxicity with respect to hERG inhibition.

Graphical Abstract

*Correspondence to: dwatt@uky.edu or sylviegttsodikova@uky.edu.

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DISCLOSURE

D.S.W. has partial ownership of a new-startup company, Epionc, Inc., that seeks to develop compounds discovered at the University of Kentucky as commercial agents. D.S.W. disclosed this information and complied with requirements to mitigate any potential conflicts of interest in accord with University of Kentucky policy.

X = 16 mono/disubstituted phenyl, *t*-Bu, or C(N=H)NH₂

Keywords

Aspergillus sp.; *Candida albicans*; cytotoxicity; hemolysis; hERG channel; non-*albicans* *Candida*

INTRODUCTION

The growing practice of organ and hematopoietic cell transplantation^{1, 2} and the increasing use of immunosuppressive, antiviral, and antineoplastic therapies opens the door to adventitious systemic fungal infections. The high mortality rates for mycoses such as candidiasis^{3–7} and aspergillosis^{8–10} underscore the need for new antifungal therapies. The challenges in the development of new systemic antifungal agents¹¹ involve finding a balance between the scope and potency of any new agents *versus* the need for acceptable pharmacokinetic, pharmacodynamic, and toxicology profiles consistent with modern medicines.¹² Compounding these challenges are the proclivity of opportunistic and dimorphic fungi to develop resistance¹³ and the growing concerns about the financial burden associated with the use of new antifungal drugs.

These challenges attracted the attention of medicinal chemists and prompted the search for new antifungal agents.^{14–17} In recent years, we and others investigated derivatives of chemical scaffolds that include aminoglycosides,^{18–26} benzimidazoles,^{21, 27, 28} azoles,^{29–34} haloperidol,³⁵ and ebselen/ebsulfur^{36–38} as potential antifungal agents. Most recently, we reported the development of bishydrazones **1** and **2** (Fig. 1A) bearing either *N*-amidino and/or *N*-aryl groups, respectively, as new classes of antibacterial and antifungal agents.³⁹ Among these agents, we were particularly interested in the spectrum of activity and absence of resistance to *N*-amidino-*N'*-aryl bishydrazones **2** in which the aryl substituent possessed either *ortho*- or *ortho,para*-halogens and in which the platform connecting the hydrazine groups was either a biphenyl or fluorenyl group. Although these first-generation bishydrazones **1** and **2** displayed good activity against various bacterial and fungal strains, we also observed some unwanted inhibition of the human *Ether-à-go-go*-related (hERG) potassium ion channel associated with QT interval prolongation in the electrocardiogram and adverse cardiac events.⁴⁰ Additional structure-activity studies designed to eliminate this potential hERG toxicity identified alkoxy-substituted *N,N'*-diaryl groups attached to

bishydrazones **3** (Fig. 1A) on either 3,4'-biphenyl or 4,4'-biphenyl platforms. These alkoxy-substituted *N,N'*-diaryl-bishydrazones **3** exhibited particularly effective control of a broad spectrum of fungi relative to the previously reported bishydrazones **2**, displayed minimal antibacterial activity, and possessed minimal hemolysis and toxicity with respect to hERG inhibition.

RESULTS AND DISCUSSION

Synthesis of *N,N'*-diaryl-bishydrazones.

The acid-catalyzed condensation of [1,1'-biphenyl]-3,4'-dicarboxaldehyde (**4**), [1,1'-biphenyl]-4,4'-dicarboxaldehyde (**5**), or 4,4'-bisacetyl-1,1-biphenyl (**6**) with two equivalents of *N*-arylhydrazines at 80 °C furnished the *N,N'*-diaryl bishydrazones **7–22** (Fig. 1B). For comparison with these aryl-substituted bishydrazones, we included several bishydrazones with *N,N'*-dialkyl or *N,N'*-diamidino substituents in place of the *N,N'*-diaryl groups. The condensations of either *tert*-butylhydrazine or *N*-aminoguanidine secured the *N,N'*-dialkyl-bishydrazones **23b** or the *N,N'*-diamidino-bishydrazones **24a-c**, respectively. Yields varied over a considerable range, but we invested no effort in yield optimization. The (*E,E*)-stereochemistry of the bishydrazones **7–24** was anticipated on the basis of steric considerations and consistent with literature precedent^{41, 42} in which heating benzaldehydes or acetophenones with arylhydrazines in an acidic medium produced the thermodynamically favored product.

Antibacterial activity.

We explored the activity of *N,N'*-diaryl-bishydrazones **7–22** against various bacterial strains, including *Listeria monocytogenes*, methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant *enterococci*. In contrast with the *N,N'*-diamidinobishydrazones **24a-c** as well as related compounds in our prior study³⁹ that displayed good antibacterial activity against these same strains, neither the *N,N'*-diaryl-bishydrazones **7–22** nor the *N,N'*-di-*tert*-butyl-bishydrazones **23b** displayed antibacterial activity.

Antifungal activity.

We tested the activity of *N,N'*-diaryl-bishydrazones **7–22** against a panel of seven strains of *C. albicans*: ATCC 10231(R) (**A**), ATCC 64124(R) (**B**), ATCC MYA-2876(S) (**C**), ATCC 90819(R) (**D**), ATCC MYA-2310(S) (**E**), ATCC MYA-1237(R) (**F**), and ATCC MYA-1003(R) (**G**). We also explored the activity of these bishydrazones **7–22** against a panel of three non-*albicans* *Candida* strains: *C. glabrata* ATCC 2001 (**H**), *C. krusei* ATCC 6258 (**I**), and *C. parapsilosis* ATCC 22019 (**J**). Finally, we explored the activity of these bishydrazones **7–22** against three *Aspergillus* strains: *A. flavus* ATCC MYA-3631 (**K**), *A. nidulans* ATCC 38163 (**L**), and *A. terreus* ATCC MYA-3633 (**M**). We used a concentration range of 0.03–31.3 µg/mL (Table 1) and used commercially available antifungal agents, caspofungin (CAS)⁴³ and voriconazole (VOR),⁴⁴ as positive controls. We reported MIC-0 values (*i.e.*, no visible growth) for the *N,N'*-diaryl-bishydrazones **7–22** and the control CAS, and we reported MIC-2 values (*i.e.*, 50% growth inhibition) for VOR against all fungal strains tested with the exception of strain **A**. The limited solubility of several *N,N'*-diaryl-

bishydrazones (*i.e.*, **8a**, **8b**, **9a**, **9b**, **11a**, **11b**, and **14a**) precluded determination of MIC values. For the bishydrazones with good solubility, we define antifungal activity as excellent (<3.9 µg/mL), moderate (7.8–15.6 µg/mL), or poor (>31.3 µg/mL) based on MIC values (Table 1).

The MIC data in Table 1 identified the *N,N'*-diaryl-bishydrazones **13b**, **16b**, **18b**, **19b**, **21a**, and **21b** to be inactive as antifungal agents, and this finding led us to discount bishydrazones with a symmetrical 4,4'-substitution pattern (series **b**, Fig. 1B) in the biphenyl platform in favor of those with an unsymmetrical 3,4'-substitution pattern (series **a**, Fig. 1B). Consistent with this generalization that a symmetrical 4,4'-substitution pattern produced largely inactive compounds, bishydrazones **9c**, **20**, **22b**, and **23b** also displayed poor activity against all fungal strains tested with exception of **9c**, **2b**, and **22b** against strain **A** (15.6 µg/mL, 7.8 µg/mL and 15.6 µg/mL, respectively) and **23b** against strain **H** (15.6 µg/mL). Also consistent with this generalization, bishydrazones **19a** with an unsymmetrical 3,4'-substitution pattern displayed moderate activity against two strains **F** and **H** (7.8 µg/mL), and bishydrazones **22a** displayed excellent activity against strain **A** (3.9 µg/mL) and moderate activity (15.6 µg/mL) against strain **B**.

Turning to the bishydrazones that displayed activity against multiple strains, bishydrazones **7a** exhibited excellent activity against strains **A**, **G**, **K**, and **L** (1.95–3.9 µg/mL) and moderate activity against strains **I** and **M** (7.8–15.6 µg/mL). Bishydrazones **7b** displayed excellent activity (1.95–3.9 µg/mL) against strains **K** and **L**, and bishydrazones **10a** and **10b** displayed excellent activity (1.95–3.9 µg/mL) against strains **I** and **K** and against strains **A** and **I**, respectively. Compound **11c** exhibited excellent activity (0.98–3.9 µg/mL) against strains **A**, **B**, **G**, and **I** as well as moderate activity (15.6 µg/mL) against strains **C**, **E**, **H**, and **J**. Similarly, compound **12a** displayed excellent activity (1.95–3.9 µg/mL) against strains **A**, **I**, and **K** as well as moderate activity (7.8–15.6 µg/mL) against strains **C**, **D**, **F**, **G**, and **L**. Bishydrazones **12b** exhibited excellent activity (3.9 µg/mL) against strain **J**, but only moderate activity (7.8–15.6 µg/mL) against strains **A** and **I**, a finding again in accord with the generalization regarding a preference for bishydrazones with an unsymmetrically substituted biphenyl platform.

Finally, in addition to bishydrazones **12a** mentioned above, the bishydrazones **15a-c**, **17a-c**, **18a**, and **20a** possessed the breadth of activity that we desired and displayed overall antifungal activity against all the strains tested. Bishydrazones **15a** and **17c** were among the best of the series and displayed excellent activity (1.95–3.9 µg/mL) against strains **A**, **C**, **D**, **F**, and **I** and against strains **A**, **B**, **C**, **E**, and **I**, respectively. Even though bishydrazones **15a** and **17c** were superior in terms of their activity, bishydrazones **15b**, **17a**, **18a**, and **20a** also exhibited good activity against the majority of the fungal strains tested. Bishydrazones **15b** displayed excellent activity (3.9 µg/mL) against strains **B** and **E** and exhibited moderate activity (7.8–15.6 µg/mL) against strains **A**, **C**, **D**, **F**, **G**, **H**, **I**, **J**, and **L**. In addition, bishydrazones **17a**, **18a**, and **20a** also displayed excellent activity (1.95–3.9 µg/mL) against strains **A**, **G**, and **L**; **G** and **J**; and **A** and **I**, respectively. Among the bishydrazones **15** and **17**, bishydrazones **15c** only exhibited moderate activity (7.8–15.6 µg/mL) against strains **A-C** and **E-I**, whereas compound **17b** displayed excellent activity (1.95 µg/mL) against strain **I** as

well as moderate activity (7.8–15.6 µg/mL) against strains **A-C**, **E-H**, **K**, and **L**. In comparison with the FDA approved antifungal agents, CAS and VOR, some of these bishydrazones exhibited comparable or superior activity against strains **A**, **I**, and **J** and superior activity against the filamentous strains **K-M**.

SAR analysis.

These latter findings required a refinement of the initial generalization regarding the preference for an unsymmetrical substitution pattern in the biphenyl platform over a symmetrical substitution pattern in driving the observed antifungal activities in these bishydrazones. In cases where the R group is a hydrogen (series **a**, Fig. 1B), the most active bishydrazones (*e.g.*, **12a**, **15a**, **17a**, **18a** and **20a**) possessed the unsymmetrical 3,4'-substitution pattern in the biphenyl platform, but in cases where the R group is a methyl (series **c**, Fig. 1B), the most active bishydrazones possessed the symmetrical 4,4'-substitution pattern in the biphenyl platform. As for any generalization, there were exceptions such as the bishydrazones with symmetrically substituted biphenyl platforms (*e.g.*, **15b** and **17b**) in which presumably the substituents on the aryl rings overrode any disadvantage in activity conferred by the symmetrical substitution pattern on the biphenyl platform. As a further illustration of this point, several bishydrazones where the R group is a methyl (series **c**, Fig. 1B) possessed excellent activity (*e.g.*, **15c** and **17c**) despite the symmetrical nature of the 4,4'-biphenyl platform.

Among the substituents on the *N*-aryl groups, the bishydrazones with *para*-fluorophenyl, *parachlorophenyl* and *para*-methoxyphenyl groups possessed the best spectrum of activity against various strains and the most potent activity as judged by their MIC values. Other regioisomers, such as the *meta*-fluorophenyl analogue **12a**, displayed good activity, including promising activity against several filamentous fungi (strains **K** and **L**), but overall, the *meta*-fluorophenyl analogue **12a** displayed a spectrum slightly less promising than that of the corresponding *para*-fluorophenyl analogues **15a-c**. In addition, the *meta*-fluorophenyl analogue **12a** displayed good activity only in the biphenyl platform with 3,4'-substitution (series **a**), and introducing the *meta*-fluorophenyl group in other platforms (series **b** and **c**) led to inactive compounds. The addition of multiple fluoro groups (*i.e.*, *N,N'*-bis-*ortho,para*-difluorophenyl-bishydrazone **20a** versus *N,N'*-di-*para*-fluorophenyl-bishydrazone **17a**) led to a modest improvement in activity against three strains (**D**, **F**, and **K**), but otherwise comparable activity. The *ortho,meta*- and *meta,meta*-difluorophenyl analogues **21a,b** and **22a,b**, respectively, showed no activity.

Other *para*-substituted *N*-aryl groups, including the *para*-bromo-, *para*-cyano-, *paratrifluoromethyl*-, and *para*-isopropoxyphenyl groups, led to *N,N'*-diaryl-bishydrazones with diminished activity in both scope and potency. Other *ortho*- and *meta*-substituted *N*-aryl groups, such as the *ortho*-fluoro-, *ortho*-methoxy, *meta*-methoxy-, and *ortho*-nitrophenyl groups, led to *N,N'*-diaryl-bishydrazones either with diminished solubility or diminished activity in both scope and potency. Finally, the two bishydrazones **23b** and **24a-c** with *N,N'*-dialkyl groups and *N,N'*-diamidino groups, respectively were inactive in the case of **23b** and marginally active in the cases of **24a-c**.

Antibiofilm activity.

Biofilms are complex communities of one or more species of microorganisms encased in extracellular polymeric substances and attached to not only a solid surface but also to each other. Because of their complex, matrix-like nature, antifungal agents are often unable to reach the pathogens embedded in these networks.^{45–47} The antibiofilm activity of 4,4'-bis((*E*)-1-(2-(4-fluorophenyl)hydrazono)ethyl)-1,1'-biphenyl (**17c**) and VOR were evaluated against biofilms of *C. albicans* ATCC 10231 (strain **A**) and *C. albicans* ATCC 64124 (strain **B**) by a tetrazolium salt XTT reduction assay^{48, 49} (Table 2). The SMIC₅₀ and SMIC₉₉ values (*i.e.*, drug concentration required to inhibit the metabolic activity of the biofilm by 50% and 99%, respectively) for bishydrazone **17c** and VOR ranged from 8 to 16 µg/mL for the SMIC₅₀ and were >32 µg/mL for the SMIC₉₉. The SMIC values for bishydrazone **17c** against the biofilm formed by strains **A** and **B** increased by 4- to 16-fold and 4- to 8-fold, respectively, as compared to their corresponding planktonic MIC values. This increase reflected some resistance of *Candida* biofilms against the bishydrazone **17c**. VOR displayed a similar trend against the biofilms formed by strains **A** and **B**. Our results suggested that bishydrazone **17c** would require a higher concentration to eliminate pre-formed biofilms of *C. albicans* strains **A** and **B**.

Cytotoxicity.

Having established the potent antifungal activity of the *N,N'*-diaryl-bishydrazones, it was important to consider their potential toxicity towards mammalian cells. We investigated the toxicity profile of *N,N'*-diaryl-bishydrazones **7a,b**, **10a**, **12a,b**, **15a,b**, **17a-c**, **20a** and *N,N'*-diamidino-bishydrazone **24a** against two mammalian cell lines A549 and BEAS-2B (Fig. 2). In general, against these cell lines a concentration-dependent toxicity was observed for these bishydrazones. When tested against the A459 cell line at 31 µg/mL, the most active bishydrazones **15a,b** and **17a** were found to be generally non-toxic. Similarly, the bishydrazones **12a**, **17c**, and **20a** displayed no toxicity against A549 at 15.5 µg/mL. Against the BEAS-2B cell line, a similar trend was observed with the bishydrazones **15a,b**, **17c**, and **20a** found to be nontoxic at 15.5 µg/mL. Bishydrazone **17a** was non-toxic at 31 µg/mL against the BEAS-2B cell line. It is important to note that the non-toxic nature of *N,N'*-diaryl-bishydrazones when compared to *N,N'*-diamidino-bishydrazone **24a** combined with their excellent MIC values can be used to make a case for their further evaluation. We also reported the cytotoxicity concentration values at which 50% of mammalian cells are killed (CC₅₀ values) of compounds **7a,b**, **10a**, **12a,b**, **15a,b**, **17a-c**, **20a** and **24a** against A549 and BEAS-2B in Table 3. After determining the CC₅₀ values of compounds against mammalian cell lines, we calculated the selectivity index (SI) values of these compounds against all the fungal strains (Table 3). The SI for a compound is defined as the ratio of its CC₅₀ value against a mammalian cell line (*e.g.*, A549 or BEAS-2B) to its MIC value against a specific fungal strain. The best compounds, **15a**, **15b**, **17a**, **17b**, and **17c**, were selective against *C. albicans* and non-*albicans Candida* with SI values ranging from 0.6 to 16. However, against *Aspergillus* strains, they were less specific, with the exception of compound **17a**, which exhibited SI values ranging from 1 to 8. From these SI values, it can be suggested that these compounds offer a better safety profile for treating infections caused by *C. albicans* and non-*albicans Candida*.

Hemolysis assay.

Although the *N,N'*-diaryl-bishydrazones **7a,b**, **12a,b**, **15a,b**, **17a-c**, and **20a** and the *N,N'*-diamidino-bishydrazone **24a** showed potent antifungal activities and limited toxicity, it was important to establish that these agents showed selectivity for fungal cells over mammalian cells. Thus, we investigated the hemolytic activity for these bishydrazones against murine red blood cells (mRBCs). Overall, these compounds displayed little or no hemolysis of mRBCs for concentrations as high as 15.6 µg/mL (Fig. 3 and Table S1). Bishydrazones **7a,b** displayed <25% and <20% hemolysis at concentration of 62.5 µg/mL which are 1- to 32-fold higher than their overall MIC values. Bishydrazones **12b** displayed lower hemolysis levels (<20% at 62.5 µg/mL) than those observed for **12a** (<20% at 15.6 µg/mL). In addition, at 62.5 µg/mL, bishydrazones **12b**, **15a,b** and **17a-c** only lysed less than 20% to 30% of mRBCs. These values were again 1 to 32-fold higher than the overall MIC values reported in Table 1 for these bishydrazones. Finally, bishydrazones **20a** displayed <25% hemolysis (1- to 32-fold of its overall MIC values) and bishydrazones **24a** at concentrations of 62.5 µg/mL displayed <20% hemolysis (1- to 32-fold of its overall MIC values).

Time-kill studies.

To determine the fungistatic or fungicidal nature of the compounds, we next performed time-kill assays over a 24-hour period with 4,4'-bis((*E*)-1-(2-(4-fluorophenyl)hydrazono)ethyl)-1,1'-biphenyl (**17c**). The bishydrazones **17c** and VOR,⁵⁰ which served as a positive control, were tested against fungal strains *C. albicans* ATCC 10231 (A) and *C. albicans* ATCC 64124 (B) (Fig. 4). Against strain A, bishydrazones **17c** was fungistatic and displayed levels of fungal growth reduction better than the control drug VOR at the same concentrations. Even at concentrations one-half of the MIC value, bishydrazones **17c** displayed a greater reduction in fungal growth than concentrations of VOR equal to the MIC value. The bishydrazones **17c** also displayed fungistatic activity against strain B at concentrations up to twice its MIC value. At concentrations equal to or even one-half of the MIC value, bishydrazones **17c** displayed a greater reduction in fungal growth against strain B than concentrations of VOR equal to the MIC value. Overall, the bishydrazones **17c** performed better in time-kill studies than the control drug VOR.

hERG binding studies.

Finally, we selected *N,N'*-diaryl-bishydrazones **15a,b**, **17a**, and **17c** as well as *N,N'*-diamidino-bishydrazones **24a** for evaluation of their hERG affinity. Inhibition of hERG channel can result in cardiac arrest, which emphasizes the importance of evaluation for hERG affinity for developing drug candidates according to the U.S Food and Drug administration (FDA) and European Medicines Agency (EMA). We performed [³H]-dofetilide binding assay using HEK-293 cell membranes expressing the hERG channel to evaluate the activity of our most potent *N,N'*-diaryl-bishydrazones **15a,b**, **17a**, **17c** and *N,N'*-diamidinobishydrazones **24a** for hERG affinity (Fig. 5). Amitriptyline (final concentration, 1 mM) was used as the positive control and exhibited an IC₅₀ value (9.6 ± 1.4 µM) that was in agreement with published values.⁵¹ Compounds exhibiting IC₅₀ values of less than 1 µM have high affinity for hERG channel; compounds exhibiting IC₅₀ values in the range of 1–10 µM have moderate affinity; and compounds exhibiting IC₅₀ values of greater than 10 µM

have low affinity for hERG channel. The IC₅₀ values for hERG inhibition of [³H]-dofetilide binding for *N,N'*-diaryl-bishydrazones **15a,b** and **17a**, and **17c** were >30 μM and therefore showed no hERG inhibition. By way of comparison, the IC₅₀ value for the *N,N'*-diamidino-bishydrazone **24a** was 4.66 ± 1.66 μM that indicated an intermediate level of inhibition. It is important to point out that our second generation *N,N'*-diaryl-bishydrazones **15a,b**, **17a**, and **17c** displayed low affinity for hERG channel. Combined with the fact that they are non-toxic against mammalian cells, these compounds are better as antifungal agents than our previously reported first generation of *N,N'*-diamidino-bishydrazones.

CONCLUSIONS

In summary, we developed a straightforward synthesis of *N,N'*-diaryl-bishydrazones **7–22** with (*E,E*)-stereochemistry that utilized the acid-catalyzed condensation of [1,1'-biphenyl]-3,4'-dicarboxaldehyde (**4**), [1,1'-biphenyl]-4,4'-dicarboxaldehyde (**5**), or 4,4'-bisacetyl-1,1-biphenyl (**6**) (Fig. 1B). We also included an *N,N'*-dialkyl and an *N,N'*-diamidino-bishydrazone for comparison purposes. Neither the *N,N'*-diaryl-bishydrazones **7–22** or the *N,N'*-dialkylbishydrazone **23b** displayed antibacterial activity. We performed a detailed study (Table 1) of the antifungal activity of the *N,N'*-diaryl-bishydrazones **7–22** against a panel of seven strains of *C. albicans*. We used commercially available antifungal agents, caspofungin (CAS) and voriconazole (VOR), as positive controls.

This study of structure-activity relationships identified three leading candidates based on MIC values: the *N,N'*-di-*para*-fluorophenyl-bishydrazones **17a-c** that displayed excellent activity against both yeast strains and filamentous fungi; the *N,N'*-di-*para*-methoxyphenyl-bishydrazones **15a-c** that displayed excellent activity against a range of yeast strains but only modest effects against filamentous fungi; and the *N,N'*-di-*para*-chlorophenyl-bishydrazones **18a** with a 3,4'-substitution pattern in the biphenyl platform that displayed excellent activity only against yeast strains. Among these three leading candidates, the order of preference in terms of both scope and potency for the *N*-aryl groups in these bishydrazones would be as follows: *para*-fluorophenyl > *para*-methoxyphenyl > *para*-chloro. With respect to the three, most promising candidates, the order of preference among the biphenyl platforms and the R substituent (Fig. 1B) would be as follows: for *para*-fluorophenyl: 4,4', CH₃ (series **c**) > 3,4', H (series **a**) ≈ 4,4', H (series **b**); for *para*-methoxyphenyl: 3,4', H (series **a**) ≈ 4,4', H (series **b**) > 4,4', CH₃ (series **c**); and for *parachlorophenyl*: 3,4', H (series **a**) >> 4,4', H (series **b**) > 4,4', CH₃ (series **c**).

Although scope and potency represent important criteria in the development of new antifungal agents, we also examined other factors crucial to the progression of these agents. In a study of the effect of these bishydrazones on the hemolysis of mRBCs, we observed a lack of hemolytic activity of *N,N'*-diaryl-bishydrazones **7a,b**, **12a,b**, **15a,b**, **17a-c**, and **20a** and *N,N'*-diamidinobishydrazones **24a** (Table S1) in comparison with the FDA-approved drug VOR (83% and 100% lysis of mRBCs at 31.3 μg/mL and 62.5 μg/mL, respectively). A time-kill assay over a 24-hour period using 4,4'-bis((*E*)-1-(2-(4-fluorophenyl)hydrazono)ethyl)-1,1'-biphenyl (**17c**) and VOR against fungal strains *C. albicans* ATCC 10231 (**A**) and *C. albicans* ATCC 64124 (**B**) (Fig. 4) indicated that bishydrazones **17c** was more fungistatic than the control drug VOR at the same

concentrations or even at reduced concentrations. Finally, drug candidates must avoid hERG activation to progress toward Investigational New Drug (IND) status, and using a dofetilide binding assay, we found that *N,N'*-diaryl-bishydrazones **15a,b** and **17a,b** possessed the desired property of not inhibiting the human hERG potassium ion channel using a [³H]-dofetilide binding assay to evaluate the interaction of a subset of these bishydrazones with hERG. Various hydrazone-containing and guanidine-containing biscationic compounds with range of promising biological activities, such as antitubercular, anti-HIV, anticancer, anti-inflammatory, antibacterial, and antifungal activities have been reported in the literature.^{52–56} Based on the recently published data, a possible mechanism by which these *N,N'*-diaryl-bishydrazones might exert their pharmacological effects is by interfering with DNA-protein interactions. In summary, *N,N'*-diaryl-bishydrazones display promise as a new family of antifungal agents, and additional studies of these agents will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- [1]. Hahn T, McCarthy PL, Jr., Hassebroek A, Bredeson C, Gajewski JL, Hale GA, Isola LM, Lazarus HM, Lee SJ, Lemaistre CF, Loberiza F, Maziarz RT, Rizzo JD, Joffe S, Parsons S, and Majhail NS (2013) Significant improvement in survival after allogeneic hematopoietic cell transplantation during a period of significantly increased use, older recipient age, and use of unrelated donors, *J. Clin. Oncol* 31, 2437–2449. [PubMed: 23715573]
- [2]. Van Thiel DH, George M, and Moore CM (2012) Fungal infections: their diagnosis and treatment in transplant recipients, *Int. J. Hepatol* 2012, 106923. [PubMed: 22966464]
- [3]. Eggimann P, Que YA, Revelly JP, and Pagani JL (2015) Preventing invasive Candida infections. Where could we do better?, *J. Hosp. Infect* 89, 302–308. [PubMed: 25592726]
- [4]. Mavor AL, Thewes S, and Hube B (2005) Systemic fungal infections caused by Candida species: epidemiology, infection process and virulence attributes, *Curr. Drug Targets* 6, 863–874. [PubMed: 16375670]
- [5]. Girishkumar H, Yousuf AM, Chivate J, and Geisler E (1999) Experience with invasive Candida infections, *Postgrad. Med. J* 75, 151–153. [PubMed: 10448492]
- [6]. Nolla-Salas J, Sitges-Serra A, Leon-Gil C, Martinez-Gonzalez J, Leon-Regidor MA, Ibanez-Lucia P, and Torres-Rodriguez JM (1997) Candidemia in non-neutropenic critically ill patients: analysis of prognostic factors and assessment of systemic antifungal therapy. Study Group of Fungal Infection in the ICU, *Intensive Care Med* 23, 23–30. [PubMed: 9037636]
- [7]. Leon C, Ostrosky-Zeichner L, and Schuster M (2014) What's new in the clinical and diagnostic management of invasive candidiasis in critically ill patients, *Intensive Care Med* 40, 808–819. [PubMed: 24718642]

- [8]. Denning DW (1996) Therapeutic outcome in invasive aspergillosis, *Clin. Infect. Dis* 23, 608–615. [PubMed: 8879787]
- [9]. Denning DW, and Stevens DA (1990) Antifungal and surgical treatment of invasive aspergillosis: review of 2,121 published cases, *Rev. Infect. Dis* 12, 1147–1201. [PubMed: 2267490]
- [10]. Mousavi SA, and Robson GD (2003) Entry into the stationary phase is associated with a rapid loss of viability and an apoptotic-like phenotype in the opportunistic pathogen *Aspergillus fumigatus*, *Fungal Genet. Biol* 39, 221–229. [PubMed: 12892635]
- [11]. Rautemaa-Richardson R, and Richardson MD (2017) Systemic fungal infections, *Medicine* 45, 757–762.
- [12]. Dodds Ashley ES, Lewis R, Lewis JS, Martin C, and Andes D (2006) Pharmacology of systemic antifungal agents, *Clin. Infect. Dis* 43, S28–S39.
- [13]. Perlin DS, Rautemaa-Richardson R, and Alastruey-Izquierdo A (2017) The global problem of antifungal resistance: prevalence, mechanisms, and management, *Lancet Infect. Dis* 17, e383–e392. [PubMed: 28774698]
- [14]. Thamban Chandrika N, and Garneau-Tsodikova S (2018) Comprehensive review of chemical strategies for the preparation of new aminoglycosides and their biological activities, *Chem. Soc. Rev* 47, 1189–1249. [PubMed: 29296992]
- [15]. Ngo HX, Garneau-Tsodikova S, and Green KD (2016) A complex game of hide and seek: the search for new antifungals, *MedChemComm* 7, 1285–1306. [PubMed: 27766140]
- [16]. Chandrika NT, and Garneau-Tsodikova S (2016) A review of patents (2011–2015) towards combating resistance to and toxicity of aminoglycosides, *MedChemComm* 7, 50–68. [PubMed: 27019689]
- [17]. Fosso MY, Li Y, and Garneau-Tsodikova S (2014) New trends in aminoglycosides use, *MedChemComm* 5, 1075–1091. [PubMed: 25071928]
- [18]. Fosso MY, Shrestha SK, Green KD, and Garneau-Tsodikova S (2015) Synthesis and bioactivities of kanamycin B-derived cationic amphiphiles, *J. Med. Chem* 58, 9124–9132. [PubMed: 26592740]
- [19]. Shrestha SK, Fosso MY, and Garneau-Tsodikova S (2015) A combination approach to treating fungal infections, *Sci. Rep* 5, 17070. [PubMed: 26594050]
- [20]. Shrestha SK, Fosso MY, Green KD, and Garneau-Tsodikova S (2015) Amphiphilic tobramycin analogues as antibacterial and antifungal agents, *Antimicrob. Agents Chemother* 59, 4861–4869. [PubMed: 26033722]
- [21]. Thamban Chandrika N, Shrestha SK, Ranjan N, Sharma A, Arya DP, and Garneau-Tsodikova S (2018) New application of neomycin B-bisbenzimidazole hybrids as antifungal agents, *ACS Infect. Dis* 4, 196–207. [PubMed: 29227087]
- [22]. Shrestha SK, Grilley M, Anderson T, Dhiman C, Oblad J, Chang CW, Sorensen KN, and Takemoto JY (2015) In vitro antifungal synergy between amphiphilic aminoglycoside K20 and azoles against *Candida* species and *Cryptococcus neoformans*, *Med. Mycol* 53, 837–844. [PubMed: 26260746]
- [23]. Benhamou RI, Steinbuch KB, and Fridman M (2016) Antifungal imidazole-decorated cationic amphiphiles with markedly low hemolytic activity, *Chem. Eur. J* 22, 1148–1151.
- [24]. Shrestha SK, Chang CW, Meissner N, Oblad J, Shrestha JP, Sorensen KN, Grilley MM, and Takemoto JY (2014) Antifungal amphiphilic aminoglycoside K20: bioactivities and mechanism of action, *Front. Microbiol* 5, 671. [PubMed: 25538692]
- [25]. Shrestha S, Grilley M, Fosso MY, Chang CW, and Takemoto JY (2013) Membrane lipid-modulated mechanism of action and non-cytotoxicity of novel fungicide aminoglycoside FG08, *PLoS One* 8, e73843. [PubMed: 24040088]
- [26]. Fosso M, AlFindee MN, Zhang Q, Nziko Vde P, Kawasaki Y, Shrestha SK, Bearss J, Gregory R, Takemoto JY, and Chang CW (2015) Structure-activity relationships for antibacterial to antifungal conversion of kanamycin to amphiphilic analogues, *J. Org. Chem* 80, 4398–4411. [PubMed: 25826012]
- [27]. Thamban Chandrika N, Shrestha SK, Ngo HX, and Garneau-Tsodikova S (2016) Synthesis and investigation of novel benzimidazole derivatives as antifungal agents, *Bioorg. Med. Chem* 24, 3680–3686. [PubMed: 27301676]

- [28]. Keller P, Muller C, Engelhardt I, Hiller E, Lemuth K, Eickhoff H, Wiesmuller KH, Burger-Kentischer A, Bracher F, and Rupp S (2015) An antifungal benzimidazole derivative inhibits ergosterol biosynthesis and reveals novel sterols, *Antimicrob. Agents Chemother* 59, 6296–6307. [PubMed: 26248360]
- [29]. Shrestha SK, Garzan A, and Garneau-Tsodikova S (2017) Novel alkylated azoles as potent antifungals, *Eur. J. Med. Chem* 133, 309–318. [PubMed: 28395217]
- [30]. Thamban Chandrika N, Shrestha SK, Ngo HX, Tsodikov OV, Howard KC, and Garneau-Tsodikova S (2018) Alkylated piperazines and piperazine-azole hybrids as antifungal agents, *J. Med. Chem* 61, 158–173. [PubMed: 29256601]
- [31]. Thamban Chandrika N, Shrestha SK, Ngo HX, Howard KC, and Garneau-Tsodikova S (2018) Novel fluconazole derivatives with promising antifungal activity, *Bioorg. Med. Chem* 26, 573–580. [PubMed: 29279242]
- [32]. Allen D, Wilson D, Drew R, and Perfect J (2015) Azole antifungals: 35 years of invasive fungal infection management, *Expert Rev. Anti Infect. Ther* 13, 787–798. [PubMed: 25843556]
- [33]. Bendaha H, Yu L, Touzani R, Souane R, Giaever G, Nislow C, Boone C, El Kadiri S, Brown GW, and Bellaoui M (2011) New azole antifungal agents with novel modes of action: synthesis and biological studies of new tridentate ligands based on pyrazole and triazole, *Eur. J. Med. Chem* 46, 4117–4124. [PubMed: 21723647]
- [34]. Fakhim H, Emami S, Vaezi A, Hashemi SM, Faeli L, Diba K, Dannaoui E, and Badali H (2017) In vitro activities of novel azole compounds ATTAF-1 and ATTAF-2 against fluconazole-susceptible and -resistant isolates of *Candida* species, *Antimicrob. Agents Chemother* 61, e01106–01116.
- [35]. Holbrook SYL, Garzan A, Dennis EK, Shrestha SK, and Garneau-Tsodikova S (2017) Repurposing antipsychotic drugs into antifungal agents: Synergistic combinations of azoles and bromperidol derivatives in the treatment of various fungal infections, *Eur. J. Med. Chem* 139, 12–21. [PubMed: 28797882]
- [36]. Ngo HX, Shrestha SK, and Garneau-Tsodikova S (2016) Identification of ebsulfur analogues with broad-spectrum antifungal activity, *ChemMedChem* 11, 1507–1516. [PubMed: 27334363]
- [37]. Thangamani S, Eldesouky HE, Mohammad H, Pascuzzi PE, Avramova L, Hazbun TR, and Seleem MN (2017) Ebselen exerts antifungal activity by regulating glutathione (GSH) and reactive oxygen species (ROS) production in fungal cells, *Biochim. Biophys. Acta* 1861, 3002–3010.
- [38]. Venturini TP, Chassot F, Loreto ES, Keller JT, Azevedo MI, Zeni G, Santurio JM, and Alves SH (2016) Antifungal activities of diphenyl diselenide and ebselen alone and in combination with antifungal agents against *Fusarium* spp, *Med. Mycol* 54, 550–555. [PubMed: 26773133]
- [39]. Shrestha SK, Kril LM, Green KD, Kwiatkowski S, Sviripa VM, Nickell JR, Dwoskin LP, Watt DS, and Garneau-Tsodikova S (2017) Bis(N-amidinohydrazones) and N-(amidino)-N'-aryl-bishydrazones: New classes of antibacterial/antifungal agents, *Bioorg. Med. Chem* 25, 58–66. [PubMed: 27769670]
- [40]. Kumar DEP (2010) Principles and practice of clinical cardiovascular genetics, Oxford University Press, New York.
- [41]. Trabelsi M, Salem M, and Champagne B (2003) Investigation of the configuration of alkyl phenyl ketone phenylhydrazones from ab initio ^1H NMR chemical shifts, *Org. Biomol. Chem* 1, 3839–3844. [PubMed: 14649916]
- [42]. Bellamy AJ, and Hunter J (1976) EZ-isomerism in alkyl phenyl ketone phenylhydrazones and acetaldehyde phenylhydrazone, *J. Chem. Soc., Perkin Trans 1* 4, 456–458.
- [43]. Balkovec JM, Bouffard FA, and Black RM (1995) AZA cyclohexapeptide compounds, (Patent U, Ed.), USA.
- [44]. Donnelly JP, and De Pauw BE (2004) Voriconazole-a new therapeutic agent with an extended spectrum of antifungal activity, *Clin. Microbiol. Infect* 10 Suppl 1, 107–117. [PubMed: 14748807]
- [45]. Ramage G, Rajendran R, Sherry L, and Williams C (2012) Fungal biofilm resistance, *Int. J. Microbiol* 2012, 528521. [PubMed: 22518145]

- [46]. Borghi E, Morace G, Borgo F, Rajendran R, Sherry L, Nile C, and Ramage G (2015) New strategic insights into managing fungal biofilms, *Front. Microbiol* 6, 1077. [PubMed: 26500623]
- [47]. Fanning S, and Mitchell AP (2012) Fungal biofilms, *PLoS Pathog* 8, e1002585. [PubMed: 22496639]
- [48]. Nett JE, Cain MT, Crawford K, and Andes DR (2011) Optimizing a *Candida* biofilm microtiter plate model for measurement of antifungal susceptibility by tetrazolium salt assay, *J. Clin. Microbiol* 49, 1426–1433. [PubMed: 21227984]
- [49]. Pierce CG, Uppuluri P, Tristan AR, Wormley FL, Jr., Mowat E, Ramage G, and Lopez-Ribot JL (2008) A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing, *Nat. Protoc* 3, 1494–1500. [PubMed: 18772877]
- [50]. Klepser ME, Malone D, Lewis RE, Ernst EJ, and Pfaller MA (2000) Evaluation of voriconazole pharmacodynamics using time-kill methodology, *Antimicrob. Agents Chemother* 44, 1917–1920. [PubMed: 10858354]
- [51]. Teschemacher AG, Seward EP, Hancox JC, and Witchel HJ (1999) Inhibition of the current of heterologously expressed HERG potassium channels by imipramine and amitriptyline, *Br. J. Pharmacol* 128, 479–485. [PubMed: 10510461]
- [52]. Nguewa PA, Fuertes MA, Cepeda V, Iborra S, Carrion J, Valladares B, Alonso C, and Perez JM (2005) Pentamidine is an antiparasitic and apoptotic drug that selectively modifies ubiquitin, *Chem. Biodivers* 2, 1387–1400. [PubMed: 17191940]
- [53]. Bilik P, Tanious F, Kumar A, Wilson WD, Boykin DW, Colson P, Houssier C, Facompre M, Tardy C, and Bailly C (2001) Novel dications with unfused aromatic systems: trithiophene and trifuran derivatives of furimidazoline, *ChemBioChem* 2, 559–569. [PubMed: 11828489]
- [54]. Rollas S, and Kucukguzel SG (2007) Biological activities of hydrazone derivatives, *Molecules* 12, 1910–1939. [PubMed: 17960096]
- [55]. Sidoryk K, Switalska M, Jaromin A, Cmoch P, Bujak I, Kaczmarek M, Wietrzyk J, Dominguez EG, Zarnowski R, Andes DR, Bankowski K, Cybulski M, and Kaczmarek L (2015) The synthesis of indolo[2,3-b]quinoline derivatives with a guanidine group: highly selective cytotoxic agents, *Eur. J. Med. Chem* 105, 208–219. [PubMed: 26496013]
- [56]. Neidle S (2001) DNA minor-groove recognition by small molecules, *Nat. Prod. Rep* 18, 291–309. [PubMed: 11476483]

Highlights

- Novel *N,N'*-diaryl-bishydrazones display broad spectrum antifungal activity against various yeast and filamentous fungi.
- The most active *N,N'*-diaryl-bishydrazones display minimal hemolysis of murine red blood cells and possessed no toxicity with respect to hERG inhibition.
- The most active *N,N'*-diaryl-bishydrazones display activity against pre-formed biofilms of *C. albicans*.

Fig. 1.

A. Representative structures for bishydrazones bearing *N*-amidino or *N*-aryl groups. B. Synthetic scheme for the preparation of compounds **7a-24c**.

Fig. 2.
Mammalian cell cytotoxicity of compounds **7a**, **7b**, **10a**, **12a**, **12b**, **15a**, **15b**, **17a**, **17b**, **17c**, **20a**, and **24a** against (A) A549 and (B) BEAS-2B cell lines.

Fig. 3.

3D bar graph depicting the dose-dependent hemolytic activity of **7a**, **7b**, **12a**, **12b**, **15a**, **15b**, **17a**, **17b**, **17c**, **20a**, **24a**, and VOR against mRBCs. mRBCs were treated and incubated for 1 h at 37 °C with bishydrazones and VOR at concentrations ranging from 0.48 to 62.5 µg/mL. Triton X-100 (1% v/v) was used as a positive control (100% hemolysis, not shown).

Fig. 4.

Representative time-kill curves for VOR and compound **17c** against **A.** *C. albicans* ATCC 10231 (strain **A**) and **B.** *C. albicans* ATCC 64124 (strain **B**). Fungal strains were treated with no drug (black circles), VOR at 1× MIC (white circle), and compound **17c** at 0.5× MIC (inverted black triangle), 1× MIC (white triangle), and 2× MIC (black square). After 24 hour, these cultures were further treated with resazurin for fungal growth detection and are shown below the growth curves for each strain (a = sterile control; b = no drug; c = VOR at 1× MIC; d = compound **17c** at 0.5× MIC; e = compound **17c** at 1× MIC; and f = compound **17c** at 2× MIC).

Fig. 5.
IC₅₀ curves for hERG interaction by compounds **15a**, **15b**, **17a**, **17c**, **24a**, and amitriptyline, which are presented as mean ± SDEV.

Table 1.

MIC values (in µg/mL) determined for compounds **7a-24c** as well as for two control antifungal agents (CAS and VOR) against various yeast strains and filamentous fungi.

Cpd #	Yeast strains										Filamentous fungi		
	A	B	C	D	E	F	G	H	I	J	K	L	M
7a	1.95	31.3	31.3	31.3	31.3	31.3	3.9	>31.3	7.8	>31.3	3.9	1.95	15.6
7b	7.8	>31.3	>31.3	31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	3.9	1.95	15.6
9c	15.6	>31.3	>31.3	>31.3	>31.3	>31.3	31.3	>31.3	31.3	31.3	>31.3	>31.3	>31.3
10a	7.8	7.8	>31.3	15.6	31.3	>31.3	7.8	>31.3	1.95	15.6	3.9	7.8	>31.3
10b	3.9	>31.3	15.6	31.3	>31.3	>31.3	15.6	>31.3	3.9	>31.3	>31.3	31.3	>31.3
11c	0.98	0.98	15.6	31.3	15.6	31.3	3.9	15.6	0.98	15.6	>31.3	>31.3	>31.3
12a	1.95	>31.3	7.8	15.6	>31.3	15.6	7.8	>31.3	1.95	31.3	3.9	7.8	>31.3
12b	7.8	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	15.6	3.9	31.3	>31.3	>31.3
13b	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	31.3	>31.3	>31.3	>31.3	>31.3	>31.3	31.3
15a	1.95	15.6	3.9	3.9	7.8	3.9	7.8	7.8	3.9	>31.3	>31.3	31.3	>31.3
15b	7.8	3.9	7.8	7.8	3.9	15.6	7.8	7.8	7.8	15.6	>31.3	7.8	>31.3
15c	15.6	15.6	15.6	31.3	15.6	7.8	7.8	7.8	15.6	>31.3	>31.3	>31.3	>31.3
16b	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3
17a	1.95–3.9	15.6	7.8	>31.3	7.8	15.6	3.9	>31.3	>31.3	31.3	15.6	3.9	>31.3
17b	7.8	15.6	7.8	31.3	15.6	7.8	7.8	15.6	1.95	31.3	7.8	7.8	>31.3
17c	1.95	3.9	3.9	7.8	1.95	7.8	7.8	15.6	1.95	15.6	>31.3	7.8	>31.3
18a	7.8	7.8	15.6	15.6	31.3	15.6	3.9	15.6	31.3	3.9	>31.3	>31.3	>31.3
18b	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	31.3	31.3	>31.3	>31.3	31.3
19a	31.3	31.3	31.3	>31.3	>31.3	7.8	31.3	7.8	>31.3	>31.3	31.3	>31.3	>31.3
19b	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	31.3	>31.3	>31.3	>31.3
20a	3.9	7.8	15.6	15.6	15.6	7.8	7.8	15.6	1.95	31.3	7.8	7.8	31.3
20b	7.8	>31.3	>31.3	31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3
21a	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3
21b	31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3
22a	3.9	15.6	31.3	>31.3	>31.3	31.3	31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3
22b	15.6	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3
23b	31.3	31.3	>31.3	>31.3	31.3	31.3	>31.3	15.6	31.3	>31.3	>31.3	31.3	>31.3
24a	1.95–3.9	3.9	3.9	>31.3	3.9	7.8	>31.3	3.9	1.95	1.95	>31.3	7.8	7.8
24b	31.3	>31.3	>31.3	>31.3	>31.3	>31.3	15.6	>31.3	15.6	3.9	>31.3	>31.3	>31.3
24c	3.9	3.9	15.6	3.9	15.6	15.6	15.6	7.8	3.9	1.95	>31.3	15.6	>31.3
CAS	0.975	0.24	0.06	0.12	0.12	0.24	0.48	0.06	0.48	1.95	>31.3	>31.3	>31.3
VOR	0.975	0.24	0.06	0.12	0.12	0.24	0.48	0.06	0.48	1.95	>31.3	>31.3	>31.3

Yeast strains: **A** = *Candida albicans* ATCC 10231(R), **B** = *C. albicans* ATCC 64124(R), **C** = *C. albicans* ATCC MYA-2876(S), **D** = *C. albicans* ATCC 90819(R), **E** = *C. albicans* ATCC MYA-2310(S), **F** = *C. albicans* ATCC MYA-1237(R), **G** = *C. albicans* ATCC MYA-1003(R), **H** = *Candida glabrata* ATCC 2001, **I** = *Candida krusei* ATCC 6258, **J** = *Candida parapsilosis* ATCC 22019. NOTE: Here, the (S) and (R) indicate that ATCC reports these strains to be susceptible (S) and resistant (R) to ITC and FLC.

Filamentous fungi: **K** = *Aspergillus flavus* ATCC MYA-3631, **L** = *Aspergillus nidulans* ATCC 38163, **M** = *Aspergillus terreus* ATCC MYA-3633.

Known antifungal agents: **CAS** = caspofungin, **VOR** = voriconazole.

^aFor yeast strains: MIC-0 values are reported for all compounds tested and CAS and VOR against strain **A**. MIC-2 value is reported for against strains **B-J**. For filamentous fungi (strains **K-M**), MIC-0 values are reported for all compounds.

Note: The MIC values for compounds **8a**, **8b**, **9a**, **9b**, **11a**, **11b**, and **14a** were not determined as these compounds are not soluble.

Table 2.

Antibiofilm activity of compound **17c** and VOR against *C. albicans* 10231 (strain **A**) and *C. albicans* 64124 (strain **B**) biofilms.

	<i>C. albicans</i> 10231 (strain A)		<i>C. albicans</i> 64124 (strain B)	
Cpd #	SMIC ₅₀ (µg/mL)	SMIC ₉₉ (µg/mL)	SMIC ₅₀ (µg/mL)	SMIC ₉₉ (µg/mL)
17c	8	>32	16	>32
VOR	16	>32	16	>32

Table 3.

CC₅₀ (in µg/mL) and selectivity index (SI) values of compounds **7a**, **7b**, **10a**, **12a**, **12b**, **15a**, **15b**, **17a**, **17b**, **17c**, **20a**, and **24a** against mammalian cell lines and various yeast strains and filamentous fungi.

Cpd #	CC ₅₀		SI (Yeast strains)										SI (Filamentous fungi)		
	A549	BEAS-2B	A	B	C	D	E	F	G	H	I	J	K	L	M
7a	>31	>31	16	1.0	1.0	1.0	1.0	1.0	4.0	1.0	4.0	1.0	8.0	16	2.0
7b	17.7	11.0	2.3	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	4.5	9.1	1.1
10a	16.4	>31	2.1	2.1	0.5	1.1	0.5	0.5	2.1	0.5	8.4	1.1	4.2	2.1	0.5
12a	11.7	11.8	6.0	0.4	1.5	0.8	0.4	0.8	1.5	0.4	6.0	0.4	3.0	1.5	0.4
12b	>31	16.0	4.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	8.0	1.0	1.0	1.0
15a	18.2	14.1	9.3	1.2	4.7	4.7	2.3	4.7	2.3	2.3	4.7	0.6	0.6	0.6	0.6
15b	>31	16.4	4.0	8.0	4.0	4.0	8.0	2.0	4.0	4.0	4.0	2.0	1.0	4.0	1.0
17a	>31	>31	16	2.0	4.0	1.0	4.0	2.0	8.0	1.0	1.0	1.0	2.0	8.0	1.0
17b	17.7	8.5	2.3	1.1	2.3	0.6	1.1	2.3	2.3	1.1	9.1	0.6	2.3	2.3	0.6
17c	16.6	10.7	8.5	4.3	4.3	2.1	8.5	2.1	2.1	1.1	8.5	1.1	0.5	2.1	0.5
20a	16.4	16.9	4.2	2.1	1.1	1.1	1.1	2.1	2.1	1.1	8.4	0.5	2.1	2.1	0.5
24a	5.3	2.9	2.7	1.4	1.4	0.7	1.4	0.7	0.7	1.4	2.7	2.7	0.2	0.7	0.7

Yeast strains: **A** = *Candida albicans* ATCC 10231(R), **B** = *C. albicans* ATCC 64124(R), **C** = *C. albicans* ATCC MYA-2876(S), **D** = *C. albicans* ATCC 90819(R), **E** = *C. albicans* ATCC MYA-2310(S), **F** = *C. albicans* ATCC MYA-1237(R), **G** = *C. albicans* ATCC MYA-1003(R)1003(R), **H** = *Candida glabrata* ATCC 2001, **I** = *Candida krusei* ATCC 6258, **J** = *Candida parapsilosis* ATCC 22019. NOTE: Here, the (S) and (R) indicate that ATCC reports these strains to be susceptible (S) and resistant (R) to ITC and FLC.

Filamentous fungi: **K** = *Aspergillus flavus* ATCC MYA-3631, **L** = *Aspergillus nidulans* ATCC 38163, **M** = *Aspergillus terreus* ATCC MYA-3633.