Very Important Paper

Broad-Spectrum Antifungal Agents: Fluorinated Aryl- and Heteroaryl-Substituted Hydrazones

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Fluorinated aryl- and heteroaryl-substituted monohydrazones displayed excellent broad-spectrum activity against various fungal strains, including a panel of clinically relevant *Candida auris* strains relative to a control antifungal agent, voriconazole (VRC). These monohydrazones displayed less hemolysis of murine red blood cells than that of VRC at the same concentrations, possessed fungicidal activity in a time-kill study, and exhibited no mammalian cell cytotoxicity. In addition, these monohydrazones prevented the formation of biofilms that otherwise block antibiotic effectiveness and did not trigger the development of resistance when exposed to *C. auris* AR Bank # 0390 over 15 passages.

Introduction

Nosocomial fungal infections[1] represent continuing threats to medical advances conjoined with immunosuppression and often afflict nursing home residents or hospitalized patients undergoing transplantation,[2] antiviral,[3] and antineoplastic therapies.[4] In addition, the emergence of new strains of fungal pathogens that resist[5] current drug therapies and possess high mortality rates compound these ongoing threats.[6] Appearing first in 2009 in Japan,[7] *Candida auris* represents an archetypical, fungal infection that presents challenges in terms of its diagnosis[8] and its treatment because some strains are resistant to the three available classes of current antifungals: the azoles (*e.g.*, fluconazole (FLC) and voriconazole (VRC)), the echinocandins (*e.g.*, caspofungin (CFG)), and the polyenes (*e.g.*, amphotericin B (AmB)). Front-page articles in *The New York Times*[9] delineate the dangers that *C. auris* represents and call for research to address this healthcare problem at a time of declining investment in antimicrobial drug development within the pharmaceutical industry.[10]

Paramount among the challenges facing investigators intent on antifungal drug development are the issues of potency, breadth of selectivity, biofilm penetration or prevention, cytotoxicity including erythrocyte hemolysis, and the development of resistance. Despite this gamut of hurdles, the complexity of fungal cell architecture offers an array of as yet, unexplored targets for drug development. Prior efforts by multiple investigators focused on antifungal agents[6a,11] possessing chemically diverse scaffolds including aminoglycosides,[12] benzimidazoles,[12d,13] azoles,[14] haloperidols,[15] gold(I) complexes,[16] and ebselen/ebsulfur.[17] We now report the development of fluorinated, aryl- and heteroaryl-substituted hydrazones as compounds that meet these challenges and represent a new class of potential agents for the selective treatment of candidiasis.[18] Of particular interest, these new agents show particular promise for the treatment of *C. auris* infections that now afflict an increasing number of patients in nursing homes and hospitals.[19]

We previously reported the development of bishydrazones **I** and **II** (Figure 1) bearing either *N*-amidino or *N*-aryl groups, respectively, as potential antibacterial and antifungal agents.[20] Subsequent structure-activity studies revealed that alkoxy-substituted, aryl groups attached to bishydrazones **III** with biphenyl linkers had greater potency as *in vitro* antifungal agents than as antibacterial agents, possessed minimal toxicity, and exhibited no resistance through multiple generations.[21]

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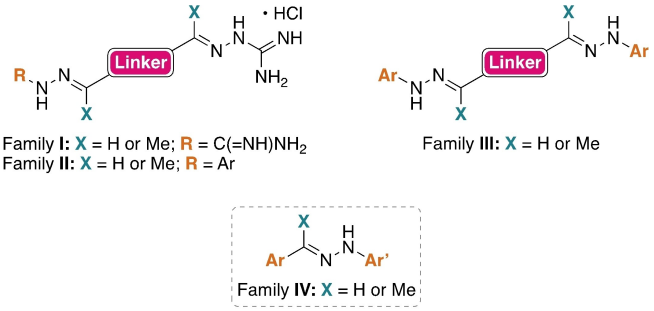


Figure 1. General structures of *N*-aryl and *N*-amidino-substituted bishydrazones (families **I**–**III**), and monohydrazones (family **IV**). The square represents the hydrocarbon platform separating these hydrazone groups in families **I**–**III**.

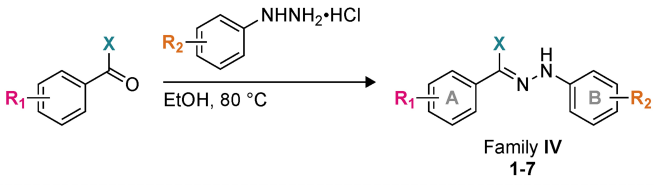
Further work disclosed that no particular advantage accrued to symmetrical bishydrazones relative to comparable monohydrazones in which an aryl or heteroaryl group replaced the biphenyl linker in **III**. The monohydrazones **IV** (*i.e.*, compounds **1**–**7** in Figure 2) bearing fluorinated aryl or heteroaryl groups possessed not only the positive spectrum of drug attributes seen for the bishydrazones **III** but also surprising potency and selectivity for ten fungal strains in the *C. auris* family.

Results and Discussion

Synthesis. The synthesis of 35 family **IV** monohydrazones in seven series (**1**–**7**), each comprised of six compounds with varied R_2 groups (**a**–**f**) entailed the condensation of either substituted aldehydes (*i.e.*, benzaldehyde, 3-fluorobenzaldehyde, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde, 2,4-difluorobenzaldehyde) or acetophenone with 1–2 equivalents of substituted phenylhydrazines at 80 °C to yield **1a**–**7f** in 26–98% yields (Figure 2).

Antifungal activity by determination of minimum inhibitory concentration (MIC) values. To provide comparable data for monohydrazones in family **IV** relative to their previously reported counterparts, namely the bishydrazones in family **III**, we first tested the 35 monohydrazones **1a**–**7f** against a panel of seven strains (*A*–*G*) of *Candida 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*) (Table 1). We also explored their activity against a panel of three non-*albicans Candida* strains: *Candida glabrata* ATCC 2001 (*H*), *Candida krusei* ATCC 6258 (*I*), and *Candida parapsilosis* ATCC 22019 (*J*) (Table 1). Throughout this study, we employed a range of concentrations varying from 0.03 to 31.3 μg/mL for monohydrazones **1a**–**7f**, as well as for the commercially available, positive antifungal controls, amphotericin B (AmB), caspofungin (CFG), fluconazole (FLC), and voriconazole (VRC). In Table 1, MIC-0 values (*i.e.*, no visible growth) were reported for monohydrazones **1a**–**7f** and for the positive controls AmB and CFG, and MIC-2 values (*i.e.*, 50% growth inhibition) were reported for FLC and VRC against all fungal strains tested. Herein, we defined antifungal activity as excellent (≤1.95 μg/mL), good (3.9–7.8 μg/mL), or poor (≥15.6 μg/mL) based on MIC values, and we utilized a color scheme (excellent, green; good, yellow; and poor, pink) to provide an overall visual picture of the performance of individual monohydrazones *versus* positive controls.

From a quick glance at the data reported in Table 1, we observed that compounds **5a** and **7a** generally displayed poor activity against the ten strains (*A*–*J*) tested, and we excluded them from additional biological studies but not additional MIC values determination. The 33 remaining monohydrazones synthesized displayed excellent to good activity against these ten fungal strains. A detailed analysis of the seven series (*i.e.*, series **1**–**7**) led to the following conclusions. Monohydrazones **1a**–**1f** with no substituents in the benzylidine portion of the monohydrazones (*i.e.*, R_1 = H; Figure 2) displayed excellent to good activity against strains *A*–*J* (0.49–7.8 μg/mL) with the exception of compounds **1a** (MIC = 15.6 μg/mL against *H*), **1c** (MIC ≥15.6 μg/mL against *E* and *H*), **1d** (MIC = 15.6 μg/mL against *E*), and **1e** (MIC ≥15.6 μg/mL against *E* and *J*). Monohydrazones **2a**–**2f** with *meta*-fluorobenzylidene structures (*i.e.*, R_1 = *m*-F; Figure 2) displayed excellent to good activity (0.06–7.8 μg/mL) against all fungal strains tested with exception of compounds **2a**, **2b**, **2c**, **2e**, and **2f** against strains *E* and *H* (15.6 μg/mL), strain *F* (15.6 μg/mL), strains *D* and *G* (15.6 μg/mL), strains *H* and *J* (15.6 and 31.3 μg/mL), and strains *B* and *F*



1a: R_1 = H, X = H, R_2 = H (66%)	**4c**: R_1 = *p*-Cl, X = H, R_2 = *p*-F (87%)
1b: R_1 = H, X = H, R_2 = *m*-F (x)	**4d**: R_1 = *p*-Cl, X = H, R_2 = *p*-Cl (83%)
1c: R_1 = H, X = H, R_2 = *p*-F (46%)	**4e**: R_1 = *p*-Cl, X = H, R_2 = *p*-OMe (94%)
1d: R_1 = H, X = H, R_2 = *p*-Cl (47%)	**4f**: R_1 = *p*-Cl, X = H, R_2 = *o,p*-diF (84%)
1e: R_1 = H, X = H, R_2 = *p*-OMe (62%)	**5a**: R_1 = *p*-OMe, X = H, R_2 = H (66%)
1f: R_1 = H, X = H, R_2 = *o,p*-diF (78%)	**5b**: R_1 = *p*-OMe, X = H, R_2 = *m*-F (x)
2a: R_1 = *m*-F, X = H, R_2 = H (96%)	**5c**: R_1 = *p*-OMe, X = H, R_2 = *p*-F (98%)
2b: R_1 = *m*-F, X = H, R_2 = *m*-F (92%)	**5d**: R_1 = *p*-OMe, X = H, R_2 = *p*-Cl (87%)
2c: R_1 = *m*-F, X = H, R_2 = *p*-F (88%)	**5e**: R_1 = *p*-OMe, X = H, R_2 = *p*-OMe (49%)
2d: R_1 = *m*-F, X = H, R_2 = *p*-Cl (82%)	**5f**: R_1 = *p*-OMe, X = H, R_2 = *o,p*-diF (89%)
2e: R_1 = *m*-F, X = H, R_2 = *p*-OMe (96%)	**6a**: R_1 = *o,p*-diF, X = H, R_2 = H (94%)
2f: R_1 = *m*-F, X = H, R_2 = *o,p*-diF (94%)	**6b**: R_1 = *o,p*-diF, X = H, R_2 = *m*-F (95%)
3a: R_1 = *p*-F, X = H, R_2 = H (84%)	**6c**: R_1 = *o,p*-diF, X = H, R_2 = *p*-F (91%)
3b: R_1 = *p*-F, X = H, R_2 = *m*-F (x)	**6d**: R_1 = *o,p*-diF, X = H, R_2 = *p*-Cl (82%)
3c: R_1 = *p*-F, X = H, R_2 = *p*-F (91%)	**6e**: R_1 = *o,p*-diF, X = H, R_2 = *p*-OMe (x)
3d: R_1 = *p*-F, X = H, R_2 = *p*-Cl (81%)	**6f**: R_1 = *o,p*-diF, X = H, R_2 = *o,p*-diF (97%)
3e: R_1 = *p*-F, X = H, R_2 = *p*-OMe (x)	**6g**: R_1 = *o,p*-diF, X = H, R_2 = *o,m*-diF (96%)
3f: R_1 = *p*-F, X = H, R_2 = *o,p*-diF (98%)	**6h**: R_1 = *o,p*-diF, X = H, R_2 = *m,m*-diF (95%)
4a: R_1 = *p*-Cl, X = H, R_2 = H (79%)	**7a**: R_1 = H, X = Me, R_2 = H (26%)
4b: R_1 = *p*-Cl, X = H, R_2 = *m*-F (89%)	**7f**: R_1 = H, X = Me, R_2 = *o,p*-diF (77%)

Figure 2. Synthetic scheme for the preparation of compounds **1a**–**7f** in family **IV**.

Table 1. MIC values (μg/mL) determined for compounds **1a–7a** as well as the antifungal controls AmB, CFG, FLC, and VRC against seven *Candida albicans* strains (*A–G*) and three non-*albicans Candida* strains (*H–J*). The LogP values calculated in ChemDraw and Molinspiration are also provided.

Cpd #	LogP value		Strain									
	ChemDraw	Molinspiration	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*
1a	3.42	4.76	0.49	7.8	3.9	3.9	7.8	7.8	1.95	15.6	0.98	7.8
1c	3.58	4.93	0.49	7.8	3.9	7.8	31.3	7.8	1.95	15.6	1.95	7.8
1d	3.98	5.44	0.98	3.9	3.9	7.8	15.6	1.95	1.95	3.9	0.98	7.8
1e	3.29	4.82	0.98	3.9	3.9	7.8	15.6	3.9	1.95	3.9	1.95	31.3
1f	3.73	5.02	0.49	3.9	7.8	7.8	3.9	1.95	3.9	3.9	0.49	7.8
2a	3.58	4.90	0.98	7.8	7.8	7.8	15.6	7.8	3.9	15.6	3.9	7.8
2b	3.73	5.04	0.49	0.98	1.95	3.9	1.95	15.6	1.95	3.9	1.95	3.9
2c	3.73	5.07	0.06	7.8	0.98	15.6	3.9	3.9	15.6	7.8	3.9	7.8
2d	4.13	5.58	1.95	3.9	3.9	3.9	0.98	3.9	1.95	1.95	3.9	3.9
2e	3.45	4.96	1.95	7.8	3.9	7.8	1.95	3.9	1.95	15.6	0.12	31.3
2f	3.89	5.16	0.24	15.6	3.9	7.8	7.8	15.6	0.98	7.8	0.98	7.8
3a	3.58	4.93	0.24	1.95	1.95	1.95	0.98	1.95	0.98	3.9	0.24	3.9
3c	3.73	5.09	0.12	7.8	1.95	15.6	7.8	3.9	15.6	15.6	3.9	7.8
3d	4.13	5.61	7.8	7.8	1.95	7.8	15.6	7.8	1.95	3.9	1.95	31.3
3f	3.89	5.18	0.12	1.95	3.9	1.95	15.6	1.95	0.98	3.9	0.49	7.8
4a	3.98	5.44	0.98	7.8	3.9	15.6	31.3	7.8	1.95	>31.3	1.95	15.6
4b	4.13	5.58	0.49	7.8	1.95	7.8	3.9	7.8	1.95	15.6	0.49	7.8
4c	4.13	5.61	0.49	7.8	3.9	31.3	7.8	7.8	1.95	15.6	15.6	7.8
4d	4.53	6.12	3.9	3.9	7.8	15.6	31.3	7.8	3.9	7.8	7.8	31.3
4e	3.85	5.50	0.98	15.6	15.6	15.6	7.8	7.8	15.6	7.8	7.8	31.3
4f	4.29	5.70	0.98	7.8	7.8	15.6	31.3	7.8	3.9	15.6	0.98	15.6
5a	3.29	4.82	0.98	>31.3	>31.3	15.6	>31.3	>31.3	>31.3	>31.3	>31.3	>31.3
5c	3.45	4.98	0.98	1.95	1.95	3.9	0.98	1.95	0.98	3.9	0.24	3.9
5d	3.85	5.50	0.12	3.9	3.9	1.95	15.6	7.8	1.95	1.95	0.49	7.8
5e	3.16	4.88	15.6	7.8	3.9	15.6	15.6	3.9	3.9	31.3	3.9	>31.3
5f	3.61	5.08	0.49	0.24	3.9	0.98	3.9	0.98	0.49	1.95	0.24	1.95
6a	3.73	5.02	0.98	7.8	3.9	3.9	7.8	7.8	3.9	15.6	0.98	7.8
6b	3.89	5.16	0.98	0.98	7.8	15.6	0.98	7.8	0.49	3.9	0.12	7.8
6c	3.89	5.18	0.24	15.6	1.95	15.6	7.8	7.8	0.98	15.6	1.95	15.6
6d	4.29	5.70	0.49	1.95	0.98	15.6	1.95	3.9	0.98	1.95	1.95	7.8
6f	4.05	5.27	0.98	7.8	3.9	3.9	7.8	7.8	1.95	7.8	0.98	7.8
6g	4.05	5.27	0.12	31.3	1.95	15.6	7.8	15.6	31.3	31.3	1.95	7.8
6h	4.05	5.27	0.06	15.6	0.98	15.6	3.9	3.9	7.8	7.8	0.98	3.9
7a	2.98	5.21	7.8	>31.3	>31.3	>31.3	31.3	>31.3	1.95	31.3	15.6	>31.3
7f	3.3	5.46	0.24	0.49	0.98	>31.3	0.98	7.8	0.49	31.3	0.98	3.9
AmB[a]			3.9	3.9	1.95	0.98	1.95	3.9	3.9	1.95	3.9	1.95
CFG[a]			0.98	0.24	0.06	0.12	0.12	0.24	0.49	0.06	0.49	1.95
FLC[a]			62.5	>125	15.6	>125	>125	62.5	62.5	>31.3	>31.3	1.95
VRC[a]			0.24	3.9	1.95	1.95	0.98	7.8	1.95	0.06	0.12	0.03

Strains: *A* = *C. albicans* ATCC 10231, *B* = *C. albicans* ATCC 64124, *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* = *C. glabrata* ATCC 2001, *I* = *C. krusei* ATCC 6258, and *J* = *C. parapsilosis* ATCC 22019. NOTE: Here, the (S) and (R) indicate that ATCC reports these strains to be susceptible (S) and resistant (R) to itraconazole (ITC) and FLC. Known antifungal agents: AmB = amphotericin B, CFG = caspofungin, FLC = fluconazole, and VRC = voriconazole. MIC-0 values are reported for compounds **1a-7f**, AmB, and CFG; and MIC-2 values are reported for azoles.

[a] These values were previously reported in ref [12d], and are here for comparison purpose.

MIC ≤1.95 μg/mL (excellent antifungal activity)

MIC = 3.9-7.8 μg/mL (good antifungal activity)

MIC ≥15.6 μg/mL (poor antifungal activity)

(15.6 μg/mL), respectively. Monohydrazones **3a**, **3c**, **3d**, and **3f** with *para*-fluorobenzylidene structures (*i.e.*, R_1 = *p*-F; Figure 2) displayed excellent to good activity against strains *A-J* (0.12–7.8 μg/mL), but compounds **3c**, **3d**, and **3f** displayed poor activity (15.6–31.3 μg/mL) against strains (*D*, *G*, and *H*), strains (*E* and *J*), and strain *E*, respectively. In the case of monohydrazones **4a–4f** with *para*-chlorobenzylidene structures (*i.e.*, R_1 = *p*-Cl; Figure 2), these compounds displayed excellent to good activity against strains *A–C*, *F*, *G*, and *I* (0.49–7.8 μg/mL) with the exception of compounds **4c** and **4e**, which displayed poor activity against strains *I*, and strains *B*, *C*, and *G*, respectively. In the case of monohydrazones **5a–5f** with *para*-methoxybenzylidene structures (*i.e.*, R_1 = *p*-OMe; Figure 2), compounds **5c**, **5d**, and **5f** exhibited excellent to good activity (0.24–7.8 μg/mL) against the whole panel of ten fungal strains tested, with the exception of compound **5d** against strain *E* (15.6 μg/mL). Compound **5e** displayed good activity against strains *B*, *C*, *F*, *G*, and *I* (3.9–7.8 μg/mL). In the case of monohydrazones **6a–6h** with *ortho,para*-difluorobenzylidene structures (*i.e.*, R_1 = *o,p*-diF; Figure 2) compounds **6a**, **6b**, **6d**, **6f**, and **6h** exhibited excellent to good activity (0.06–7.8 μg/mL) against strains *A–J* with the exception of compounds **6a**, **6b**, **6d**, and **6h** against strain *H* (15.6 μg/mL), strain *D* (15.6 μg/mL), strain *D* (15.6 μg/mL), and strains *B* and *D* (15.6 μg/mL),

respectively. Compounds **6c** and **6g** exhibited excellent to good activity against strains *A*, *C*, *E*–*G*, and *I* (0.24–7.8 μg/mL) and strains *A*, *C*, *E*, *I*, and *J* (0.12–7.8 μg/mL), respectively. Finally, monohydrazone **7f** with a 1-phenylethylidene structure (*i.e.*, R_1 = H; Figure 2) displayed excellent to good activity (0.24–7.8 μg/mL) against strains *A*–*C*, *E*–*G*, *I*, and *J*. In summary, perhaps best grasped from the green and yellow colors in Table 1, a comparison with the FDA-approved antifungal agents, AmB, CFG, FLC, and VRC with some of these monohydrazones revealed that monohydrazones exhibited comparable or superior activity against strains *A*, *B*, *F*, *G*, and *I*.

We next explored the activity of representatives monohydrazones (*i.e.*, **1a**, **1c**, **1d**, **1e**, **1f**, **2b**, **2d**, **2f**, **4c**, **4d**, **5e**, **6b**, and **7a**) against three *Aspergillus* strains: *Aspergillus flavus* ATCC MYA-3631 (*K*), *Aspergillus nidulans* ATCC 38163 (*L*), and *Aspergillus terreus* ATCC MYA-3633 (*M*) (Table S1). We found all of the representative monohydrazones tested to be generally inactive as antifungal agents against *Aspergillus* strains. As a result, we decided against testing the remaining 22 compounds against these three *Aspergillus* strains. From all of the observations made on compounds **1a**–**7f**, we concluded that compounds **1d**, **2b**, **2d**, **2e**, **3a**, **3f**, **4b**, **5c**, **5d**, **5f**, **6b**, **6c**, **6d**, and **7f** displayed the best overall activity. It is important to point out that these compounds maintained activity against the FLC-resistant *C. albicans* strain.

Based on the promising antifungal activities observed in Table 1, we selected seven of the best compounds (*i.e.*, **2b**, **3f**, **4b**, **5f**, **6b**, **6d**, and **7f**) and two of the worst (*i.e.*, **5a** and **7a** as negative controls) for further testing against a panel of ten *C. auris* strains (AR Bank # 0381–0390) and ten other fungal strains including three *Candida duobushaemulonii* strains (AR Bank # 0391, AR Bank # 0392, and AR Bank # 0394), two *Candida haemulonii* strains (AR Bank # 0393, and AR Bank # 0395), two *Saccharomyces cerevisiae* strains (AR Bank # 0399 and AR Bank # 0400), and one each of the following strains: *Kodameae ohmeri* (AR Bank # 0396), *Candida krusei* (AR Bank # 0397), and *Candida lusitaniae* (AR Bank # 0398) (Table 2). Using a concentration range of 0.015–31.3 μg/mL for the nine selected monohydrazones and using AmB, CFG, FLC, and VRC as positive controls, we obtained MIC-0 values (*i.e.*, no visible growth) for the monohydrazones and the control AmB, and the MIC-2 values (*i.e.*, 50% growth inhibition) for CFG, FLC, and VRC. Monohydrazones **2b**, **3f**, **4b**, **5f**, **6d**, and **7f** displayed excellent to good activity (0.015–7.8 μg/mL) against all 20 strains tested. Compound **6b** exhibited excellent to good activity (0.24–7.8 μg/mL) against most of the strains tested, with the exception of strains AR Bank # 0383–0385, AR Bank # 0387, AR Bank # 0389, AR Bank # 0399, and AR Bank # 0400 (15.6–31.3 μg/mL). As expected based on their poor activity against *C. albicans*, compounds **5a** and **7a** displayed poor activity against the ten *C. auris* strains tested. However, compound **5a** displayed excellent to good activity (0.12–7.8 μg/mL) against *C. duobushaemulonii*, *C. haemulonii*, *S. cerevisiae*, *K. ohmeri*, *C. krusei*, and *C. lusitaniae*. On the other hand, compound **7a** only displayed excellent activity (0.49–0.98 μg/mL) against strains AR Bank # 0393, AR Bank # 0395, and AR Bank # 0397. Overall, as shown in Table 2, the most active monohydrazones, namely **2b**, **3f**, **4b**, **5f**, **6b**, **6d**, and **7f**, displayed excellent activity against a panel of ten *C. auris* (AR Bank # 0381–0390) and ten other fungal strains (AR Bank # 0391–0400). Excluding the *C. auris* strains, monohydrazones **5a** and **7a** showed promise against other *K. ohmeri* and other *Candida* strains.

Table 2. MIC values (μg/mL) determined for compounds **1a**–**7a** as well as the antifungal controls AmB, CFG, FLC, and VRC against ten *Candida auris* strains (AR Bank # 0381-0390) and ten other fungal strains (AR Bank # 0391-0400).

Strain	AR #	Cpd #									Controls		
		2b	3f	4b	5a	5f	6b	6d	7a	7f	AmB	CFG	FLC	VRC
C. auris	0381	0.49	0.24	0.24	31.3	0.24	3.9	0.98	31.3	0.24	0.98	≤0.06	0.49	0.06
	0382	0.12	0.24	0.24	3.9	0.49	1.95	0.98	31.3	0.49	1.95	≤0.06	0.49	0.06
	0383	0.49	1.95	0.49	31.3	0.98	15.6	1.95	>31.3	7.8	3.9	≤0.06	62.6	1.95
	0384	3.9	3.9	1.95	>31.3	3.9	15.6	3.9	>31.3	7.8	1.95	0.12	31.3	0.24
	0385	0.49	0.49	0.49	>31.3	0.98	15.6	0.98	>31.3	0.98	3.9	≤0.06	62.6	1.95
	0386	0.24	0.24	0.24	7.8	0.24	1.95	0.24	31.3	0.49	0.98	≤0.06	>62.6	3.9
	0387	0.12	0.12	0.12	31.3	0.12	15.6	0.49	31.3	0.12	1.95	≤0.06	>62.6	3.9
	0388	0.12	0.12	0.12	15.6	0.12	7.8	0.49	>31.3	0.12	1.95	≤0.06	0.98	0.06
	0389	0.12	0.12	0.12	15.6	0.24	15.6	0.98	>31.3	≤0.06	0.98	≤0.06	>62.6	0.49
	0390	0.98	1.95	0.98	15.6	1.95	7.8	7.8	>31.3	1.95	1.95	≤0.06	>62.6	0.98
C. duobushaemulonii	0391	0.12	0.12	0.12	0.98	0.12	0.98	0.12	3.9	0.12	0.98	0.06	>62.6	>31.3
C. duobushaemulonii	0392	0.49	1.95	0.49	0.24	0.06	0.49	0.24	7.8	>3.9	3.9	0.06	62.6	>31.3
C. haemulonii	0393	0.24	0.24	0.49	0.24	0.24	0.98	0.12	0.98	0.24	1.95	0.12	>62.6	31.3
C. duobushaemulonii	0394	0.12	0.12	0.12	3.9	0.98	7.8	0.12	31.3	0.49	0.98	0.06	62.6	>31.3
C. haemulonii	0395	0.12	0.12	0.12	0.12	0.12	0.24	0.24	0.49	0.12	3.9	0.12	>62.6	3.9
K. ohmeri	0396	0.49	0.49	0.98	0.24	0.49	0.98	0.98	3.9	0.98	1.95	0.12	7.8	0.06
C. krusei	0397	0.015	0.03	0.015	0.12	0.03	0.24	0.06	0.49	0.015	0.49	0.12	>62.6	3.9
C. lusitaniae	0398	1.95	3.9	1.95	1.95	0.98	3.9	0.98	31.3	3.9	3.9	0.12	1.95	0.06
S. cerevisiae	0399	1.95	3.9	1.95	7.8	0.98	31.3	0.98	31.3	3.9	1.95	0.12	1.95	0.24
S. cerevisiae	0400	1.95	1.95	1.95	3.9	0.98	15.6	0.98	15.6	1.95	1.95	0.24	3.9	0.12

Known antifungal agents: AmB = amphotericin B, CFG = caspofungin, FLC = fluconazole, and VRC = voriconazole. MIC-0 values are reported for compounds **1a-7f** as well as AmB. MIC-2 values are reported for CFG at 24 h and for the azoles, FLC and VRC, at 48 h.

MIC ≤1.95 μg/mL (excellent antifungal activity)

MIC = 3.9-7.8 μg/mL (good antifungal activity)

MIC ≥15.6 mg/μL (poor antifungal activity)

The monohydrazones used in this study were synthesized by condensation reaction between substituted aldehydes with substituted phenylhydrazines. We performed a checkerboard assay where benzaldehyde derivatives and phenylhydrazine derivatives were tested in combination to evaluate the activity of the components used for the synthesis of monohydrazones (Table S2). For checkerboard assay we considered monohydrazones **3f**, **4b**, and **6d**. Thus, the corresponding benzaldehyde derivatives and phenylhydrazine derivatives used for their synthesis were utilized in the assay. The aldehyde derivatives by themselves did not exhibit any antifungal activity at 31.3 μg/mL. The phenylhydrazine derivatives exhibited excellent antifungal activity by themselves with MIC values in the range of 0.06–1.95 μg/mL, which are comparable to their monohydrazone counterparts. When tested in combination, these benzaldehyde derivatives and phenylhydrazine derivatives exhibited an additive effect. These data indicate that the activity that we observe for our monohydrazones do not result from molecules that would have been degraded. Additionally, we explored the stability of monohydrazone **4b** as a representative by incubating it in the RPMI medium that was used for antifungal testing. Compound **4b** was stable even after 6 days, as observed by liquid chromatography-mass spectrometry (LC-MS) (Figure S109).

Structure-activity relationship (SAR) analysis. The substitution pattern and identity of the substituent(s) in rings A (R_1) and B (R_2) had a considerable influence on the activity of these monohydrazones. When investigating the effect of the R_2 substituent while keeping R_1 constant (*i.e.*, comparing the monohydrazones within each series), we observed that when R_1 = H (series **1**, Figure 2), the introduction of either *o*,*p*-diF (**1f**), *p*-Cl (**1d**), or H (**1a**) as R_2 substituents resulted in better antifungal activity than other substituents (*i.e.*, *m*-F, *p*-F and *p*-OMe). For series **2** (R_1 = *m*-F), compounds **2b**, **2d**, and **2e** that displayed excellent activity had *m*-F, *p*-Cl, and *p*-OMe as R_2 substituents. In addition, when the R_1 groups = *p*-F, *p*-Cl, or *p*-OMe (series **3**, series **4**, or series **5**, Figure 2), the most active monohydrazones in each series (*i.e.*, (**3a**, **3f**, and **3d**), (**4b**, **4a**, and **4c**), and (**5f**, **5c**, and **5d**)) possessed (H, *o*,*p*-diF, and *p*-Cl), (*m*-F, H, and *p*-F), and (*o*,*p*-diF, *m*-F, and *p*-Cl) as R_2 substituents, respectively. In series **6** (R_1 = *o*,*p*-diF), the R_2 substituents *p*-Cl (**6d**), *m*-F (**6b**), and *o*,*p*-diF (**6f**) resulted in better activity than the activity observed with other substituents (*i.e.*, H, *p*-F, *p*-OMe, *o*,*m*-diF, and *m*,*m*-diF). In general, we observed that in each series (**1**–**6**), the majority of the most active monohydrazones had either *p*-Cl (**d**) and/or *o*,*p*-diF (**f**) as R_2 substituents.

When looking at the effect of the R_1 substituent while keeping R_2 constant (*i.e.*, comparing **1a**–**6a**, **1b**–**6b**, etc.), we found that the monohydrazones displaying the best antifungal activity generally did not have the same R_1 substituents. In the case of compounds with R_2 = H (**1a**, **2a**, **3a**, **4a**, **5a**, and **6a**), the most active compounds **3a**, **1a**, and **6a** had *p*-F, H, and *o*,*p*-diF as R_1 substituents. For monohydrazones with R_2 = *m*-F (**b**), the introduction of *m*-F and *o*,*p*-diF as R_1 substituents resulted in compounds **2b** and **6b** with better overall antifungal activity than those with other substitution patterns. The most active compounds in the case of monohydrazones with R_2 = *p*-F, compounds **5c**, **6c**, and **1c** possessed *p*-OMe, *o*,*p*-diF, and H as R_1 substituents. When R_2 group = *p*-Cl, the most active compounds **6d**, **5d**, and **2d** possessed *o*,*p*-diF, *p*-OMe, and *m*-F as R_1 substituents. In addition, for compounds with R_2 = *p*-OMe or *o*,*p*-diF the most active monohydrazones had *m*-F or H (**2e** and **1e**), and *p*-OMe, *p*-F, and *o*,*p*-diF (**5f**, **3f**, and **6f**) as R_1 substituents. Overall, we observed that most of the monohydrazones with the best antifungal activity with diverse R_2 groups were from series **6** with R_1 = *o*,*p*-diF with the sole exception of the monohydrazone with R_2 = *p*-OMe (**6e**).

Since the monohydrazones with *o*,*p*-diF groups as either R_1 or R_2 substituent displayed broad-spectrum activity against the fungal strains tested, we explored the effect of R_2 = *o*,*m*-diF and *m*,*m*-diF substituents on antifungal activity. The monohydrazones **6g** (R_1 = *o*,*p*-diF, R_2 = *o*,*m*-diF) and **6h** (R_1 = *o*,*p*-diF, R_2 = *m*,*m*-diF) were compared to **6f** (R_1 = *o*,*p*-diF, R_2 = *o*,*p*-diF). In both cases, the introduction of *o*,*m*-diF and *m*,*m*-diF as R_2 substituents led to a decrease in activity profile against the whole panel of fungal strains compared to the *o*,*p*-diF analogue **6f**. Next, we explored the effect of regioisomers by comparing series **2** (R_1 = *m*-F) with series **3** (R_1 = *p*-F). We observed that the compounds **3a** (R_1 = *p*-F, R_2 = H) and **3f** (R_1 = *p*-F, R_2 = *o*,*p*-diF) performed better than their counterparts **2a** (R_1 = *m*-F, R_2 = H) and **2f** (R_1 = *m*-F, R_2 = *o*,*p*-diF), whereas compounds **2c** (R_1 = *m*-F, R_2 = *p*-F) and **2d** (R_1 = *m*-F, R_2 = *p*-Cl) displayed better activity compared to **3c** (R_1 = *p*-F, R_2 = *p*-F) and **3d** (R_1 = *p*-F, R_2 = *p*-Cl). From the data reported above, we were unable to point to the superiority of one regioisomeric series over another. We then evaluated the impact of the specific halogen on antifungal activity by comparing series **3** and series **4** (*p*-F *vs* *p*-Cl). Compounds **3a** (R_1 = *p*-F, R_2 = H), **3d** (R_1 = *p*-F, R_2 = *p*-Cl), and **3f** (R_1 = *p*-F, R_2 = *o*,*p*-diF) exhibited better broad-spectrum activity against various strains than their corresponding counterparts **4a** (R_1 = *p*-Cl, R_2 = H), **4d** (R_1 = *p*-Cl, R_2 = *p*-Cl), and **4f** (R_1 = *p*-Cl, R_2 = *o*,*p*-diF). In this case, monohydrazones where R_1 = *p*-F performed significantly better their counterparts where R_1 = *p*-Cl. Finally, we explored the effect of a methyl group (where X=Me) on the monohydrazone activity by comparing **7a** with **1a** and **1f** with **7f**. The addition of a methyl group in compound **1a** (where X=H) resulted in compound **7a** (where X=Me) with decreased antifungal activity against all the strains tested. In contrast, when we compared **1f** with **7f**, the addition of a methyl group resulted in a considerable increase in antifungal activity. Overall, compounds **3a**, **5f**, **6d**, **5c**, **2b**, **3f**, **7f**, **6b**, **5d**, **1d**, **2d**, **2e**, **4b**, and **6c** displayed the broadest spectrum of activity based on their MIC values.

Hemolysis assay. Potency and SAR development are only one facet of antimicrobial drug development. Since the monohydrazones displayed potency and broad-spectrum antifungal activity, it was additionally important to establish that these agents showed selectivity for fungal cells over mammalian cells. Thus, we investigated the hemolytic activity for the most promising monohydrazones **2b**, **3f**, **4b**, **5f**, **6d**, and **7f**, as well as controls AmB and VRC against murine red blood cells (mRBCs) (Figure 3 and Table S3). In order to simplify the discussion, a specific threshold value of 10% hemolysis was considered. Monohydrazones **2b** and **5f** displayed 10%

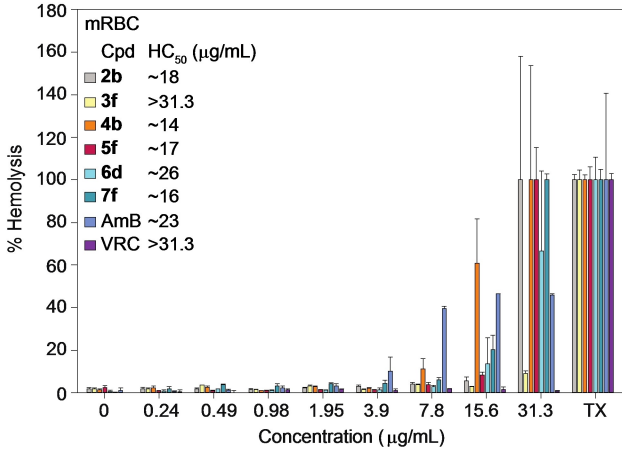


Figure 3. 2D bar graph normalized at 100% depicting the dose-dependent hemolytic activity and calculated HC_{50} values of monohydrazones **2b**, **3f**, **4b**, **5f**, **6d**, **7f**, as well as AmB and VRC against mRBCs. mRBCs were treated and incubated for 1 h at 37 °C with monohydrazones, AmB, and VRC at concentrations ranging from 0.24 to 31.3 μg/mL. Triton X-100® (TX) (1% *v/v*) was used as a positive control (100% hemolysis). *Note*: The corresponding non-normalized data are presented in Figure S104.

hemolysis at values >15.6 μg/mL (1- to 1040-fold of their overall MIC values), whereas monohydrazone **6d** displayed 10% hemolysis at a value of 15.6 μg/mL (1- to 260-fold of its overall MIC values). Monohydrazone **3f** displayed <10% hemolysis at concentrations of 31.3 μg/mL (2- to 260-fold of its overall MIC values). Finally, monohydrazones **4b** and **7f** displayed 10% hemolysis at 7.8 and >7.8 μg/mL (0- to 520-fold of their overall MIC values). Overall, the monohydrazones **2b**, **3f**, **5f**, **6d**, and **7f** displayed little to no hemolysis of mRBCs at either concentrations of 15.6 or 31.3 μg/mL that lie well above their MIC values. The 50% hemolysis (HC_{50}) values were calculated for these compounds and are presented in Figure 3. For all the compounds tested the calculated HC_{50} values were >14 μg/mL, which emphasizes the observed low hemolysis values for these compounds.

Cytotoxicity. It was also important to consider the toxicity of these monohydrazones towards mammalian cell lines. The toxicity profile of compounds **2b**, **3f**, **4b**, **5f**, **6d**, and **7f**, as well as controls AmB and VRC (within a concentration range of 0.12–31.3 μg/mL) was investigated against three mammalian cell lines A549, J774A.1, and HEK-293 (Figure 4). When tested against all three mammalian cell lines at 31.3 μg/mL, none of the monohydrazones tested displayed toxicity (with the exception of **4b** against J774A.1 that displayed 86% cell survival at that concentration). The excellent MIC values of these monohydrazones combined with the fact that none of them exhibited toxicity to mammalian cell lines at the highest concentration provided still further evidence that these agents warranted additional biological evaluation.

Time-kill studies. A time-kill kinetics assay was used to determine whether the monohydrazones are fungistatic and simply inhibit growth, or are fungicidal and kill fungal cells. Compounds **2b**, **3f**, **4b**, **5f**, **6d**, and **7f** were tested at $^1/_2\times$, 1×, and 4× MIC against *C. albicans* ATCC 10231 (strain *A*) to observe

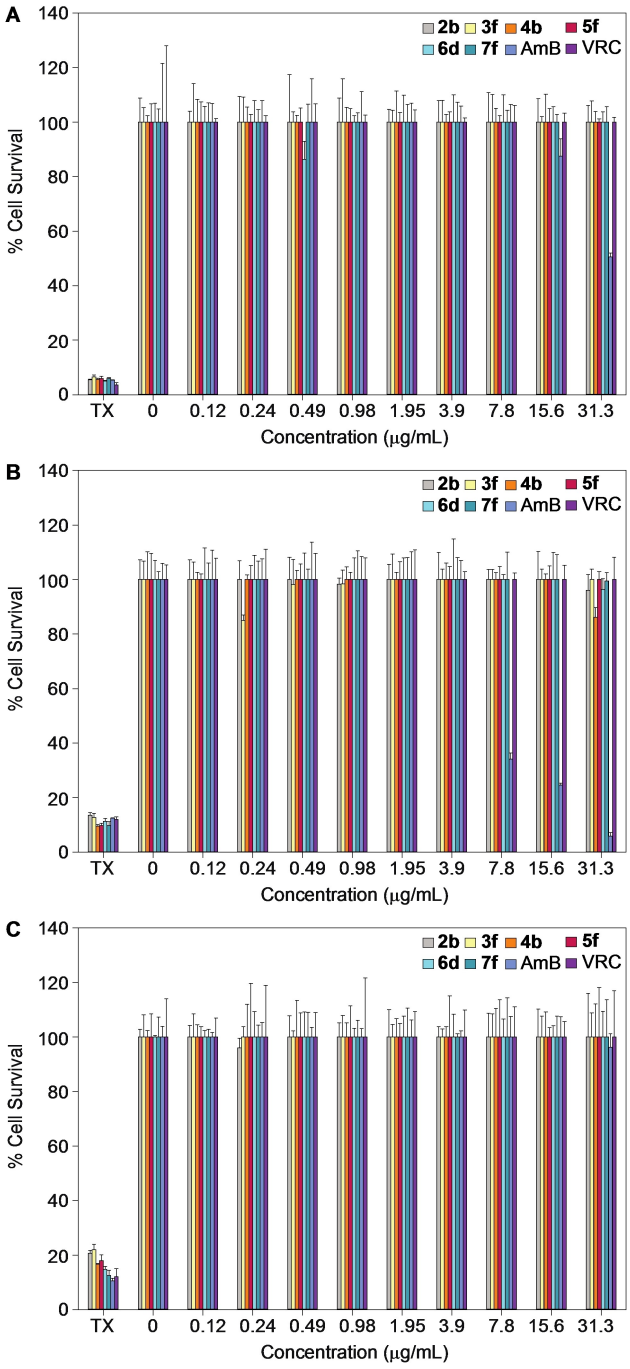


Figure 4. 2D bar graph normalized at 100% depicting the dose-dependent cytotoxic activity of monohydrazones **2b**, **3f**, **4b**, **5f**, **6d**, **7f**, as well as AmB and VRC against **A**. A549, **B**. J774A.1, and **C**. HEK-293 cell lines. *Note*: For Triton X-100® (TX) the eight bars are colored differently which corresponds to colors of the respective compounds for which TX was used as a positive control. *Note*: The corresponding non-normalized data are presented in Figure S105.

the dose-dependent effect and also to account for any normal variations in MIC values (Figures 5 and S106). In addition, AmB at 1× MIC and VRC at 1× MIC-0 against *C. albicans* ATCC (strain *A*) were also evaluated for comparison purposes. Fungicidal activity is defined as at least a three $\log_{10}$-fold decrease in

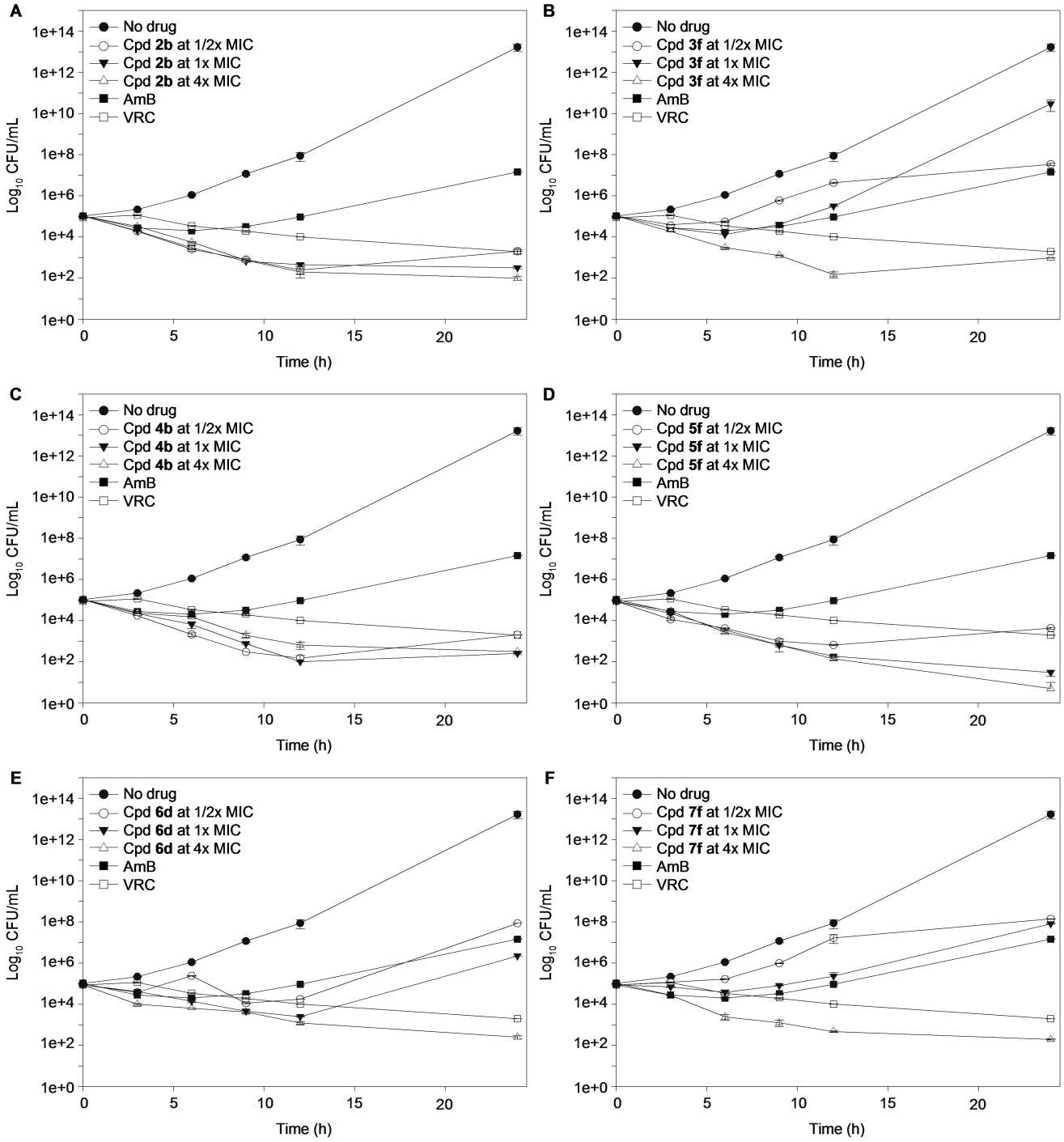


Figure 5. Time-kill kinetics for compounds **A. 2b**, **B. 3f**, **C. 4b**, **D. 5f**, **E. 6d**, and **F. 7f** at $^{1}/_{2}\times$, 1×, and 4× MIC as well as AmB 1× MIC and VRC at 1× MIC-0 against *C. albicans* ATCC (strain *A*). To confirm CFU counts, the metabolic dye, resazurin, was added at 24 h. The corresponding images with resazurin for 24 h, 48 h, and 72 h are presented in Figure S106. A screen against *C. auris* AR # 0384 and *C. auris* AR # 0390 is also presented in Figure S106.

colony forming units (CFU), and this level of decrease is observed with the monohydrazones at the 1× MIC concentration. A three $\log_{10}$-fold decrease was observed for compound **2b** at the $^{1}/_{2}\times$ MIC concentration (half of the MIC concentration of this compound against this particular fungal strain; *e.g.*, If MIC is 0.49 μg/mL, then $^{1}/_{2}\times$ MIC is 0.24 μg/mL), compounds **2b**, **4f**, and **5f** at the 1× MIC concentration, and all tested at the 4× MIC concentration. CFU counts were confirmed by adding the metabolic dye resazurin at the 24 h time point and observing the amount of color change over the following two days where no growth is indicated by a blue color (Figure S106, panel A). By 72 h, only four sample tubes with *C. albicans* remain blue and these samples include **2b** at 4×, **5f** at 1× and 4×, and **7f** at 4×. A screen with the resazurin was performed

with *C. auris* AR Bank # 0384 and # 0390 (Figure S106, panels B and C). Except for compound **6d**, which had growth at 72 h at the 1× concentration, all monohydrazones at 1× and 4× concentrations inhibited *C. auris* growth up to 72 h. The fungicidal activity at and above MICs shows the potential of the monohydrazones to clear, not just halt, a fungal infection.

Efficacy of monohydrazones against biofilms. Fungal biofilms[6a] are a protective mechanism that allow fungal cells to survive harsh conditions including those that may exist within the human body. These biofilms commonly occur, for example, on medical devices such as catheters. Once biofilms are formed, it is significantly more difficult to eliminate the fungal cells than in their absence. The ability of compounds **2b**, **3f**, **4b**, **5f**, **6d**, and **7f**, as well as controls AmB and VRC were assessed against *C. albicans* ATCC 10231 (strain *A*), *C. auris* AR Bank # 0384, and *C. auris* AR Bank # 0390 in two biofilm studies: (i) prevention of biofilm formation and (ii) destruction of pre-formed biofilms.

Prevention of biofilm formation. A large fungal load was exposed to the monohydrazones at time 0 h to evaluate the ability of the compounds to prevent *Candida* biofilm formation (Table 3, Figure S107). We determined the sessile MIC (SMIC) for 50% and 90% inhibition of biofilm formation as compared to the no drug control. Monohydrazones **2b** and **4b** displayed excellent activity similar to VRC with $SMIC_{50}$ values of 1.95 μg/mL against *C. albicans* ATCC 10231 (strain *A*), which is 4-fold greater than the MIC. Monohydrazones **5f** and **6d** also had good activity against *C. albicans* with $SMIC_{50}$ of 7.8 and 3.9 μg/mL, respectively, and **7f** also had some activity as well. Two monohydrazones, **4b** and **7f**, displayed good activity against one *C. auris* strain, *C. auris* AR Bank # 0384, with $SMIC_{50}$ values of 7.8 μg/mL. Monohydrazones **2b**, **5f**, and **6d** also displayed poor activity against *C. auris* AR Bank # 0384 while compounds **4b**, **5f**, **6d**, and **7f** displayed poor activity against *C. auris* AR Bank # 0390 with $SMIC_{50}$ values of 15.6 μg/mL. Monohydrazones **4b** and **6d** were the most promising as they display $SMIC_{90}$ values of 7.8 μg/mL against *C. albicans* (16-fold greater than MIC) and 15.6–31.3 μg/mL against the *C. auris* strains which is 4- to 16-fold greater than their MIC values against the same strains.

Destruction of pre-formed biofilms. Although the monohydrazones were able to prevent biofilm formation, we also evaluated their ability to destroy a pre-formed biofilm (treatment after 24 h) (Table 4). Overcoming the problem a biofilm presents is challenging, and reflecting this challenge, we report $SMIC_{50}$ values because no monohydrazones were able to decrease biofilm activity by 90%. Against *C. albicans* ATCC 10231 (strain *A*), compounds **4b** and **7f** display $SMIC_{50}$ values of 31.3 μg/mL, which matched the value for VRC. Against both *C. auris* strains, compounds **2b**, **4b**, and **6d** displayed $SMIC_{50}$ values of 15.6–31.3 μg/mL, which were better than VRC. Overall, compounds **4b** and **6d** performed the best against biofilms. In both the *prevention of biofilm formation* and *destruction of a pre-formed biofilm* assays, the monohydrazones appear to have similar activity to VRC, but very little activity compared to AmB.

Resistance development. To evaluate the potential of fungi to develop resistance to the monohydrazones, we repeatedly exposed *C. auris* AR Bank # 0390 to the monohydrazones at $^1/_2$× MIC to simulate fungal drug exposure in a clinical setting (Figure 6). Compounds **2b**, **3f**, **4b**, **5f**, and **6d**, as well as controls AmB were investigated. Compound **7f** was not included due to degradation of the compound when kept in solution. While normal variations in MIC values occurred, no significant changes in MIC values were observed as the MIC values remained within 8-fold of the original MIC value. Considering the generally long duration of treatment with antifungal drugs, this is a promising result that suggests that a

Table 3. $SMIC_{50}$ and $SMIC_{90}$ values (μg/mL) for prevention of biofilm formation determined for compounds **2b**, **3f**, **4b**, **5f**, **6d**, **7f** as well as the antifungal controls AmB and VRC against three fungal strains.

		$SMIC_{50}$ (μg/mL)							
Strain		**2b**	**3f**	**4b**	**5f**	**6d**	**7f**	AmB	VRC
C. albicans ATCC 10231 (*A*)	$SMIC_{50}$	1.95	31.3	1.95	7.8	3.9	15.6	0.12	1.95
	$SMIC_{90}$	31.3	>31.3	7.8	31.3	7.8	15.6	0.98	3.9
C. auris AR Bank # 0384	$SMIC_{50}$	15.6	31.3	7.8	15.6	15.6	7.8	1.95	3.9
	$SMIC_{90}$	31.3	>31.3	15.6	31.3	15.6	15.6	7.8	>31.3
C. auris AR Bank # 0390	$SMIC_{50}$	31.3	>31.3	15.6	15.6	15.6	15.6	1.95	1.95
	$SMIC_{90}$	31.3	>31.3	15.6	31.3	31.3	31.3	3.9	15.6
	MIC ≤1.95 μg/mL (excellent antifungal activity)								
	MIC = 3.9-7.8 μg/mL (good antifungal activity)								
	MIC ≥15.6 mg/μL (poor antifungal activity)								

Table 4. $SMIC_{50}$ values (μg/mL) for destruction of a pre-formed biofilm determined for compounds **2b**, **3f**, **4b**, **5f**, **6d**, **7f** as well as the antifungal controls AmB and VRC against three fungal strains.

	$SMIC_{50}$ (μg/mL)							
Strain	**2b**	**3f**	**4b**	**5f**	**6d**	**7f**	AmB	VRC
C. albicans ATCC 10231 (*A*)	>31.3	>31.3	31.3	>31.3	>31.3	31.3	0.24	31.3
C. auris AR Bank # 0384	15.6	>31.3	31.3	>31.3	15.6	>31.3	0.98	>31.3
C. auris AR Bank # 0390	31.3	>31.3	15.6	>31.3	15.6	>31.3	1.95	>31.3
	MIC ≤1.95 μg/mL (excellent antifungal activity)							
	MIC ≥15.6 mg/μL (poor antifungal activity)							

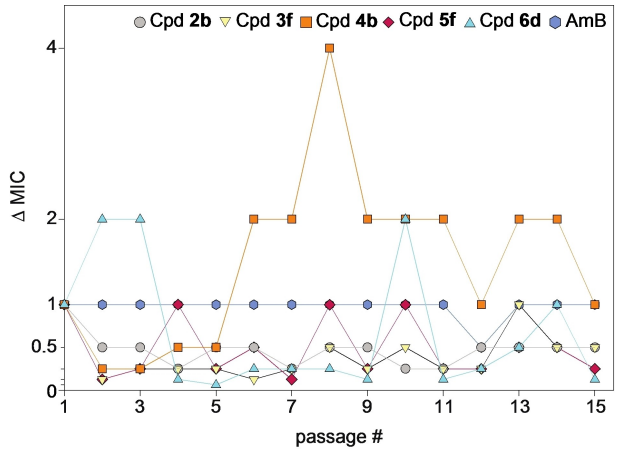


Figure 6. Graph illustrating fold changes in MIC values (Δ MIC) over 15 serial passages for monohydrazones **2b**, **3f**, **4b**, **5f**, **6d**, as well as AmB against *C. auris* AR Bank # 0390. *Note*: starting MIC values were 0.24, 0.49, 0.49, 0.49, 1.95, and 0.98 μg/mL, respectively.

fungal strain is not likely to develop resistance to the monohydrazones, even after repeated exposures.

Conclusions

In summary, we developed a synthesis of substituted monohydrazones **1a**–**7f** and performed a detailed study of antifungal activity of the compounds **1a**–**7f** against a panel of seven strains of *C. albicans* and three strains of non-*albicans Candida*. Commercially available antifungal agents, AmB, CFG, FLC, and VRC were used as positive controls. These SAR studies identified compounds **3a**, **5f**, **6d**, **5c**, **2b**, **3f**, **7f**, **6b**, **5d**, **1d**, **2d**, **2e**, **4b**, and **6c** as having the broadest spectrum of activities based on their MIC values. The seven best compounds (**2b**, **3f**, **4b**, **5f**, **6b**, **6d**, and **7f**) and two of the worse (**5a** and **7a** to serve as negative controls) were further tested against a panel of ten *C. auris* and ten other fungal strains. The monohydrazones **2b**, **3f**, **4b**, **5f**, **6d**, and **7f** displayed excellent to good activity (0.015–7.8 μg/mL) against all 20 strains tested. In comparison with the FDA-approved drug VRC, monohydrazones **2b**, **3f**, **5f**, **6d**, and **7f** displayed little to no hemolysis of mRBCs at concentrations of either 15.6 or 31.3 μg/mL. In addition, none of the monohydrazones **2b**, **3f**, **5f**, **6d**, and **7f** exhibited toxicity against three mammalian cell lines, A549, J774A.1, and HEK-293. A time-kill assay over a 24 h period using compounds **2b**, **3f**, **4b**, **5f**, **6d**, and **7f** against *C. albicans* ATCC 10231 (strain *A*) indicated the monohydrazones were fungicidal at and/or above their MIC values. Compounds **2b**, **3f**, **4b**, **5f**, **6d**, and **7f**, as well as controls AmB and VRC were assessed against *C. albicans* ATCC 10231 (strain *A*), *C. auris* AR Bank # 0384, and *C. auris* AR Bank # 0390 in two biofilm studies: (i) prevention of biofilm formation and (ii) destruction of pre-formed biofilms. The monohydrazones were able to prevent the formation of biofilm against these representative strains. When exposed to compounds **2b**, **3f**, **4b**, **5f**, and **6d** over 15 passages, *C. auris* AR Bank # 0390 developed no resistance. A possible mechanism by which these fluorinated aryl- and heteroaryl-substituted hydrazones might exert their pharmacological effects is by interfering with DNA-protein interactions. Additional studies to determine the mechanism of the action of these compounds will be reported in due course. In conclusion, the fluorinated aryl-and heteroaryl-substituted monohydrazones reported herein display promise as a new family of antifungal agents.

Experimental Section

All experimental procedures along with compound characterizations are reported in the Supporting Information.

Abbreviations

AmB	amphotericin B
ATCC	American Type Culture Collection
CFG	caspofungin
FLC	fluconazole
LC–MS	liquid chromatography-mass spectrometry
MIC	minimum inhibitory concentration
mRBCs	murine red blood cells
SAR	structure-activity relationship
SMIC	sessile minimum inhibitory concentration
VRC	voriconazole

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Conflict of Interest

D.S.W. has partial ownership of a new-start 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.

Keywords: Biofilm · *Candida auris* · Cytotoxicity · Drug resistance · Hemolysis · Monohydrazones

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FULL PAPERS

Antifungal drug development: The synthesis and biological activity of fluorinated aryl- and heteroaryl-substituted monohydrazones are presented. In most cases, the antifungal activity of these new compounds exceeded those of reference drugs against various fungal strains tested. All the lead compounds exhibited better safety profiles and there was low incident of resistance development. Thus, these fluorinated aryl- and heteroaryl-substituted monohydrazones could lead to the development of novel antifungal agents.