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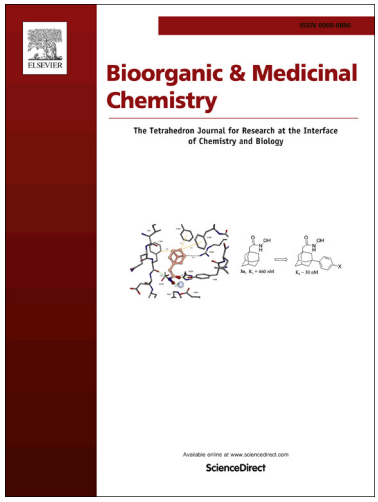

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Potent Antimicrobial Agents Against Azole-Resistant Fungi Based on Pyridinohydrazide and Hydrazomethylpyridine Structural Motifs

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Abstract

Schiff base derivatives have recently been shown to possess antimicrobial activity, and these derivatives include a limited number of salicylaldehyde hydrazones. To further explore this structure-activity relationship between salicylaldehyde hydrazones and antifungal activity, we previously synthesized and analyzed a large series of salicylaldehyde and formylpyridinetrione hydrazones for their ability to inhibit fungal growth of both azole-susceptible and azole-resistant species of *Candida.* While many of these analogs showed excellent growth inhibition with low mammalian cell toxicity, their activity did not extend to azole-resistant species of *Candida.* To further dissect the structural features necessary to inhibit azole-resistant fungal species, we synthesized a new class of modified salicylaldehyde derivatives and subsequently identified a series of modified pyridine-based hydrazones that had potent fungicidal antifungal activity against multiple *Candida* spp. Here we would like to present our synthetic procedures as well as the results from fungal growth inhibition assays, mammalian cell toxicity assays, time-kill assays and synergy studies of these novel pyridine-based hydrazones on both azole-susceptible and azole-resistant fungal species.

Introduction

The incidence rate for fungal infections has increased dramatically in the last few decades. For instance, recent analyses of hospital data in France revealed that the annual rate of invasive fungal infections increased by 16% and the annual rate of deaths from invasive fungal infections increased 33% in the last decade (1), and it is reasonable to assume that world trends are similar. The most common culprits for the onset of invasive infections arise from the *Candida* spp. Candidiasis is most commonly associated with *Candida albicans, Candida glabrata* and *Candida krusei* microorganisms (2;3). The immune-compromised -- specifically transplant recipients, cancer patients and those infected with AIDS – are particularly susceptible to candidiasis infections (4;5). While classes of antifungal therapies, such as the polyenes, azoles and the echinocandins, are available for the treatment of invasive *Candida* spp. infections, many have adverse effects or other drawbacks associated with their use, including nephrotoxicity, hepatotoxicity and the development of resistance in the target microorganism (6;7). In addition, oral candidiasis is one of the most frequent opportunistic infections associated with HIV and AIDS, and these infections have largely been controlled with the use of topical polyenes or systemic azoles (8). However, the overuse of azoles in the treatment of oral candidiasis has ultimately led to the development of drug resistance. For example, it has been reported that up to 33% of AIDS patients have azole-resistant oral candidiasis infections (8). This resistance to the azoles stems not only from the drugs' fungistatic effects, but also from overexpression of efflux pumps (9) and point mutations in the *ERG11* gene (10) in *Candida albicans.* In addition, *Candida glabrata* develops resistance during prolonged treatment with azole antifungals (11). For these reasons, there is a continuous demand for the discovery of novel therapeutics to treat *Candida* spp. infections, particularly if they have a different method of action than the azoles.

Some literature data suggest that simple Schiff bases possess antimicrobial activity and might have different biological targets than currently available antifungal agents (12; 13). Others have previously studied the antimicrobial activity of a limited number of salicylaldehyde hydrazones, however the mechanism of action for these derivatives has not been clearly defined (14). It was suggested that the activity of these molecules may be based on the formation of Schiff bases with important amino groups of the microbial cells. To further explore this structure-activity relationship and to identify possible pharmacophores, we previously synthesized and analyzed a large series of salicylaldehyde (15) and formylpyridinetrione hydrazones (16) for their ability to inhibit fungal growth of both azole-susceptible and azole-resistant species of *Candida*. Additionally, we explored potential mechanisms of action for these compounds. Many of these analogs showed excellent growth inhibition with low mammalian cell toxicity. However, this activity did not extend to azole-resistant species of *Candida.* Furthermore, our preliminary mechanistic studies indicated that these compounds were likely fungistatic, as with azoles. Therefore, a new structure-activity relationship study was designed using modified salicylaldehyde derivatives not previously studied, with the goal of determining the pharmacophore required to produce antifungal activity against azole-resistant fungi. We subsequently identified a series of modified pyridine-based hydrazones that had potent fungicidal antifungal activity against multiple *Candida* spp. Here we would like to present our synthetic procedures as well as the results from fungal growth inhibition assays, mammalian cell toxicity assays, time-kill assays and synergy studies of these novel pyridine-based hydrazones on both azole-susceptible and azole-resistant fungal species (8;17). In summary, we have identified the structural components that illicit potent fungal growth inhibition of both azole-susceptible and azole-resistant fungal species and have synthesized a broad class of structural analogs.

Furthermore, these analogs show limited toxicity against mammalian cells *in vitro* and several exhibit fungicidal activity. Considering these data, the analogs presented here may be a promising alternative route for the development of novel antifungal agents.

Results

1.A Chemistry

All hydrazone derivatives were prepared from the corresponding aromatic aldehydes and hydrazine derivatives (phenylhydrazines, hydrazides, or sulfonohydrazides). All aromatic aldehydes, phenylhydrazine, 4-nitrophenylhydrazine, and 2,4-dinitrophenylhydrazine were purchased from either Sigma-Aldrich or Ark Pharm. All hydrazines were prepared from the corresponding carboxylic acid through Amberlyst-15 catalyzed ethanol esterification, as previously reported (18). Following esterification, the catalyst was removed by filtration. The filtrate was then mixed with hydrazine hydrate and refluxed for several hours to yield the desirable acid hydrazide (**Scheme 1**). Because the catalyst is recyclable and isolation of the intermediate ester is not necessary, this synthetic procedure is a one-pot reaction. This synthetic method also tolerates the presence of the phenol group and pyridine moiety. The sulfonohydrazides were synthesized using the large scale preparation method that we previously published (**Scheme 2**) (15). Hydroxybenzylidenepyridinohydrazides were prepared from 5-substituted salicylaldehyde and pyridinohydrazides in refluxing ethanol without an acid catalyst (**Scheme 3**) (19;20). This reaction was driven by increasing the reactant concentration, allowing the product to selectively precipitate from the reaction mixture. To efficiently synthesize these derivatives in greater quantities, we developed a large scale synthetic method (3 L round bottom flask using 1.5 L of ethanol to make 100g of product) using a modified

Hickman still head with an attached condenser and distillation receiving flask (**Scheme 3**). Following this scheme, individual reactions were prepared in 50 ml round bottom flasks to produce about 300 mg of each member of the diverse pyridine-based hydrazone library. This was accomplished by slow distillation of the mixture to remove the ethanol. The rate of distillation was controlled by both the size of pre-column and the temperature of the reaction mixture. Distillation continued until precipitate started to form in the reaction flask. The distilled ethanol was recycled and reused in the preparation of the more than fifty members of our hydrazone library. The same procedure was used for the preparation of individual members of the hydrazonomethylpyridine library (**Scheme 4**).

1.B Antifungal activity

1.b.1 Minimum Inhibitory Concentration (MIC) studies

The antifungal activity of the pyridinohydrazide and hydrazomethylpyridine derivatives described in the above schemes were evaluated *in vitro* using *Candida albicans* (ATCC no. 10231) and *Candida glabrata* (ATCC no. 48435). In addition, the compounds were evaluated for activity against a previously-described clinical isolate of *C. albicans* known to have developed azole-resistance (a kind gift from Dr. Glen Palmer, LSUHSC). These isolates were obtained over a two year period from a single AIDS patient and the development of the azole-resistance mechanisms have been extensively characterized (8). MIC assays were performed on the clinical isolates 1 and 17 (annotated TW1 and TW17, respectively), where isolate 1 was used as an azole-susceptible control and isolate 17 was considered azole-resistant. All assays were done in accordance with NCCLS reference documents (21). The results of these screenings are summarized in **Tables 1-9** as the minimum concentrations that inhibited more than 80% of

fungal growth (MIC_{80}) as compared to positive controls containing 1% DMSO. Assays were performed in HEPES-buffered RPMI-1640 media. All MIC screens used a visual scoring method as opposed to spectroscopic analysis, due to the altered absorbance spectra of many of the compounds.

Table 1 lists the MICs of the picolinohydrazides (**AR1-AR6)**. These derivatives are all hydrazones of salicylaldehyde and five-substituted salicylaldehyde. All of the synthesized derivatives in this group consistently inhibited fungal growth of the azole-susceptible fungal species at very low concentrations (~3-15 uM - **Table 1**). In addition, 4 of the 6 derivatives (namely **AR2, AR4, AR5 and AR6- Table 1**) inhibited fungal growth in the azole-resistant fungi TW17 and *C. glabrata* at a concentration range of 3-30 uM (**Table 1**). Furthermore, the position of the nitrogen in the pyridine ring proved to have little effect on the ability of the resulting hydrazone analog to act as a fungal growth inhibitor. For example, 5-substituted salicylaldehyde hydrazones of nicotinohydrazide **(Table 2)** also consistently inhibited fungal growth, even though several derivatives were less active against azole-resistant isolates (**AR8 and AR12).** Additionally, substituted salicylaldehyde hydrazones of isonicotinohydrazide all possessed antifungal activity with the most potent again being the 5-nitrosalicylaldehyde hydrazone **(Table 3; AR18**).

To determine the effects of an OH-group or oxidizing pyridine moiety on fungal growth inhibition, these two functionalities were individually incorporated on the nicotinohydrazide derivatives. Unlike the previous nicotinohydrazide tested where all derivatives were active (see **Table 2)** the incorporation of the OH-functionality in the two position of the pyridine ring led to inconsistent results. Introducing the hydroxyl group diminished or even eliminated the antifungal activity previously observed (**Table 4**). However, we did observe growth inhibition at

very low concentrations across all *Candida albicans* isolates treated with **AR19.** In addition, **AR22** was one of our most potent compounds against *Candida glabrata.* In the case of the pyridine oxides presented in Table 5, activity of the analogs was consistent, with all derivatives exhibiting inhibition of fungal growth, regardless of the azole-sensitivity or resistance. However, in general the MICs of these analogs were higher than other classes of compounds (**Table 5**). The most potent analog in this class against azole-resistant TW17 was **AR28** (activity at 2 μM**)**.

Our data suggested that the pyridine structural motif might be essential for antifungal activity, particularly for azole-resistant isolates. Various hydrazones of 2-pyridinecarbaldehyde were prepared for further analysis of this motif (**Table 6 and Table 7**). Each of these analogs lacks the corresponding salicylaldehyde moiety present on the active derivatives presented in Tables **1-5**. The phenylhydrazone **AR29**, and the picolinohydrazide **AR34 (Table 6)** showed moderate activity**,** likely due to structural similarities to compounds shown in Table 1, but in general, the loss of the salicyl moiety resulted in a significant loss of antifungal activity. Furthermore, sulfohydrazide derivatives showed no antifungal activity (**Table 7**) which is consistent with our previously published data showing that compounds bearing a sulfonyl group are essentially inactive (15).

Because phenylhydrazone **AR29** showed moderate activity, two new groups of phenylhydrazones were prepared and tested to determine if we could increase the antifungal activity. One set of analogs was synthesized from 3-pyridinecarbaldehyde (**Table 8**) and the other from 4-pyridinecarbaldehyde (**Table 9**). As in the case of 2-pyridinecarbaldehyde, moderate antifungal activity was observed with phenylhydrazones **AR44** and **AR48,** but not with any other derivatives. Based on these data, our most consistent results with respect to growth

inhibition of both azole-susceptible and azole-resistant fungal isolates were obtained with hydrazones bearing both salicylaldehyde and pyridine structural motifs.

1.b.2 Mammalian Cell Toxicity studies

Following the fungal growth inhibition assays, compounds displaying antifungal activity at concentrations below or equal to 8 μg/mL were further subjected to *in vitro* mammalian cell toxicity studies using mammalian kidney cells and human liver cells (Vero (kidney) cells -ATCC no. CRL-1651 and Hep G2 (liver) ATCC no. HB-8065). Active analogs meeting this criterion were screened at multiple concentrations, including the lowest MIC among the *Candida* isolates as well as at multiples of 5, 10 and 100 times the MIC. Cytotoxicity studies were performed in accordance with Promega CellTiter 96 Non-RadioactivCell Proliferation Assay (cat # G4000). All cytotoxicity studies used a negative control (wells of either kidney or liver cells containing 1% DMSO but lacking the analog), a positive control (wells of either kidney or liver cells treated with the known cytotoxic agent, saponin) and the serial dilutions of the novel analogs. All control (negative) wells were normalized to 100% viable cells to accurately show the percentage of either liver or kidney cells that remained alive and viable following either overnight incubation with the analogs or 5-minute treatment with 0.1% saponin. We tested representative active compounds from each of the structural classes synthesized. We found that the picolinohydrazide **AR2** (**Table 1**) showed only minimal toxicity, even at the highest concentration of 100 times greater than the MIC (**Fig 1**, **Panel A**). Similar trends in toxicity were observed with other active compounds in Table 1, as shown in the *Supplemental Materials.* However, the nicotinohydrazides, represented by **AR12** and shown in Table 2, had elevated toxicity in the kidney cells, even at the lower concentrations of 5 times the MIC (Fig 1 Panel B

and *Supplemental Material*). Isonicotinohydrazide **AR18** (Table 3) was not toxic through 10X and showed minimal cytotoxicity at the 100X concentration. Similarly, the 2-hydroxy nicotinohydrazide **AR19** showed minimal toxicity at concentrations through 10X (Fig1 panels C and D). Unfortunately, one of the more promising pyridine oxide analogs (Table 5) was cytotoxic to liver and kidney cells, even at the 5X concentrations (**AR27** Fig 1 Panel E). Finally, the aryl hydrazone pyridines of **AR44** and **AR48** (Tables 8 and 9) showed no cell toxicity through 10X concentrations of the MIC, with the 100X concentrations being toxic to both kidney and liver cells (Fig 1). In summary, while some toxicity was observed with a few of the analogs tested, the majority of compounds analyzed had limited cytotoxicity in both mammalian cell lines. All results from the toxicity screenings outside of **Fig 1** are provided in the *Supplemental Information.*

1.b.3 Time-Kill Assay

One of the objectives of this study was to determine the cidal or static nature of the analogs that were active against TW17, the azole-resistant clinical isolate. Azoles affect *Candida* spp. by inhibiting ergosterol biosynthesis, which ultimately prevents their reproduction. This effect has led to the classification of azoles as fungistats. Determining the fungistatic or fungicidal nature of our synthesized compounds allows a comparison to the azoles, in addition to improving our understanding of how the structural motifs might lend toward an alternate mechanism of action. Therefore, time-kill assays were performed using the representative analogs that were active against the azole-resistant TW17 at concentrations at or below 8 ug/mL. Solid drugs were dissolved in DMSO to a concentration of 10 mg/ml. This stock solution was then further diluted in water to the desired experimental concentration, where the experimental concentration was

determined to be four times that of the MIC for TW17 (as reported in the Tables). Individual colonies of TW17 were suspended in 5 ml of sterile water and adjusted to achieve a McFarland reading between 0.08 and 0.12. The inoculum was then further diluted in sterile water at a ratio of 1:100. The experimental "Kill Curve" tubes were prepared by combining 2.5 ml of above diluted inoculum, 21.7 ml of HEPES buffered RPMI-1640 media, and 800 µl of experimental drug dilution in a 50-ml tissue culture tube. A "Growth Control" tube was prepared by combining 2.5 ml of inoculum, 21.7 HEPES buffered RPMI-1640 media, and 800 µl of water-DMSO solution equal to the DMSO concentration of the experimental drug dilution. A "Sterile Control" tube was prepared by combining 24.2 ml of HEPES-buffered RPMI-1640 media and 800 µ of water-DMSO solution as in the "Growth Control". All tubes were incubated for 48 hours at 35°C, 50 RPM. 500 µl samples were taken from the tubes at timepoints of predetermined intervals during this incubation.

Timepoints for the "Kill-Curve" and "Growth Control" tubes were taken at 0, 1, 2, 4, 6, 8, 24, and 48 hours after the start of incubation. Timepoints for "Sterile Control" were taken at 0 and 48 hours. Each timepoint sample was serially diluted 1:10 in sterile 0.9% NaCl solution up to 5 times. 100 µl of each sample was spread onto YM agar plates with a sterile bent spreading rod. Plates were incubated at 35°C overnight and colonies were then counted on each plate. Colonies on plates from diluted timepoints were used to estimate uncountable undiluted samples. The number of colonies in undiluted samples was estimated to be 10^n times greater than the diluted sample, where n was the number of dilutions made.

From our time-kill assays, we identified several compounds that were fungistatic. In general, analogs with the picolinohydrazide structure (Figure 2), the nicotinohydrazide structure (supplemental material) and the isonicotinohydrazide structure were fungistatic and no increase

in fungal growth could be observed after 48 hours in the presence of these analogs. An exception to this generalization regards the presence of a chloro- or bromo-substituent in the 4-position of the salicylaldehyde moiety; these analogs showed fungicidal activity (see Figure 2, Panel B **AR5**). With respect to the active pyridine oxide compound **AR27** (Table 5), the presence of the bromo-substituent did not render the analog fungicidal; this compound instead showed fungistatic activity. Finally, the aryl substituted pyridines lacking a salicylaldehyde moiety, **AR44** and **AR48** (Tables 8 and 9) were fungicidal, with no observed colonies after 24 hours, making these derivatives promising analogs for future exploration. Considering their high cell toxicity and their fungistatic nature, the pyridine oxide derivatives show the least promise for future antifungal use.

1.b.4 Synergy studies: Checkerboard Assay

Considering that two different modes of activity (cidal and static) were observed depending on the structural motifs present in our analogs, we performed a series of checkerboard assays to determine if representative active compounds could act in synergy with the azole, fluconazole. Each of these assays tested multiple concentrations of the selected analog in the presence of multiple concentrations of fluconazole. All checkerboard assays were prepared in 96-well plates. 200 µl of previously prepared drug solutions were added to each well in column 2. Using a multichannel pipette, 100 µl from each well in column 2 was serially diluted (1:2) horizontally across the plate, stopping at column 10. Contents of each well were mixed thoroughly. Column 11 was reserved for fluconazole MIC control and contained no other drug. 100 µl of media was added to each well in columns 2-10 except row B. Each well was mixed thoroughly with a multichannel pipette. 100 µl was then removed and discarded from these

wells. 100 µl of previously prepared fluconazole dilution was added to each well in row B. Using a multichannel pipette, 100 ul from each well was serially diluted (1:2) vertically down the plate, stopping at row F. Contents of each well were mixed thoroughly. Row G was reserved for tested drug MIC control and contained no fluconazole. Well G11 contained neither drug and served as a drug-free growth control.

The fungal samples were prepared from glycerol stocks of *Candida albicans* ATCC. No 10231. The inoculum's concentration was adjusted to a McFarland standard between 0.08 and 0.12. Inocolum was then diluted at a ratio of 1:37.5 in buffered media. 100 µl of inoculum-media solution was added to each well. Following inoculation, the 96-well plates were wrapped in parafilm to prevent evaporation and incubated for overnight at 35°C. After 18 hours, the wells showing growth were recorded.

Synergistic activity was determined based on the MIC read from these plates. The fractional inhibitory concentration (FIC) for a given well is the ratio of the minimum inhibitory concentration of one drug A in the presence another drug B to the minimum inhibitory concentration with only drug A. The FIC was calculated for the test drug in each of the wells along the growth/no-growth interface at varying concentrations of fluconazole, then these values were averaged to determine the average FIC for the test drug. The same process was used to calculate the average FIC for fluconazole at varying concentrations of the test drug. The sum of these two FICs gives the FIC index which can be used to determine the synergistic effect between the two drugs (22)**.** An FIC index value less than 1 indicates synergistic interactions between the two drugs while an FIC index value greater than or equal to 1 indicates antagonistic interactions. Table 10 shows the results from our checkerboard assays, indicating no synergy exists between fluconazole and our new analogs.

Chemistry Experimental

Thin-layer chromatographic analysis (TLC) was performed on each compound using silica gel on aluminum foil-backed glass plates and was detected under ultraviolet (UV) light. The ^{1}H and ^{13}C NMR spectra were run on Varian 400 MHz Unity instruments in DMSO-d_6. The solvent signals were used as internal NMR chemical shift references. All products were purified by crystallization from ethanol. When necessary during the preparation of the hydrazides (HD1-HD8, **Scheme 1**), some intermediate esters were purified by short silica gel (40-70 mm) filtration with various mixtures of ethyl acetate and dichloromethane as eluents. Silica gel was purchased from Sorbent Technologies. Substituted phenylhydrazines were prepared from corresponding anilines by following an existing preparation procedure (23). All solvents were purchased from Fisher Scientific. All reagents were purchased from Sigma-Aldrich and were analytical grade.

Preparation 5-nitrosalycilaldehyde. Acetic acid (20 ml) was added to a large (190x100 mm) crystallization dish equipped with a magnetic stirrer and placed under a fume hood with aqueous sodium carbonate and a nitrogen oxide trap. This acetic acid salicylaldehyde (12.2 g; 0.1 mol) was added to concentrated nitric acid (80 ml). The resulting dark red reaction mixture was stirred at room temperature for approximately 10 minutes, when the reaction started to spontaneously heat up forming a large quantity of nitric oxide (**WARNING:** Do not use a reaction scale four times or greater than this under these conditions, as the reaction mixture will explode due to the its exothermicity). The mixture was stirred at room temperature for an additional hour; no cooling of the reaction mixture was necessary. During this period, the color of the reaction mixture changed from dark red to orange. This reaction mixture was then poured on crushed ice (400 g) and left at room temperature for one hour. The resulting yellow

suspension was filtered and the solid precipitate was collected. The precipitate was washed with ice cold water (3x20 ml) and -5°C methanol (3x15 ml), then dried at room temperature to give pure product (14.6 g; 82%).

Typical procedure for the preparation of hydrazides. **Preparation of 2-Hydroxybenzohydrazide (HD2)**. An ethanol (300 ml) suspension of salicylic acid (13.8 g; 0.1 mol) and strongly acidic ion-exchange resin, Amberlyst-15 (5g) were stirred with refluxing for three days. Insoluble catalyst was separated by filtration, and washed with ethanol (3x20 ml). Combined ethanol filtrates were mixed with hydrazine hydrate (20 ml; 20.5 g; 0.4 mol) and refluxed with slow solvent distillation using the modified Hickman still apparatus (**Scheme 3**). After three hours of refluxing, the volume of the reaction mixture was reduced to about 50 ml and white precipitate started to form. The white suspension was cooled to room temperature and then left at -5°C for one hour. Insoluble product was separated by filtration, washed with ice cold ethanol, and dried on air to give pure product (13.2 g; 87%). ^{1}H-NMR (DMSO-d_6)) δ 12.3 (1H, broad s, OH), 10.0 (1H, broad s, NH), 7.0 (1H, d of d, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 6-H), 7.33 (1H, t, J =8. 4 Hz. 4-H), 6.89 (1H, d, J =8.4 Hz, 3-H), 6.86 (1H, t, J = 8.4 Hz, 5-H), and 4.70 (2H, brad s, NH_2) ppm. ^{13}C-NMR (DMSO-d_6) δ 168.7, 160.3, 134.1, 127.8, 119.3, 118.0, and 115.1 ppm.

Picolinohydrazide (HD4). Isolated yield 81% (11.1 g). ^{1}H-NMR (DMSO-d_6) δ 9.23 (1H, broad s, NH), 8.56 (1H, d, J = 4.8 Hz, 6-H), 7.98 (1H, d, J = Hz, 3-C), 7.93 (1H, t = 7.6 Hz, 5-H), 7.51 (1H, t, J = 4.8 Hz, 4-H), and 4.64 (2H, broad s, NH_2) ppm. ^{13}C-NMR (DMSO-d_6) δ 163.4, 150.5, 149.2, 138.3, 126.9, and 122.5 ppm.

Nicotinohydrazide (HD5). Isolated yield 92% (12.6 g). ^{1}H-NMR (DMSO-d_6) δ 9.99 (1H, s, NH), 8.95 (1H, s, 2-H), 8.64 (1H, m, 4-H), 8.13 (1H, m, 6-H), 7.44 (1H, m, H-5), and 4.61 (2H, NH_2) ppm. ^{13}C-NMR (DMSO-d_6) δ 165.2, 152.4, 148.8, 135.4, 129.5, and 124.2 ppm.

Isonicotinohydrazide (HD6). Isolated yield 94% (12.9 g). ^{1}H-NMR (DMSO-d_6) δ 10.13, 8.66 (2H, d, *J* = 6.0 Hz, 3-H), 7.71 (2H, d, *J* = 6.0 Hz, 2-H), and 4.69 (2H, NH_2) ppm. ^{13}C-NMR (DMSO-d_6) δ 164.8, 150.8, 140.9, and 121.7 ppm.

2-Hydroxynicotinohydrazide (HD7). Isolated yield 84% (12.9 g). ^{1}H-NMR (DMSO-d_6) δ 7.93 (1H, d, *J* = 6.0 Hz), 7.85-7.80 (2H, m), and 8.32 (1H, d, *J* = 9.6 Hz) ppm. ^{13}C-NMR (DMSO-d_6) δ 164.3, 163.1, 142.0, 139.4, 137.7, 119.8, and 111.9 ppm.

3-(Hydrazinecarbonyl)pyridine 1-oxide (HD8). Isolated yield (12.1 g; 79%). ^{1}H-NMR (DMSO-d_6) δ 10.06 (1H, broad s, NH), 8.50 (1H, s, 2-H), 8.32 (1H, d, *J* = 6.4 Hz, 4-H), 7.69 (1H, d, *J* = 8.0 Hz, 6-H), 7.49 (1H, t, *J* = 7.2 Hz, 5-H), and 4.60 (2H, broad s, NH_2) ppm. ^{13}C-NMR (DMSO-d_6) δ 162.6, 141.3, 137.9, 133.2, 127.2, and 124.4 ppm.

Typical Procedure for preparation of aryl sulfonohydrazides. **Preparation of 4-methoxybenzenesulfonohydrazide (SH3)**. A water (300 ml) solution of hydrazine hydrate (12.5 g; 0.25 mol) was cooled in an ice-water bath to 5°C. A tetrahydrofuran (50ml) solution containing 4-methoxybenzenesulfonyl chloride (5.16 g, 0.025 mol) was slowly added while stirring slowly. Following this addition, the reaction mixture was stirred at 5°C for an additional thirty minutes, and then the tetrahydrofuran was evaporated at reduced pressure and room temperature. The white solid product was separated by filtration, washed with cold water (3x15 ml) and dried on air overnight to give pure product (4.4 g; 88%). ^{1}H-NMR (DMSO-d_6) δ 8.20

(1H, s, NH), 7.72 (2H, d, *J* = 8.8 Hz, 2-H), 7.09 (2H, d, *J* = 8.8 Hz), 3.99 (2H, s, NH_2), and 3.81 (3H, s OCH3) ppm. ^{13}C-NMR (DMSO-d_6) δ 163.1, 130.5, 130.0, 114.9, and 56.3 ppm.

4-bromobenzenesulfonohydrazide (SH4). Isolated yield 5.6 g (89%). ^{1}H-NMR (DMSO-d_6) δ 8.47 (1H, s, NH), 7.78 (2H, d, *J* = 8.0 Hz, 2-H), 7.70 (2H, d, *J* = 8.0 Hz, 3-H), and 4.16 (2H, s, NH2) ppm. ^{13}C-NMR (DMSO-d_6) δ 138.1, 132.8, 130.4, and 127.3 ppm.

4-Nitrobenzenesulfonohydrazide (SH5). Isolated yield 4.86 g (89%). ^{1}H-NMR (DMSO-d_6) δ 8.67 (1H, broad s, NH), 8.39 (2H, d, *J* = 7.6 Hz, 3-H), 8.04 (2H, d, *J* = 7.6 Hz, 2-H), and 4.30 (2H, broad s, NH_2) ppm. ^{13}C-NMR (DMSO-d_6) δ 150.4, 144.8, 129.9, and 124.9 ppm.

2-Naphthalenesulfonohydrazide (SH6). Isolated yield 5.2 g (93%). ^{1}H-NMR (DMSO-d_6) δ 8.53 (1H, s, NH), 8.47 (1H, s), 8.12 (2H, m), 8.01 (1H, d, *J* = 7.6 Hz), 7.84 (1H, m), 7.67 (2H, m), and 4.00 (2H, s, NH_2) ppm. ^{13}C-NMR (DMSO-d_6) δ 135.9, 135.1, 132.4, 129.9, 129.8, 129.7, 129.4, 128.5, 128.2, and 123.8 pmm.

Typical procedure for preparation of individual members of pyridinohydrazide (**Scheme 3**) and hydrazonomethylpyridine (**Scheme 4**) libraries (Only NMR characterization is presented for **AR1-AR6**, Table 1). Preparation of **(*E*)-*N*'-(2-hydroxybenzylidene)picolinohydrazide (AR1)**. An ethanol (15 ml) mixture of salycilaldehyde (122 mg; 1 mmol) and picolinohydrazide (137 mg; 1 mmol) was refluxed with slow solvent distillation using the modified Hickman still (Sheme 3). After the volume of the reaction mixture was reduced to about 3-5 ml white precipitate started to form. This suspension was left at room temperature for one hour followed by an additional hour at 0ºC. Insoluble product was removed by filtration, washed with ice cold ethanol (3x2 ml) and dried on air to give pure product (220 mg) with a yield of 91%. ^{1}H-NMR (DMSO-d_6) δ 12.51 (1H, s, NH), 11.42 (1H, s, OH), 8.83 (1H, s, CH=N), 8.71 (1H, d, *J* = 4.4

Hz, picolino 3-H), 8.12 (1H, d, *J* = 8.0 Hz, picolino 6-H), 8.04 (1H, t, *J* = 8.0 Hz, picolino 5-H), 7.66 (1H, m, picolino 4-H), 7.45 (1H, d, *J* = 7.6 Hz, salicylic 6-H), 7.29 (1H, t, *J* = 8.0 Hz, salicylic 4-H) and 6.92 (2H, m, salicylic 3-H and 5-H) ppm. ^{13}C-NMR (DMSO-d_6) δ 161.1, 158.4, 150.8, 149.8, 149.3, 138.8, 132.2, 130.6, 127.9, 123.5, 120.1, 119.3, and 117.2 ppm.

(*E*)-*N*'-(2-Hydroxy-5-methylbenzylidene)picolinohydrazide (AR2). Isolated yield 240 mg (94%). ^{1}H-NMR (DMSO-d_6) δ 12.47 (1H, s, NH), 11.14 (1H, s, OH), 8.77 (1H, s, C<u>H</u>=N), 8.71 (1H, d, *J* = 4.8 Hz, picolino 3-H), 8.12 (1H, d, *J* = 7.6 Hz, picolino 6-H), 8.05 (1H, t, *J* = 8.0 Hz, picolino 5-H), 7.67 (1H, t, *J* = 6.0 Hz, picolino 4-H), 7.26 (1H, s, salicylic 6-H), 7.10 (1H, d, *J* = 8.4 Hz, salicylic 4-H), 6.82 (1H, d, *J* = 8.4 Hz, salicylic 3-H), and 2.24 (3H, s, CH_3) ppm. ^{13}C-NMR (DMSO-d_6) δ 156.2, 150.7, 149.9, 149.3, 138.8, 132.9, 130.5, 128.6, 127.9, 123.5, 118.9, 117.0, 115.6, and 20.6 ppm.

(*E*)-*N*'-(2-Hydroxy-5-methoxybenzylidene)picolinohydrazide (AR3). Isolated yied 250 mg (92%). ^{1}H-NMR (DMSO-d_6) δ 12.47 (1H, s, NH), 10.80 (1H, s, OH), 8.79 (1H, s, C<u>H</u>=N), 8.71 (1H, d, *J* = 4.8 Hz, picolino 3-H), 8.11 (1H, d, *J* = 7.6 Hz, picolino 6-H), 8.07 (1H, t, *J* = 7.6 Hz, picolino 5-H), 7.66 (1H, m, picolino 4-H), 7.26 (1H, s, salicylic 6-H), 7.03 (1H, d, J = 2.8 Hz, salicylic 6-H), 6.91 (1H, d of d, J_1 = 8.8 Hz, J_2 = 3.2Hz, salicylic 4-H), 6.85 (1H, d, *J* = 8.8 Hz), and 3.72 (3H, s, CH_3) ppm. ^{13}C-NMR (DMSO-d_6) δ 161.2, 152.8, 152.4, 150.0, 149.3, 138.8, 135.5, 127.8, 123.5, 119.5, 119.1, 118.0, 113.3, and 56.2 ppm. EMS m/z 272.2 ($M+H^+$) and 294.2 ($M+Na^+$). Anal. Calcd for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83; N, 15.49. Found: C, 62.08; H, 4.98; N, 15.36.

(*E*)-*N*'-(5-Chloro-2-hydroxybenzylidene)picolinohydrazide (AR4). Isolated yield 255 mg (92%). ^{1}H-NMR (DMSO-d_6) δ 12.56 (1H, s, NH), 11.33 (1H, s, OH), 8.80 (1H, s, C<u>H</u>=N), 8.70 (1H, d, *J* = 4.8 Hz, picolino 3-H), 8.12 (1H, d, *J* = 7.6 Hz, picolino 6-H), 8.04 (1H, t of d, J_1= 7.6

Hz, J_2 = 1.6 Hz, picolino 5-H), 7.65 (1H, m, picolino 4-H), 7.57 (1H, d, *J* = 2.8 Hz, salicylic 6-H, 7.30 (1H, d of d, J_1 = 8.8 Hz, J_2 = 2.8 Hz, salicylic 4-H), and 6.93 (1H, d, J = 8.8 Hz) ppm. ^{13}C-NMR (DMSO-d_6) δ 161.3, 156.9, 149.8, 149.3, 148.3, 138.7, 131.6, 128.6, 127.9, 123.6, 123.5, 121.3, and 118.9 ppm. EMS m/z 276.2 ($M+H^+$) and 298.1 ($M+Na^+$). Anal. Calcd for $C_{13}H_{10}ClN_3O_2xH_2O$: C, 53.49; H, 4.07; N, 14.40. Found: C, 53.40; H, 4.05; N, 14.49.

(*E*)-*N*'-(5-bromo-2-hydroxybenzylidene)picolinohydrazide (AR5). Isolated yield 300 mg (94%). ^{1}H-NMR (DMSO-d_6) δ 12.55 (1H, s, NH), 11.34 (1H, s, OH), 8.79 (1H, s, CH=N), 8.69 (1H, d, *J* = 4 Hz, picolino 3-H), 8.11 (1H, d, *J* = 7.6 Hz, picolino 6-H), 8.03 (1H, t, *J* = 7.6 Hz, picolino 5-H), 7.69 (1H, d, *J* = 2.4 Hz, salicylic 6-H), 7.65 (1H, m, picolino 4-H), 7.40 (1H, d od d, J_1 = 8.8 Hz, J_2 = 2.4 Hz, salicylic 4-H), and 6.88 (1H, d, *J* = 8.8 Hz, salicylic 3-H) ppm. ^{13}C-NMR (DMSO-d_6) δ 161.3, 157.3, 149.8, 149.3, 148.1, 138.7, 134.4, 131.5, 127.9, 123.6, 121.9, 119.4, and 111.1 ppm. EMS m/z 320.2 (^{79}Br-$M+H^+$), 322.1 (^{81}Br-$M+H^+$), 342.1 (^{79}Br-$M+Na^+$), and 344.0 (^{81}Br-$M+H^+$) and 298.1 ($M+Na^+$). Anal. Calcd for $C_{13}H_{10}BrN_3O_2x0.2H_2O$: C, 48.23; H, 3.17; N, 12.98. Found: C, 48.22; H, 3.19; N, 13.01

(*E*)-*N*'-(2-hydroxy-5-nitrobenzylidene)picolinohydrazide (AR6). Isolated yield 260 mg (90%). ^{1}H-NMR (DMSO-d_6) δ 12.62 (1H, s, NH), 12.30 (1H, s, OH), 8.88 (1H, s, CH=N), 8.68 (1H, d, *J* = 4 Hz, picolino 3-H), 8.45 (1H, d, J = 2.8 Hz, salicylic 6-H), 8.11 (2H, m), 8.03 (1H, m), 7.64 (1H, m) and 7.40 (1H, d, *J* = 9.2 Hz, salicylic 3-H) ppm. ^{13}C-NMR (DMSO-d_6) δ 163.5, 161.4, 149.7, 149.3, 146.8, 140.5, 138.7, 127.3, 127.3, 124.8, 123.6, 120.5, and 117.8 ppm.

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(LSU Department of Ophthalmology-LSUHSC). Isolates TW1 and TW17 were a kind gift from Dr. Glen Palmer, LSUHSC.

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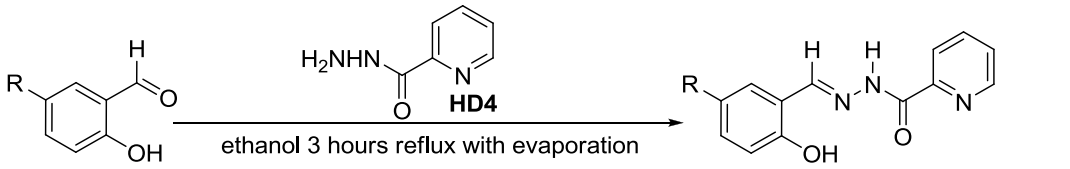
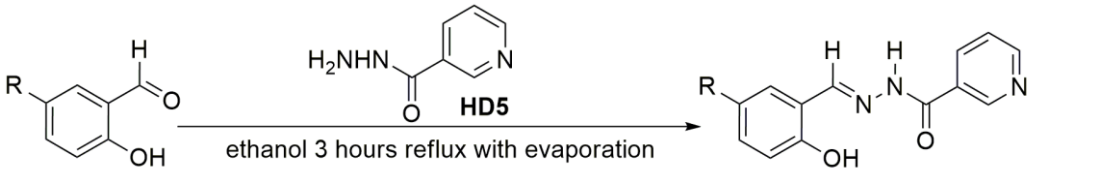


N'-(2-hydroxybenzylidene)picolinohydrazides

Table 1. Antifungal Activity of *N*'-(2-hydroxybenzylidene)picolinohydrazides

Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$** ***TW1*** **(μg/ml)**	**[2]MIC$_{80}$** ***TW17*** **(μg/ml)**	**[3]MIC$_{80}$** ***C. albicans*** **(μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$** ***C. glabrata*** **(μg/ml)** ATCC no. 48435
1	H	**AR1**	91%	62	NA	16	8
2	CH_3	**AR2**	94%	4	8	2	2
3	CH_3O	**AR3**	92%	8	31	4	1
4	Cl	**AR4**	92%	4	8	1	1
5	Br	**AR5**	94%	2	4	1	4
6	NO_2	**AR6**	90%	8	8	4	8

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant



N'-(2-hydroxybenzylidene)nicotinohydrazides

Table 2. Antifungal Activity of N'-(2-hydroxybenzylidene)nicotinohydrazides

Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$** ***TW1*** **(μg/ml)**	**[2]MIC$_{80}$** ***TW17*** **(μg/ml)**	**[3]MIC$_{80}$** ***C. albicans*** **(μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$** ***C. glabrata*** **(μg/ml)** ATCC no. 48435
1	H	**AR7**	91%	4	31	4	16
2	CH_3	**AR8**	93%	16	NA	4	8
3	CH_3O	**AR9**	86%	16	4	62	4
4	Cl	**AR10**	91%	8	62	4	8
5	Br	**AR11**	92%	8	16	4	16
6	NO_2	**AR12**	86%	8	8	4	NA

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant

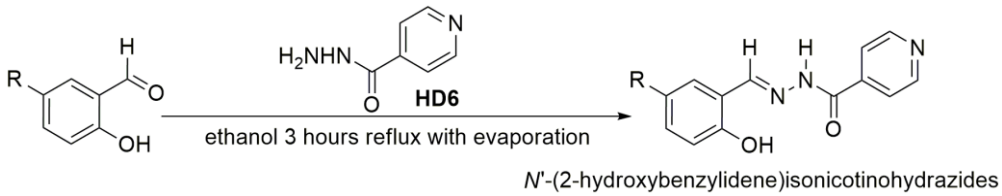
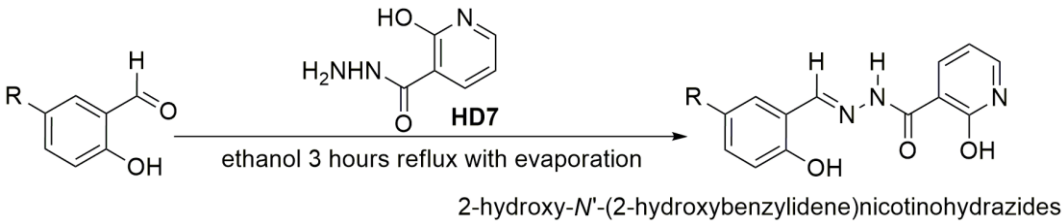


Table 3. Antifungal Activity of N'-(2-hydroxybenzylidene)isonicotinohydrazides

Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$ *TW1* (μg/ml)**	**[2]MIC$_{80}$ *TW17* (μg/ml)**	**[3]MIC$_{80}$ *C. albicans* (μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$ *C. glabrata* (μg/ml)** ATCC no. 48435
1	H	**AR13**	90%	4	31	8	16
2	CH_3	**AR14**	92%	16	31	4	31
3	CH_3O	**AR15**	91%	16	16	4	4
4	Cl	**AR16**	94%	16	125	31	16
5	Br	**AR17**	97%	16	125	16	64
6	NO_2	**AR18**	86%	8	8	4	31

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant



2-hydroxy-*N*'-(2-hydroxybenzylidene)nicotinohydrazides

Table 4. Antifungal Activity of 2-hydroxy-N'-(2-hydroxybenzylidene)nicotinohydrazides

Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$ *TW1* (μg/ml)**	**[2]MIC$_{80}$ *TW17* (μg/ml)**	**[3]MIC$_{80}$ *C. albicans* (μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$ *C. glabrata* (μg/ml)** ATCC no. 48435
1	H	**AR19**	89%	4	4	NA	1
2	CH_3	**AR20**	87%	NA	NA	NA	2
3	Cl	**AR21**	91%	NA	NA	NA	NA
4	Br	**AR22**	93%	NA	NA	NA	0.5

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant

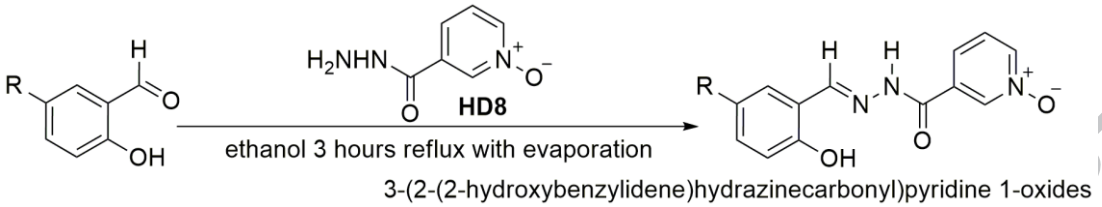
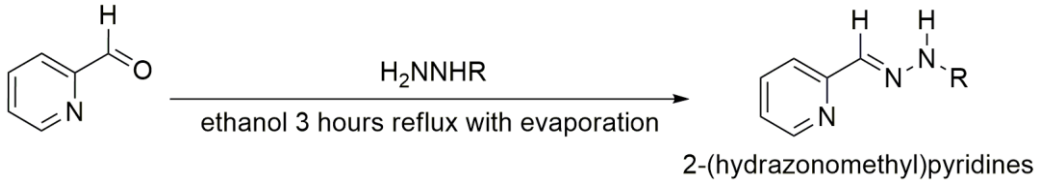


Table 5. Antifungal Activity of 3-(2-(2-hydroxybenzylidene)hydrazinecarbonyl)pyridine 1-oxides

Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$ *TW1* (μg/ml)**	**[2]MIC$_{80}$ *TW17* (μg/ml)**	**[3]MIC$_{80}$ *C. albicans* (μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$ *C. glabrata* (μg/ml)** ATCC no. 48435
1	H	**AR23**	87%	31	31	31	16
2	CH_3	**AR24**	89%	16	16	16	31
3	CH_3O	**AR25**	88%	62	31	16	4
4	Cl	**AR26**	91%	31	31	16	4
5	Br	**AR27**	93%	8	8	16	4
6	NO_2	**AR28**	85%	4	2	8	4

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant



2-(hydrazonomethyl)pyridines

Table 6. Antifungal activity of phenyl and aroyl N'-substituted 2-(hydrazonomethyl)pyridines

Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$ *TW1* (μg/ml)**	**[2]MIC$_{80}$ *TW17* (μg/ml)**	**[3]MIC$_{80}$ *C. albicans* (μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$ *C. glabrata* (μg/ml)** ATCC no. 48435
1	Phenyl	**AR29**	93%	16	16	16	31
2	2,4-dinitrophenyl	**AR30**	91%	NA	NA	NA	NA
3	benzoyl	**AR31**	90%	31	62	62	62
4	2-hydroxybenzoyl	**AR32**	88%	31	62	31	4
5	4-nitrobenzoyl	**AR33**	86%	NA	NA	NA	NA
6	Pyridine-2-carbonyl	**AR34**	92%	16	16	31	16
7	Pyridine-3-carbonyl	**AR35**	93%	62	62	125	125
8	Pyridine-4-carbonyl	**AR36**	91%	62	125	125	125
9	2-hydroxynicotinoyl	**AR37**	88%	NA	NA	NA	NA
10	1-oxidopyridine-3-carbonyl	**AR38**	90%	62	125	125	125

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant

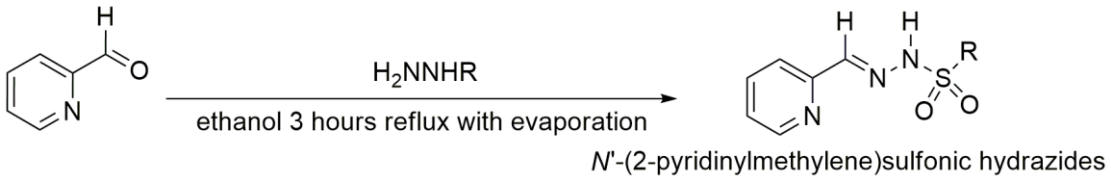
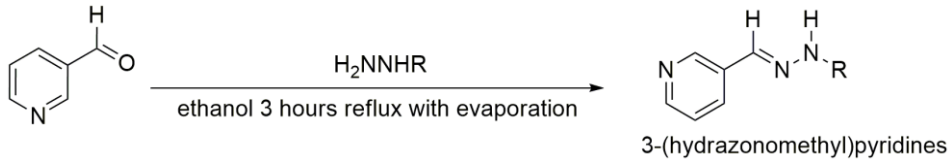


Table 7. Antifungal activity of aryl substituted *N*'-(2-pyridinylmethylene)sulfonic hydrazides

Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$ *TW1* (μg/ml)**	**[2]MIC$_{80}$ *TW17* (μg/ml)**	**[3]MIC$_{80}$ *C. albicans* (μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$ *C. glabrata* (μg/ml)** ATCC no. 48435
1	2-naphthyl	**AR39**	91%	NA	NA	NA	NA
2	4-methylphenyl	**AR40**	88%	NA	NA	NA	NA
3	4-methoxyphenyl	**AR41**	93%	NA	NA	NA	NA
4	4-bromophenyl	**AR42**	97%	NA	NA	NA	NA
5	4-nitrophenyl	**AR43**	91%	NA	NA	NA	NA

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant

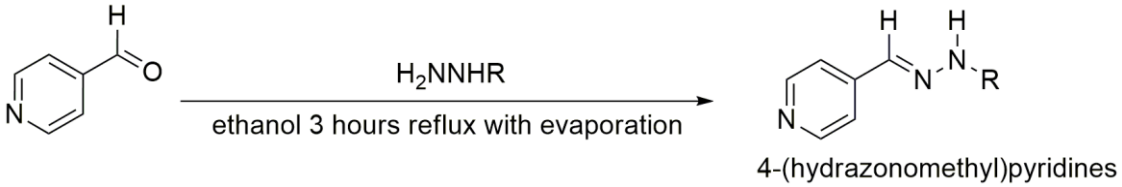


3-(hydrazonomethyl)pyridines

Table 8. Antifungal activity of aryl substituted 3-(hydrazonomethyl)pyridines

Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$ *TW1* (μg/ml)**	**[2]MIC$_{80}$ *TW17* (μg/ml)**	**[3]MIC$_{80}$ *C. albicans* (μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$ *C. glabrata* (μg/ml)** ATCC no. 48435
1	Phenyl	**AR44**	93%	16	8	16	16
2	2,4-dinitrophenyl	**AR45**	96%	NA	NA	NA	NA
3	Benzoyl	**AR46**	91%	NA	NA	NA	NA
4	4-nitrobenzoyl	**AR47**	86%	NA	NA	NA	NA

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant



4-(hydrazonomethyl)pyridines

Table 9. Antifungal activity of aryl substituted 4-(hydrazonomethyl)pyridines

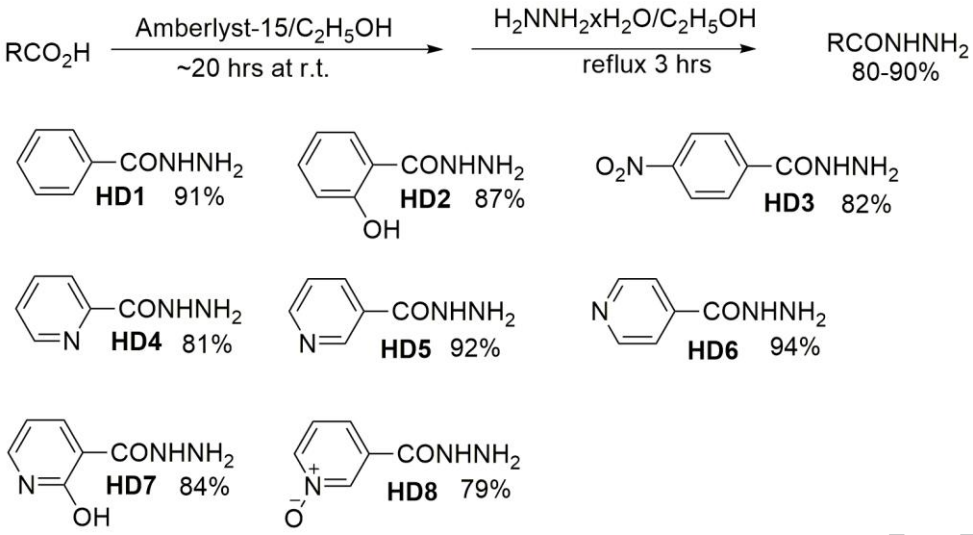
Entry	**R**	**Compound**	**Yield**	**[1]MIC$_{80}$ *TW1* (μg/ml)**	**[2]MIC$_{80}$ *TW17* (μg/ml)**	**[3]MIC$_{80}$ *C. albicans* (μg/ml)** ATCC no. 10231	**[4]MIC$_{80}$ *C. glabrata* (μg/ml)** ATCC no. 48435
1	Phenyl	**AR48**	92%	16	8	31	4
2	2,4-dinitrophenyl	**AR49**	95%	NA	NA	NA	NA
3	Benzoyl	**AR50**	89%	NA	NA	NA	NA
4	4-nitrobenzoyl	**AR51**	87%	NA	NA	NA	NA

[1]Azole-susceptible; [2]Azole-resistant; [3]Azole-susceptible; [4]Azole-resistant

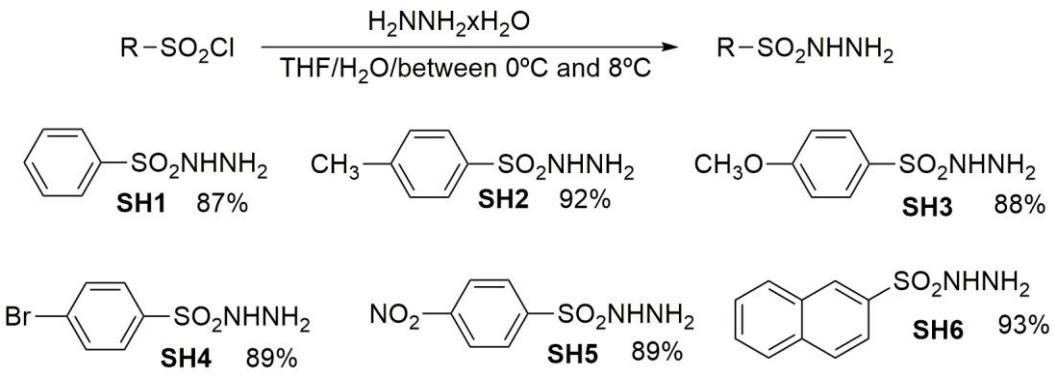
Table 10: Fractional Inhibitory Concentrations (FIC) for select analogs with fluconazole

Compound	FIC Fluc	FIC Test	FIC Index	Activity	Cidal vs Static
AR2	1.00	1.00	2.00	Antagonistic	Static
AR5	1.00	0.90	1.90	Antagonistic	Cidal
AR6	1.00	1.00	2.00	Antagonistic	Static
AR12	1.00	1.00	2.00	Antagonistic	Static
AR18	1.00	1.00	2.00	Antagonistic	Static
AR27	0.92	1.00	1.92	Antagonistic	Static
AR28	1.00	1.00	2.00	Antagonistic	Static
AR44	1.00	0.90	1.90	Antagonistic	Cidal
AR48	3.87	0.80	4.67	Antagonistic	Cidal

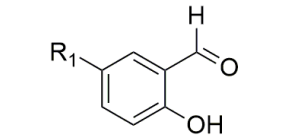
Scheme 1: Preparation of hydrazides from corresponding carboxylic acids



Scheme 2. Preparation of sulfonohydrazide

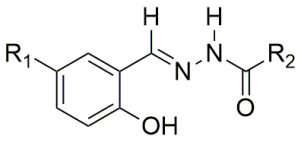


Scheme 3. Preparation method for 2-hydroxybenzylidenepyridinohydrazides and our modification of Hickman still



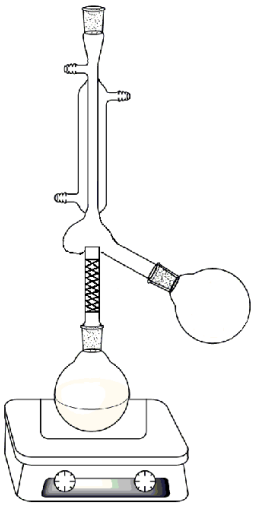
H_2NNCOR_2

slow
ethanol
distillation
~ 1 hour



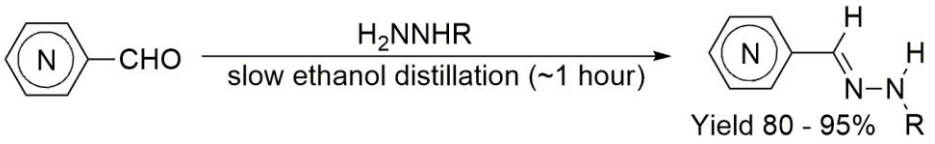
Yield 85-97%

R_1 = H, CH_3, OCH_3, Cl, Br, and NO_2
R_2 = 2-pyridinyl, 3-pyridinyl, 4-pyridinyl, 2-hydroxy-3-pyridinyl, 1-oxido-3-pyridiny



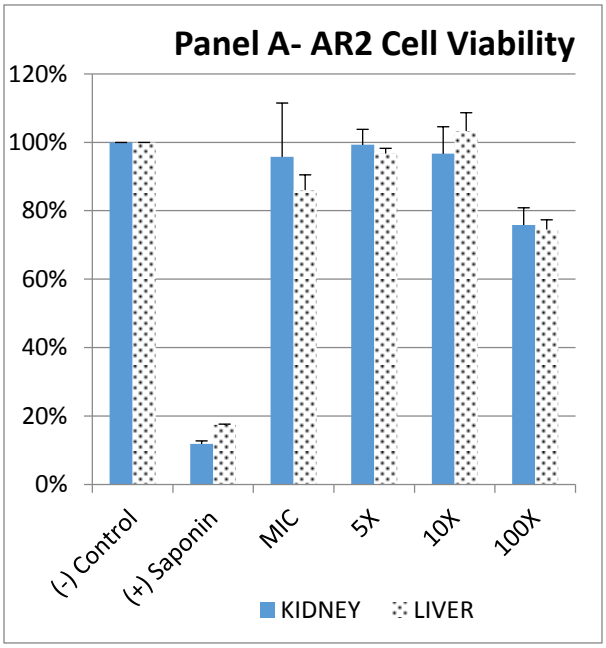
our modification of Hickman still

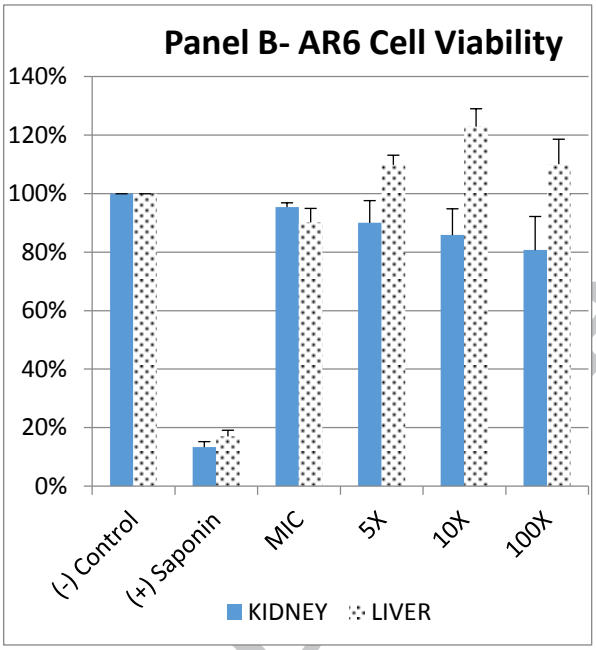
Scheme 4. Members of hydrazonomethylpyridine library

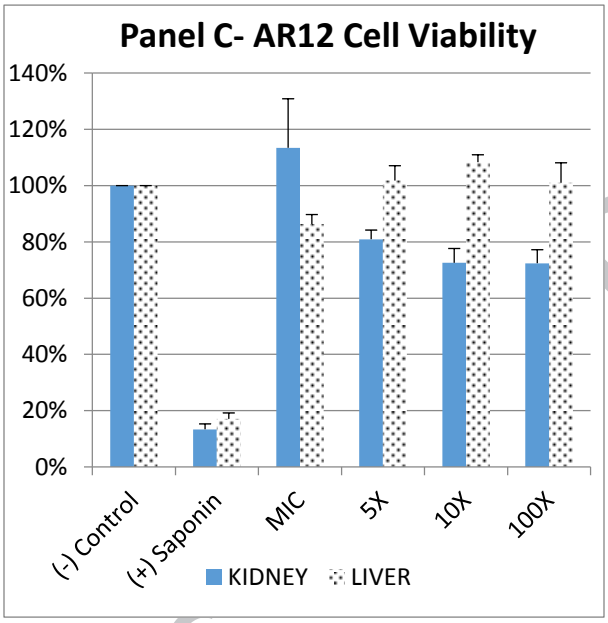


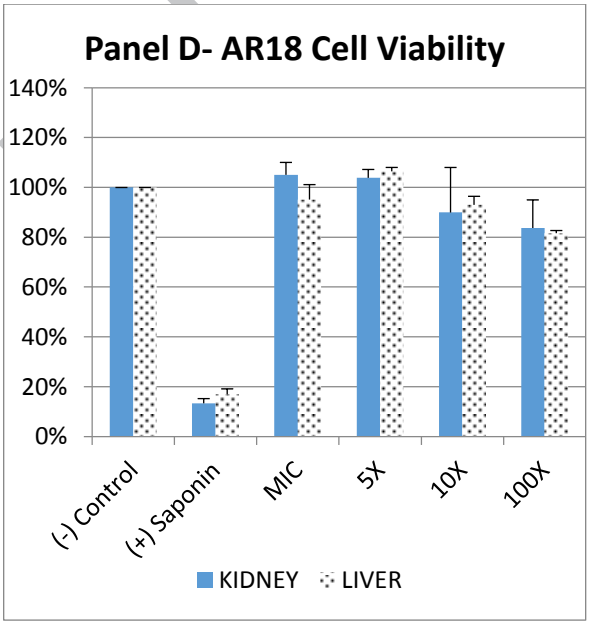
Nitrogen ring position is 2, 3, or 4 (2-pyridinecarbaldehyde, 3-pyridinecarbaldehyde, or 4-pyridinecarbaldehyde. R = phenyl, 2,4-dinitrophenyl, benzoyl, 2-hydoxybenzoyl, 4-nitrobenzoyl, 2-pyrinecarbonyl, 3-pyridinecaronyl, 4-pyrdinecarbonyl, 2-hydroxy-3-pyridinecarbonyl, 1-oxido-3-pyridinecarbonyl, 4-methylbenzenesulfonyl, 4-methoxybenzenesulfonyl, 4-bromobenzenesulfonyl, 4-nitrobenzenesulfonyl,

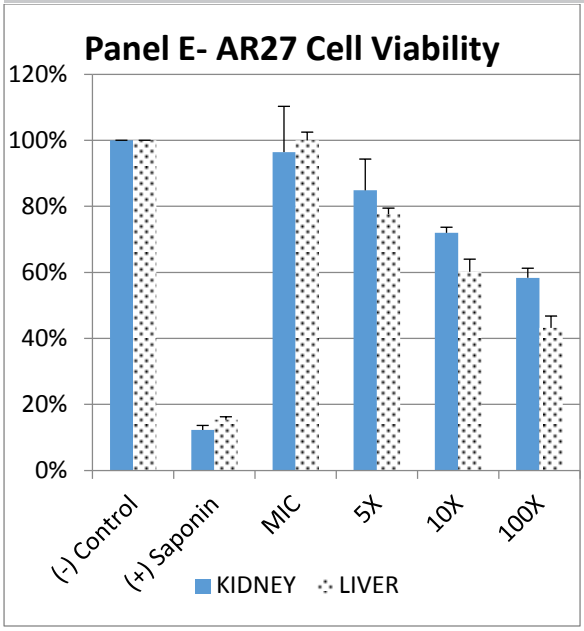
Figure 1:

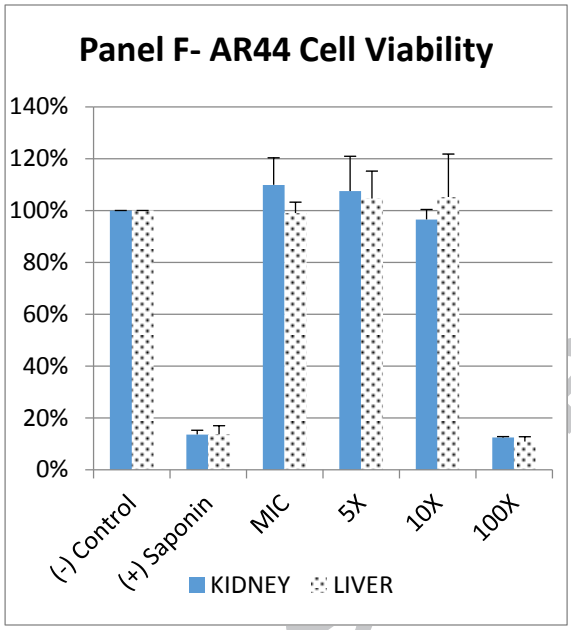












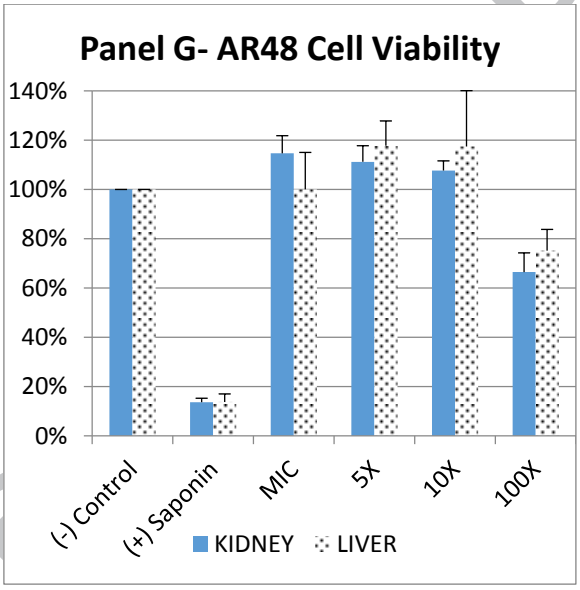
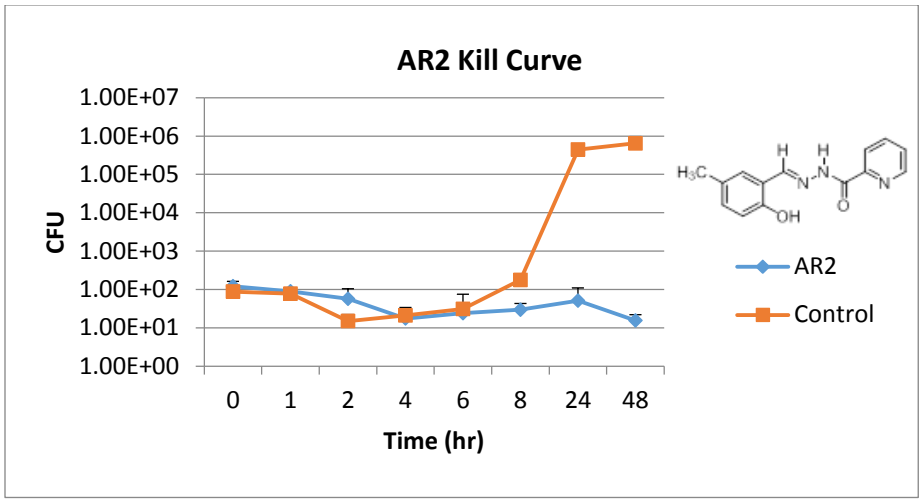
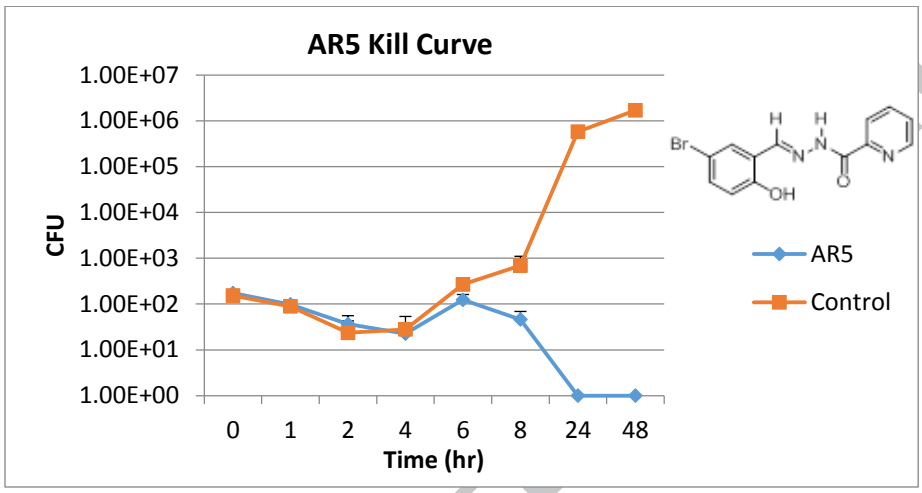
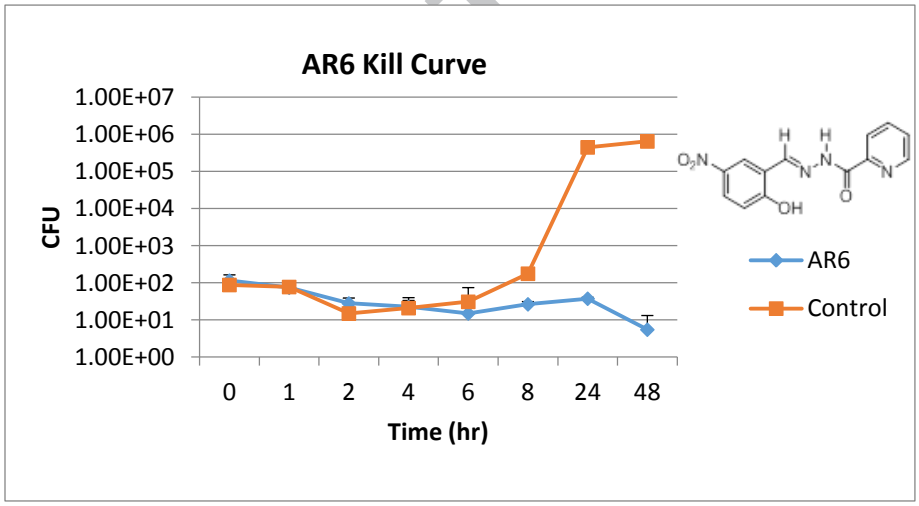


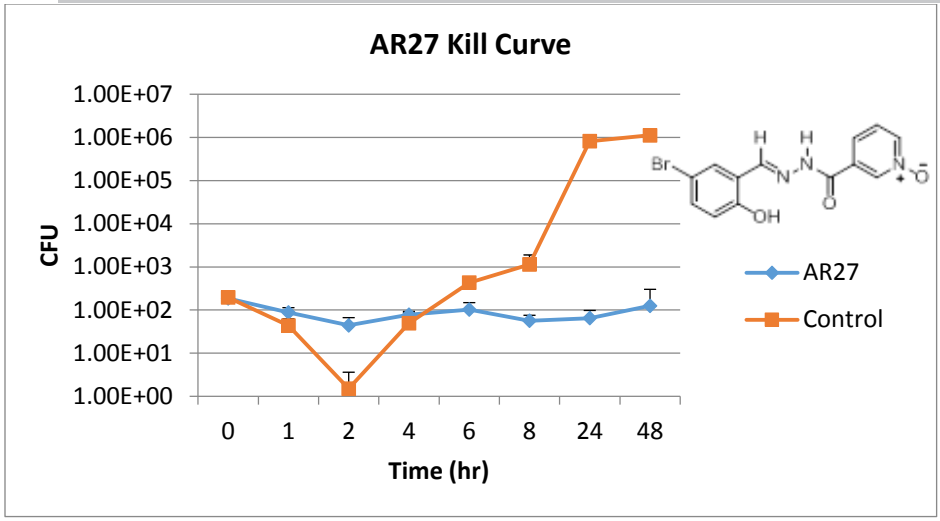
Figure 1: Evaluation of mammalian cell toxicity of select active compounds Cytotoxicity was determined using non-cancerous Vero cells (kidney-african green monkey) and liver cells (Hep-2G) in accordance with Promega CellTiter 96 Non-Radioactive Cell Proliferation Assay (cat # G4000). Compounds were diluted in media and all assays were done in triplicate. Average values are presented, with ±SD. Cells were incubated in the presence of the compounds for 24 hours at 37°C and 5% CO_2. Tetrazolium dye solution was added to each well and allowed to incubate for 1-4 h. Solubilization/Stop solution was added and allowed to sit at room temperature for 1 h. Formazan product was scored spectrophotometrically with an automatic plate reader set at 570 nm. Positive control wells for cell death were briefly treated with 0.1% saponin, immediately prior to the addition of dye solution. Wells containing 1% DMSO and no drug were used as a negative control and the average absorbance at 570 nm were set to 100%. The percent of cells viable were calculated as a ratio between the average absorption at 570 nm of the drug containing wells/ average absorption at 570 nm of the control (no drug). Ratios were multiplied by 100 to give the values as a percentage as expressed on the y-axis.

Figure 2:









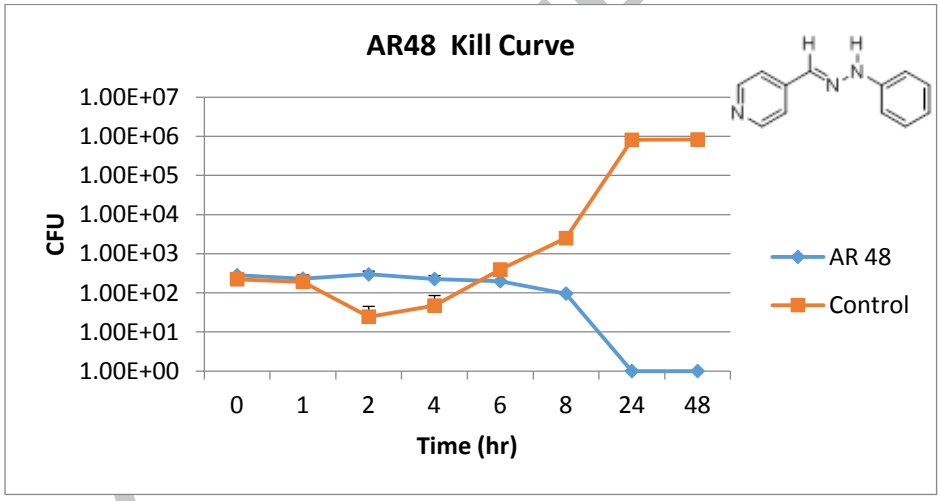


Figure 2. Time-Kill assays for representative analogs that were active against the azole-resistant TW17 were done to determine the cidal or static nature of the analog tested. 100 µl of each sample was spread onto YM agar plates with a sterile bent spreading rod. Plates were incubated at 35°C overnight and colonies were then counted on each plate.

Graphical Abstract