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Synthesis and antifungal activity of substituted salicylaldehyde hydrazones, hydrazides and sulfohydrazides

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ABSTRACT

Efficient synthetic procedures for the preparation of acid hydrazines and hydrazides were developed by converting the corresponding carboxylic acid into the methyl ester catalyzed by Amberlyst-15, followed by a reaction with hydrazine monohydrate. Sulfohydrazides were prepared from the corresponding sulfonyl chlorides and hydrazine monohydrate. Both of these group of compounds were condensed with substituted salicylaldehydes using gradient concentration methods that generated a large library of hydrazone, hydrazide and sulfohydrazide analogs. Antifungal activity of the prepared analogs showed that salicylaldehyde hydrazones and hydrazides are potent inhibitors of fungal growth with little to no mammalian cell toxicity, making these analogs promising new targets for future therapeutic development.

Opportunistic fungal infections are common and potentially life-threatening infections.[1] For example, invasive fungal infections from *Candida* spp. are a significant cause of morbidity and mortality in at-risk populations, particularly transplant recipients, cancer patients and those infected with HIV and AIDS.[2,3] Further, invasive *Candida* spp. infections rapidly develop resistance to currently marketed antifungal agents,[4] making vigilant treatment difficult at best. Clinically, candidiasis incidence is commonly associated with *Candida albicans*, *Candida glabrata* and *Candida krusei* microorganisms most frequently.[5,6] Currently, the first-line treatment for these infections are azoles, yet the *Candida* spp. have developed multiple resistance mechanisms to azoles, including overexpression of efflux pumps[1] and point mutations in the *ERG11* gene[7] in *Candida albicans*. In addition, *Candida glabrata* develops resistance during prolonged treatment with azole antifungals.[8] For these reasons, there is a continuous demand for the discovery of novel therapeutics to treat fungal infections, particularly *Candida* spp. infections.

Hydrazine and hydrazone derivatives are emerging in the literature as novel classes of potential antifungal agents with activity against numerous *Candida* spp. Recently, we reported an extensive collection of substituted pyrimidinetrione derivatives with potent antifungal activity against two human pathogenic species, *Candida albicans* and *Candida glabrata*.[9] A number of those derivatives, including phenylhydrazones of 5-acylpyrimidinetrione exhibited potent growth inhibition at or below 10 μM with minimal mammalian cell toxicity, making this class of compounds an interesting alternative for exploration and development to treat fungal infections. In addition, it has also been well documented that some substituted salicylaldehyde hydrazones also possess antimicrobial activity while salicylaldehydes by themselves do not possess antifungal activity.[10–17] Nonetheless, there are significant drawbacks that have limited development of salicylaldehyde hydrazones as a broad class of antifungal agents. These drawbacks include the lack of appropriately developed synthetic procedures that can produce a range of structural variations within the class of salicylaldehyde hydrazones for antifungal and SAR studies. In this work, we present the efficient synthetic methodology used to generate three structurally diverse groups of salicylaldehyde derivatives; salicylaldehyde phenylhydrazones (group I), benzohydrazides (group II), and sulfohydrazides (group III; Scheme 1). The antifungal activity for *Candida albicans* and *Candida glabrata* was assessed for each class of salicylaldehyde derivatives. Finally, mammalian cell toxicity screens were performed against all compounds with MICs of 8 μg/mL or less. In summary, we found that nitro salicylaldehyde hydrazones, in addition to a broad range of unsubstituted, substituted and halogenated salicylaldehyde hydrazides were potent inhibitors of fungal growth with minimal cytotoxicity to liver or kidney cells.

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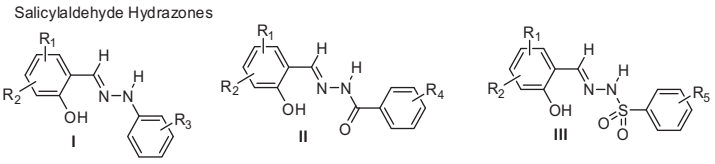
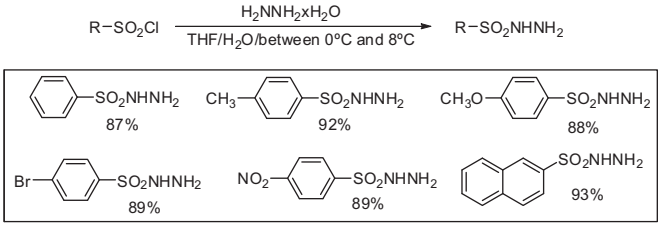


Scheme 1. Three groups of salicylaldehyde hydrazone libraries selected for antifungal studies.

1. Chemistry

To our surprise, we could find no simple and high yielding general synthetic procedures applicable to the preparation of large quantities of structurally diverse acid hydrazides[18–22] even though their application in medicine and medicinal chemistry has been well documented.[23–27] Several approaches were documented for the preparation of acid hydrazides,[18] including starting with carboxylic acids,[28] acid esters,[29] acid chlorides,[20] amides[30] and even *N*-acylbenzotriazoles.[31] In general, acid chlorides and acid anhydrides double acylate hydrazines[32] and subsequently are not viable intermediates for the preparation of acid hydrazides. On the other hand, single hydrazine acylation with esters seemed to be easier to control. For this reason, we have developed two parallel methods (Methods I and II, Scheme 2) for the preparation of acid hydrazides. The first approach uses thionyl chloride catalyzed acid esterification with ethanol, which is commonly used for the preparation of amino acid hydrazides.[33] Thus, the prepared esters were isolated, purified and used in the next reaction with hydrazine monohydrate to yield the desirable acid hydrazide (Method 1, Scheme 2). The second preparation method utilized Amberlyst-15,[34] a strong acid exchange resin catalyst for esterification with methanol. After the reaction was complete, the catalyst was removed by filtration and the same clear methanol solution that contained an ester was mixed with an excess of hydrazine monohydrate to give the targeted acid hydrazide in high isolated yields (Method II, Scheme 2). Additionally, the catalyst is recyclable[35] and the reaction could also tolerate the presence of acid sensitive moieties such as phenol, OH, *t*-butyl, or even the pyridine moiety. In this way Method II was developed as the method of choice for the preparation of a structurally diverse library of acid hydrazides.

Sulfonohydrazides (Scheme 3) were all prepared from the corresponding sulfonyl chloride by modifying procedures outlined by Friedman and co-workers.[36] The reaction involved the slow addition of a tetrahydrofuran solution of the corresponding sulfonyl chloride into ice-water cooled aqueous hydrazine. It was important to maintain the reaction mixture temperature close to 0 °C to avoid the formation of the double sulfonation byproduct. It was also important to evaporate tetrahydrofuran at a reduced pressure without heating. Furthermore, we found that this procedure was an appropriate general method for the preparation of sulfonohydrazides, a class of compounds that are becoming



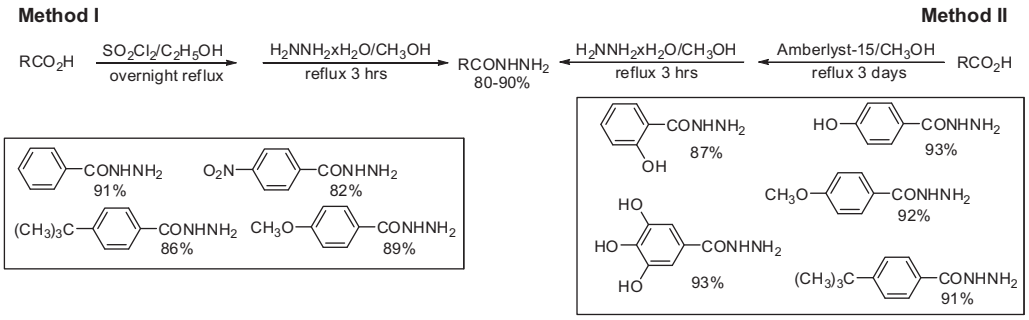
Scheme 3. Preparation of sulfonohydrazides.

increasingly important synthetic intermediates[37] for preparation of valuable biologically active compounds.[38–42]

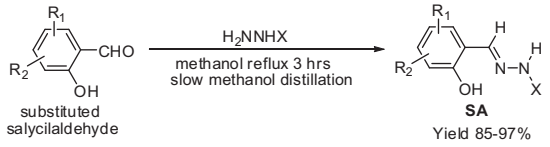
In general, hydrazones were prepared from the corresponding carbonyl (aldehyde or ketone) compound and the mono and asymmetric double substituted hydrazines[43–46] in refluxing methanol or ethanol without acid or base catalyst (Scheme 4). This reaction was generally completed after three hours. Isolated yields and purification of the final products were simplified when the reaction began as a very diluted reaction mixture (100 ml of methanol per 1 mmol of aldehyde and 1 mmol of substituted hydrazine) and then the reagent concentration was gradually increased by slowly distilling the solvent from the refluxing reaction mixture. Distillation of the solvent was stopped at the point when a solid started to form in the reaction mixture or if the reaction mixture volume was reduced to ~5 ml. At room temperature or at −5 °C, all of the prepared compounds crystallized readily. They were further purified by washing with ice cold methanol, ether, or hexane as solvents for which the corresponding products are only slightly soluble. It is important to emphasize that the formation of the hydrazone product was enhanced by gradually increasing the reactant concentration through slow solvent distillation. In this way, the isolated yields of structurally diverse hydrazones was >85% (Scheme 4).

2. Antifungal activity

The antifungal activity of the salicylaldehyde hydrazones, hydrazides and sulfohydrazides described in Schemes 2–4 were evaluated in vitro using *Candida albicans* (ATCC No. 10231) and *Candida glabrata* (ATCC No. 48435). All assays were done in



Scheme 2. Two different pathways for preparation of hydrazides.



R_1 = H, Cl, Br, CH_3, CH_3O, NO_2, OH, R_2 = H, Cl, Br, CH_3, CH_3O, NO_2, OH
R_3 = H, OH, NO_2
X = H, C_6H_5, C_6H_4-4-OCH_3, C_6H_3-2,4-$(CH_3)_2$, C_6H_4-4-NO_2, C_6H_3-2,4-$(NO_2)_2$, COC_6H_5, COC_6H_4-4-NO_2, COC_6H_4-2-OH, COC_6H_4-4-$C(CH_3)_3$, COC_6H_4-4-OCH_3, $SO_2C_6H_5$, $SO_2C_6H_4$-4-CH_3, $SO_2C_6H_4$-4-OCH_3, $SO_2C_6H_4$-4-Br, $SO_2C_6H_4$-NO_2, SO_2-2-naphthyl

Scheme 4. General route for preparation our hydrazone library.

accordance with NCCLS reference documents.[47] The results of these screenings are summarized in Tables 1–8 as the minimal inhibitory concentrations that inhibited more than 80% fungal growth as compared to the positive controls in 1% DMSO and HEPES buffered RPMI media. All MIC screens were done using a visual scoring method as opposed to spectroscopic methods of analysis, due to the physical properties of many of the compounds altering their absorbance spectra relative to the positive, negative and drug controls.

Of the analogs tested, none of the salicylaldehyde sulfohydrazides showed antifungal activity through 125 μg/mL, regardless of the nature of the aromatic ring attached (Table 8). Similarly, limited fungal growth inhibition was observed with 2-(*p*-nitrophenylhydrazonomethyl)phenols presented in Scheme 4 (Table 3). In contrast, however, virtually every salicylaldehyde hydrazone and hydrazide synthesized, regardless of whether the aromatic ring substitutions included halogens, electron donating or electron withdrawing moieties, showed antifungal activity through fungal growth inhibition (Tables 1, 2 and 4–7). The most potent inhibitors of fungal growth included the nitrobenzohydrazides **SA6** and **SA49**, Table 5 and the halogenated benzohydrazide **SA10**, Table 4, each of which had antifungal activity at a concentration below 2 μM for both fungal species tested.

To further test the mammalian cell toxicity of the salicylaldehyde analogs presented, we screened selected compounds against liver and kidney cells to establish toxicity parameters. All analogs presented in this manuscript with antifungal activity of less than 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). Multiple concentrations of the analog were used, including the concentration at which fungal inhibition was observed as well as at 5×, 10× and 100× the minimum inhibition concentration. Cytotoxicity studies were performed in accordance with Promega CellTiter 96 Non-RadioactivCell Proliferation Assay (cat. # G4000). Representative compounds are presented in Figure 1. Briefly, all cytotoxicity studies were done using a negative control (wells of either kidney or liver cells in the absence of the analog), a positive control (wells of either kidney or liver cells treated with a known cytotoxic agent known as 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 remain alive and viable following overnight incubation with either saponin or the analogs tested. Of the analogs assayed, **SA1**, **SA6**, **SA10** and **SA55** had minimal reductions in viable kidney and liver cells, where the only marked cell death was observed at concentrations 100× greater than the established MIC (Fig. 1). In contrast, the two most potent analogs, **SA48** and **SA49** exhibited toxicity toward kidney cells, with an ~20% reduction in viable cells even at the MIC relative to the negative control (Fig. 1). While this was a somewhat discouraging finding, more studies are currently underway to determine the mechanism of toxicity and possible ways to reduce kidney toxicity associated with these two analogs. Nonetheless, the majority of analogs tested showed minimal cytotoxicity in our in vitro assays. All results from the toxicity screenings outside of Figure 1 are provided in Supplemental information.

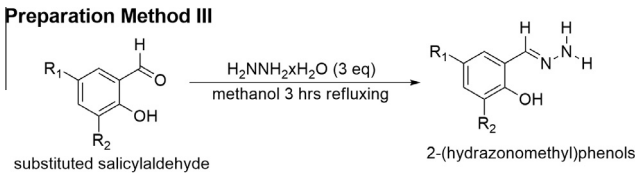
3. Chemistry experimental

Thin-layer chromatographic analysis (TLC) was performed using silica gel on aluminum foil glass plates and was detected under ultraviolet (UV) light. The ^1H and ^{13}C NMR spectra were run on Varian 400 MHz Unity instruments in $CDCl_3$ or in DMSO-d_6. Solvent signals were use as internal NMR chemical shift references. If necessary, products were purified by short silica gel (40–70 mm) filtration. Silica gel was purchased from Sorbent Technologies. Substituted phenylhydrazines were prepared from corresponding anilines by following the preparation procedure outlined in Vogel.[48] All reagents and solvents were purchased from Sigma–Aldrich and were analytical grade.

3.1. Typical procedure for preparation of aryl hydrazides. Preparation Method I (see Scheme 2)

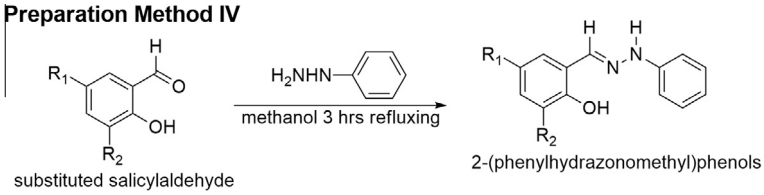
Preparation of 3,4,5-trihydroxybenzohydrazide (H1-Supplemental material). A methanol (300 ml) suspension of 3,4,5-trihydroxybenzoic acid and (17 g; 0.1 mol) and the strongly acidic ion-exchange resin Amberlyst-15 (5 g) was stirred with refluxing for three days. The insoluble catalyst was separated by filtration, and washed with methanol (3 × 10 ml). The combined methanol filtrates were mixed with hydrazine hydrate (20 ml; 20.5 g; 0.4 mol) and refluxed for 3 h. The volume of the reaction mixture

Table 1
Isolated yield and antifungal activity of 2-(hydrazonomethyl)phenols



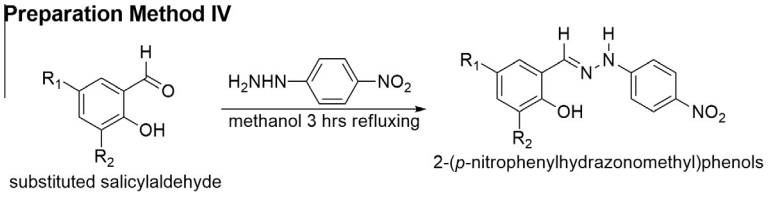
Entry	R_1	R_2	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
1	H	H	**SA1**	92	16	4
2	H	$C(CH_3)_3$	**SA50**	93	125	62
3	Cl	H	**SA19**	94	62	31
4	Cl	Cl	**SA28**	96	16	62
5	Br	Br	**SA23**	91	16	8
6	NO_2	H	**SA33**	85	NA	31

Table 2
Antifungal activity of 2-(phenylhydrazonomethyl)phenols



Entry	R_1	R_2	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
1	H	H	**SA2**	89	16	16
2	CH_3	H	**SA46**	92	62	8
3	H	$(CH_3)_3C$	**SA38**	97	NA	NA
4	CH_3O	H	**SA42**	92	31	31
5	Cl	H	**SA20**	93	31	31
6	Cl	Cl	**SA29**	92	125	125
7	Br	H	**SA15**	91	31	31
8	H	Br	**SA51**	87	8	16
9	Br	Br	**SA24**	88	NA	62
10	NO_2	H	**SA34**	91	31	16
11	NO_2	NO_2	**SA55**	83	2	4

Table 3
Antifungal activity of 2-(*p*-nitrophenylhydrazonomethyl)phenols



Entry	R_1	R_2	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
1	H	H	**SA4**	94	125	4
2	CH_3	H	**SA47**	93	NA	NA
3	H	$(CH_3)_3C$	**SA39**	89	NA	NA
4	Cl	H	**SA21**	92	NA	62
5	Cl	Cl	**SA30**	93	NA	62
6	Br	H	**SA16**	91	NA	NA
7	H	Br	**SA52**	85	NA	NA
8	Br	Br	**SA25**	88	NA	62
9	NO_2	H	**SA35**	85	125	62
10	NO_2	NO_2	**SA56**	82	125	125

was reduced to ~30 ml and left at room temperature for 1 h and then at −5 °C. The formed white precipitate was separated by filtration, washed with ice cold methanol (3 × 5 ml), followed by ice cold ether (3 × 10 ml) and dried on air to give pure product (17.1 g; 93%). ^{1}H NMR (DMSO-d_6) δ 9.34 (1H, br s, NH), 6.79 (2H, s, 2-H), and 4.33 (2H, br s, NH_2) ppm. ^{13}C NMR (DMSO-d_6) δ 167.1, 146.1, 136.9, 124.3, and 107.2 ppm.

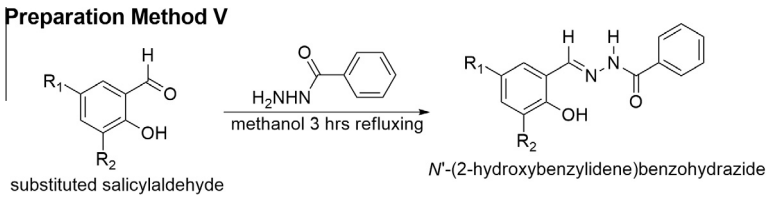
3.2. Typical procedure for preparation of aryl sulfonohydrazides. Preparation Method II (see Scheme 3)

Preparation of 4-nitrobenzenesulfonohydrazide (H5). A water (300 ml) solution of hydrazine hydrate (12.5 g; 0.25 mol) was cooled in ice-water bath to ~5 °C. The temperature was then maintained below 8 °C, while the tetrahydrofuran (50 ml) solution of 4-nitrobenzenesulfonyl chloride (5.54 g, 0.025 mol) was slowly added with stirring. After the addition was completed the reaction mixture was stirred at room temperature for thirty minutes and tetrahydrofuran was evaporated at reduced pressure. The white solid product was separated by filtration, washed with water (3 × 15 ml) and dried on air overnight to give pure product (4.86 g; 89%). ^{1}H NMR (DMSO-d_6) δ 8.67 (1H, br 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, br s, NH_2) ppm. ^{13}C NMR (DMSO-d_6) δ 150.4, 144.8, 129.9, and 124.9 ppm.

3.3. Typical procedure for preparation of hydrazinomethylphenols. Preparation Method III (see Scheme 4)

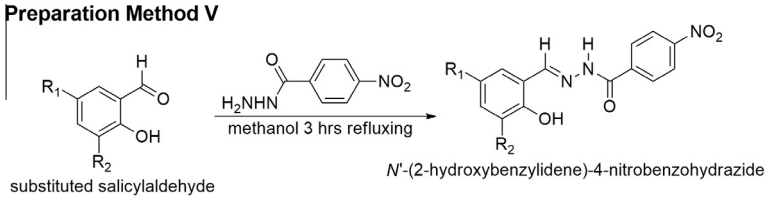
Preparation of 2-(hydrazonomethyl)-4-nitrophenol (**SA33**). A mixture of methanol (300 ml), 2-hydroxy-5-nitrobenzaldehyde (250 mg; 1.5 mmol), and hydrazine monohydrate (130 mg; 4.5 mmol) was refluxed for three hours. The solvent was evaporated to a solid residue. This residue was mixed with hot methanol (10 ml) and sonicated at 60 °C for a few minutes. The resulting mixture was mixed with water (~100 ml) and then cooled in ice bath. The resulting insoluble product was separated by filtration, washed with cold water (3 × 5 ml) and dried under reduced pressure to give pure product (230 mg; 85%). ^{1}H NMR (DMSO-d_6) δ 12.0

Table 4
Antifungal activity of *N*′-(2-hydroxybenzylidene)benzohydrazides



Entry	R_1	R_2	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
1	H	H	**SA5**	87	2	1
2	CH_3	H	**SA48**	92	1	1
3	H	$C(CH_3)_3$	**SA40**	89	NA	NA
4	CH_3O	H	**SA44**	91	4	0.5
5	Cl	H	**SA10**	95	0.5	0.5
6	Cl	Cl	**SA31**	91	8	16
7	Br	H	**SA17**	93	2	2
8	H	Br	**SA53**	94	4	2
9	Br	Br	**SA26**	86	8	31
10	NO_2	H	**SA36**	87	16	16
11	NO_2	NO_2	**SA57**	81	NA	NA

Table 5
Antifungal activity of *N*′-(2-hydroxybenzylidene)-4-nitrobenzohydrazide



Entry	R_1	R_2	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
1	H	H	**SA6**	92	0.5	0.5
2	CH_3	H	**SA49**	95	0.5	0.125
3	H	$(CH_3)_3C$	**SA41**	93	31	125
4	CH_3O	H	**SA45**	89	125	2
5	Cl	H	**SA22**	94	16	62
6	Cl	Cl	**SA32**	89	31	125
7	Br	H	**SA18**	91	1	2
8	H	Br	**SA54**	93	31	31
9	Br	Br	**SA27**	87	1	125
10	NO_2	H	**SA37**	87	NA	16
11	NO_2	NO_2	**SA58**	83	NA	NA

(1H, br s, OH), 8.22 (1H, d, J = 2.8 Hz, 6-H), 7.98 (1H, s, CH═N), 7.96 (1H, d of d, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 4-H), 7.25 (2H, br s, NH_2), and 6.95 (1H, d, J = 9.2 Hz, 3-H) ppm. ^{13}C NMR (DMSO-d_6) δ 162.6, 140.4, 138.1, 124.7, 123.5, 121.5, and 117.1 ppm.

3.4. Typical procedure for preparation phenylhydrazones. Preparation Method IV (see Scheme 4)

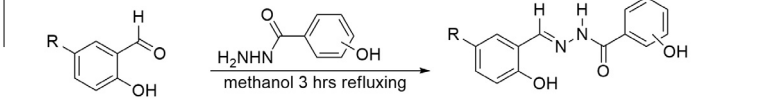
Preparation of 3,5-dichloro-6-((2-phenylhydrazono)methyl)-phenol (**SA29**). A methanol (50 ml) solution of 3,5-dichloro-2-hydroxybenzaldehyde (191 mg; 1 mmol) and phenylhydrazine (1.08 mg; 1 mmol) was refluxed for 4 h. Solvent was evaporated to an oily residue that crystalized upon cooling. This residue was dissolved in hot hexane (~100 ml) and left at room temperature. The formed crystalline product was separated by filtration, washed with hexane (3 × 10 ml) and dried on air to give pure product (260 mg; 92%). ^{1}H NMR (DMSO-d_6) δ 11.50 (1H, br s, OH), 10.81 (1H, s, NH), 8.07 (1H, s, CH═N), 7.54 (1H, d, J = 2.0 Hz, salicylic 6-H), 7.42 (1H, d, J = 2.0 Hz, salicylic 4-H), 7.26 (2H, J = 7.6 Hz, phenyl 3-H), 6.96 (2H, d, J = 8.4 Hz, phenyl 2-H), and 6.82 (1H, t, J = 7.3 Hz, phenyl 4-H) ppm. ^{13}C NMR (DMSO-d_6) δ 151.0, 144.5, 136.8, 130.1, 128.6, 126.7, 124.0, 123.7, 121.9, 120.6, and 112.6 ppm.

3.5. Typical procedure for preparation of benzohydrazones. Preparation Method V (see Scheme 4)

Preparation of *N*′-(2-hydroxy-5-methoxybenzylidene)-4-nitrobenzohydrazide (**SA45**). A methanol (100 ml) mixture of 4-nitrobenzohydrazide (181 mg; 1 mmol) and 5-methoxysalicylaldehyde (152 mg; 1 mmol) was refluxed for 4 h. The solvent volume was reduced to ~10 ml by atmospheric evaporation followed by evaporation at reduced pressure to yield a white solid residue. The solid was mixed with ether (20 ml) and sonicated with refluxing for 10 min. The suspension was left at room temperature for 30 min. An insoluble white precipitate was separated by filtration, washed with ether (3 × 5 ml) and dried on air to give pure product (280 mg; 89%). ^{1}H NMR (DMSO-d_6) δ 12.31 (1H, br s, OH) 10.52 (1H, s, NH), 8.64 (1H, s, CH═N), 8.34 (2H, d, J = 8.4 Hz,

Table 6
Antifungal activity of *N*′-(2-hydroxybenzylidene)hydroxybenzohydrazide

Preparation Method V



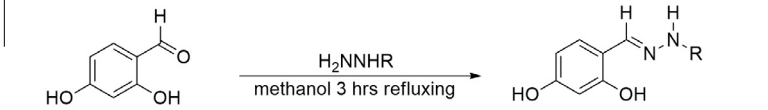
5-substituted salicylaldehyde

N′-(2-hydroxybenzylidene)hydroxybenzohydrazide

Entry	R	OH	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
1	H	2	**SA83**	94	16	8
2	CH_3	2	**SA143**	92	16	NA
3	CH_3O	2	**SA123**	89	16	16
4	Cl	2	**SA96**	95	8	NA
5	Br	2	**SA109**	91	8	62
6	H	4	**SA84**	92	NA	4
7	CH_3	4	**SA144**	91	NA	1
8	CH_3O	4	**SA127**	87	NA	4
9	Cl	4	**SA97**	89	NA	62
10	Br	4	**SA110**	91	125	62

Table 7
Antifungal activity of *N*′-substituted 4-(hydrazonomethyl)benzene-1,3-diol

Preparation Method V



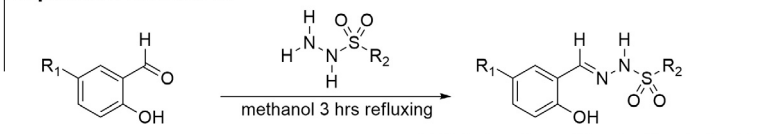
2,4-dihydroxybenzaldehyde

4-(hydrazonomethyl)benzene-1,3-diol

Entry	R	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
1	H	**SA8**	85	62	31
2	C_6H_5	**SA9**	95	31	2
3	4-$(O_2N)C_6H_4$	**SA11**	87	N/A	1
4	2,4-$(O_2N)_2C_6H_3$	**SA68**	93	NA	NA
5	C_6H_5CO	**SA12**	91	31	1
6	4-$(O_2N)C_6H_4CO$	**SA13**	88	NA	0.5

Table 8
Lack of antifungal activity for *N*′-(2-hydroxybenzylidene)sulfonohydrazides

Preparation Method VI



5-sabstituted salicylaldehyde

N′-(2-hydroxybenzylidene)sulfonohydrazide

Entry	R_1	R_2	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
1	H	C_6H_5	**SA75**	91	NA	125
2	H	4-$(CH_3)C_6H_4$	**SA74**	93	NA	NA
3	H	4-$(CH_3O)C_6H_4$	**SA73**	88	NA	125
4	H	4-BrC_6H_4	**SA76**	94	NA	NA
5	H	4-$(O_2N)C_6H_4$	**SA77**	85	NA	NA
6	H	2-Naphthyl	**SA78**	77	NA	NA
7	CH_3	C_6H_5	**SA135**	88	NA	NA
8	CH_3	4-$(CH_3)C_6H_4$	**SA134**	89	NA	NA
9	CH_3	4-$(CH_3O)C_6H_4$	**SA133**	92	NA	NA
10	CH_3	4-BrC_6H_4	**SA136**	93	NA	NA
11	CH_3	4-$(O_2N)C_6H_4$	**SA137**	88	NA	NA
12	CH_3	2-Naphthyl	**SA138**	75	NA	NA
13	OCH_3	C_6H_5	**SA132**	91	NA	NA
14	OCH_3	4-$(CH_3)C_6H_4$	**SA131**	93	NA	NA
15	OCH_3	4-BrC_6H_4r	**SA130**	89	NA	NA
16	OCH_3	4-$(O_2N)C_6H_4$	**SA128**	87	NA	NA

Table 8 (*continued*)

Entry	R_1	R_2	Compound	Yield (%)	MIC_{80} *C. albicans* (μg/mL)	MIC_{80} *C. glabrata* (μg/mL)
17	OCH_3	2-Naphthyl	**SA129**	74	NA	NA
18	Cl	C_6H_5	**SA88**	93	NA	NA
19	Cl	4-$(CH_3)C_6H_4$	**SA86**	95	NA	NA
20	Cl	4-$(CH_3O)C_6H_4$	**SA87**	93	NA	NA
21	Cl	4-BrC_6H_4	**SA89**	91	NA	NA
22	Cl	4-$(O_2N)C_6H_4$	**SA90**	88	NA	NA
23	Cl	2-Naphthyl	**SA91**	83	NA	NA
24	Br	C_6H_5	**SA101**	93	NA	NA
25	Br	4-$(CH_3)C_6H_4$	**SA100**	91	NA	NA
26	Br	4-$(CH_3O)C_6H_4$	**SA99**	92	NA	NA
27	Br	4-BrC_6H_4	**SA102**	90	NA	NA
28	Br	4-$(O_2N)C_6H_4$	**SA103**	88	NA	NA
29	Br	2-Naphthyl	**SA104**	78	NA	NA
30	NO_2	C_6H_5	**SA119**	88	NA	NA
31	NO_2	4-$(CH_3)C_6H_4$	**SA120**	89	NA	NA
32	NO_2	4-$(CH_3O)C_6H_4$	**SA121**	83	NA	NA
33	NO_2	4-BrC_6H_4	**SA118**	86	NA	NA
34	NO_2	4-$(O_2N)C_6H_4$	**SA117**	79	NA	NA
35	NO_2	2-Naphthyl	**SA116**	72	NA	NA

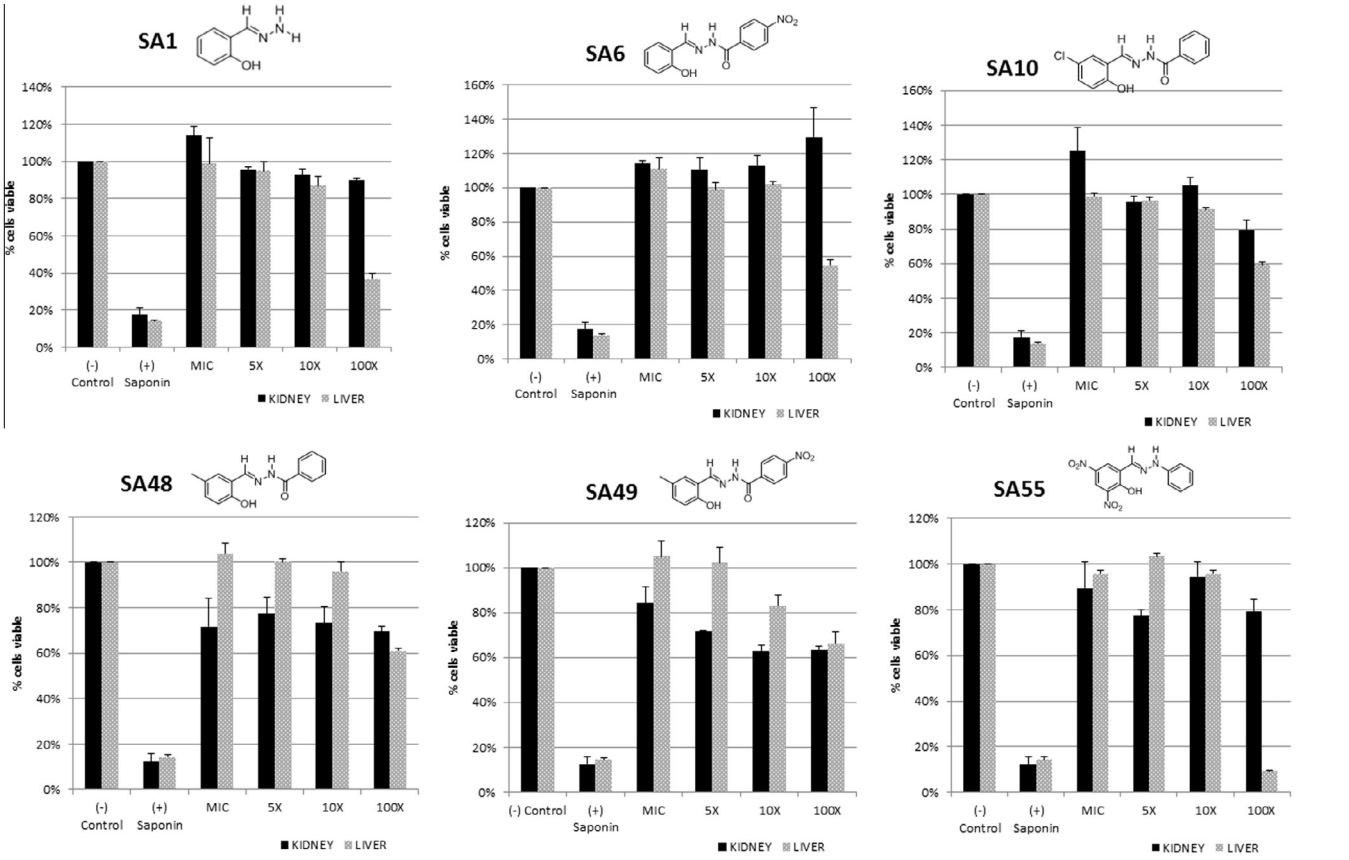


Figure 1. Evaluation of mammalian cell toxicity of select active compounds. Cytotoxicity was determined using non-cancerous Vero cells (kidney-african green monkey) and liver cells (Hep-2G) in accordance with Promega CellTiter 96 Non-RadioactivCell Proliferation Assay (cat. # G4000). Compounds were diluted 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 h at 37 °C and 5% CO_2. Tetrazolium dye solution was added to each well and allowed to incubate for 1–4 h. Stabilization/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. 0.1% saponin was used as a control to measure cell death. Wells containing 1% DMSO and no drug were used as a positive 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.

benzohydrazide 3-H), 8.15 (2H, d, J = 8.4 Hz, benzohydrazide 2-H), 7.14 (1H, d, J = 2.4 Hz, salicylic 6-H), 6.90 (1H, d of d, J_1 = 8.8 Hz, J_2 = 2.4 Hz, salicylic 4-H), 6.84 (1H, d, J = 8.8 Hz, salicylic 2-H) and 3.71 (3H, s, OCH_3) ppm. ^{13}C NMR (DMSO-d_6) δ 152.8, 152.2, 150.0, 148.9, 139.3, 129.9, 124.3, 119.6, 119.3, 118.0, 112.5, and 65.1 ppm.

3.6. Typical procedure for preparation sulfonohydrazones. Preparation Method VI (see Scheme 4)

Preparation of *N*′-(2-hydroxybenzylidene)naphthalene-2-sulfonohydrazide (**SA78**). A methanol (20 ml) solution of salicylaldehyde (122 mg; 1 mmol) and naphthalene-2-sulfonohydrazide

(222 mg; 1 mmol) was refluxed for 2 h. Solvent was evaporated at reduced pressure to an oily residue. This residue was mixed with ether (~10 ml) and sonicated at 5 °C for 5 min. Ether solution was decanted and mixed with hexane (~50 ml). The resulting slightly milky mixture was left at room temperature for 1 h. The formed white powdery product was separated by filtration, washed with hexane (3 × 5 ml) and dried on air to give pure product (250 mg; 77%). ^{1}H NMR (DMSO-d_6) δ 11.61 (1H, s, OH), 10.15 (1H, s, NH), 8.55 (1H, s, naphthalene 1-H), 8.19 (1H+1H, d+s, naphthalene 4-H+CH=N), 8.12 (1H, d, J = 8.8 Hz, naphthalene 3-H), 8.00 (1H, d, J = 8.00 Hz, naphthalene 8-H), 7.85 (1H, d of d, J_1 = 8.8 Hz, J_2 = 1.6 Hz, naphthalene 5-H), 7.66 (2H, m, naphthalene 6,7-H), 7.44 (1H, d of d, J_1 = 8.0 Hz, J_2 = 1.6 Hz salicylic 6-H), 7.17 (1H, d of t, J_1 = 8.0 Hz, J_2 = 1.6 Hz, salicylic 4-H), 6.80 (1H, d, J = 7.6 Hz, salicylic 3-H), and 6.77 (1H, t, J = 7.2 Hz, salicylic 5-H) ppm. ^{13}C NMR (DMSO-d_6) δ 157.2, 146.8, 136.5, 135.1, 132.4, 132.2, 130.1, 130.0, 129.8, 129.1, 128.6, 128.4, 127.9, 123.1, 120.1, 119.7, and 116.8 ppm.

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Supplementary data

Supplementary data (spectroscopic characterization, including electronic images of NMR data, literature data and CAS numbers and additional cytotoxicity screens are provided for all new compounds as well as compounds with biological activity) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.07.022.

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