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SAR Studies on Aromatic Acylhydrazone-Based Inhibitors of Fungal Sphingolipid Synthesis as Next-Generation Antifungal Agents

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Abstract

Recently, the fungal sphingolipid glucosylceramide (GlcCer) synthesis has emerged as a highly promising new target for drug discovery of next-generation antifungal agents, and we found two aromatic acylhydrazones as effective inhibitors of GlcCer synthesis based on HTP screening. In the present work, we have designed libraries of new aromatic acylhydrazones, evaluated their antifungal activities (MIC_{80} and time-kill profile) against *C. neoformans* and performed an extensive SAR study, which led to the identification of five promising lead compounds, exhibiting excellent fungicidal activities with very large selectivity index. Moreover, two compounds demonstrated broad spectrum antifungal activity against six other clinically relevant fungal strains. These five lead compounds were examined for their synergism/cooperativity with five clinical drugs against seven fungal strains and very encouraging results were obtained, e.g., the combination of all five lead compounds with voriconazole exhibited either synergistic or additive effect to all seven fungal strains.

Graphical Abstract

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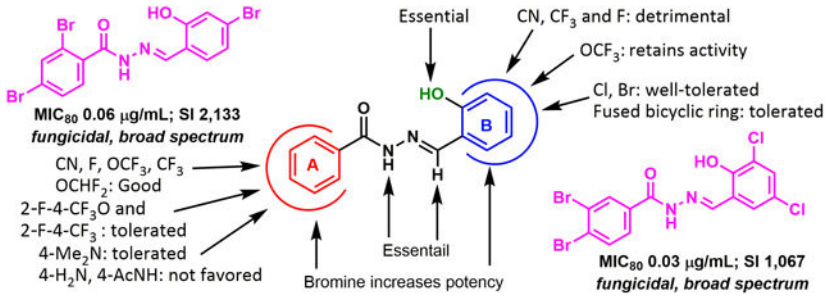
ASSOCIATED CONTENTS

Supporting Information.

^{1}H and ^{13}C spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

All other authors have no conflict of interest.



INTRODUCTION

Invasive fungal infections (IFIs), such as cryptococcosis, aspergillosis and candidiasis, are a serious threat to human health, as IFIs are associated with a large number of deaths which is similar in number to that of tuberculosis or malaria.[1] Recent statistics suggest that more than 150 million people suffer from serious fungal infections and it is estimated that annually around 1.5–2 million deaths occur as a result of these invasive fungal infections.[2, 3] IFIs are highly prevalent among individuals with low immunity such as HIV positive patients, organ transplant and cancer patients receiving immunosuppressants, as well as pediatric and geriatric patients.[4–8] *Cryptococcus neoformans* (*C. neoformans*) that causes meningoencephalitis is responsible for 600,000 deaths per year, accounting for 15% AIDS-related deaths globally.[9, 10] *Candida* species can cause invasive candidiasis that includes blood-derived and deep-tissue infections in hospitalized individuals who are treated for various conditions.[11] *Candida* species are also a major concern for immunocompromised patients.[12, 13] Candidemia caused by *Candida* species is associated with poor prognosis and contributes to ~30–60% mortality rate.[14] *Aspergillus* species was recently estimated to cause ca, 250,000 cases of invasive aspergillosis.[2]

Current treatment options for IFIs consist of three major classes of drugs which include azoles (e.g. fluconazole), polyenes (e.g. amphotericin B) and echinocandins (e.g. caspofungin).[15] These drugs are associated with serious side effects such as nephrotoxicity, narrow spectrum of activity and drug resistance.[16–19] Amphotericin B, the last resort anti-fungal agent, is associated with adverse drug-drug interactions with anticancer agents and azoles.[20, 21] In spite of all those drawbacks, the same three classes of drugs have been used to date, because no newer and more efficacious anti-fungal drugs have been approved by FDA for some time. Hence, there is a dire need for new, efficacious antifungal drugs that can overcome drug resistance with novel mechanism of action.

In this context, the fungal sphingolipid glucosylceramide (GlcCer) synthesis has emerged as a highly promising new target for the development of next-generation antifungal agents.[22–24] GlcCer is essential for the cell division of pathogenic fungi such as *C. neoformans, Candida albicans* (*C. albicans*) and *Aspergillus fumigatus* (*A. funigatus*), and responsible for their virulence.[23–27] It has been shown that fungal cells lacking GlcCer cannot replicate in neutral or alkaline environments.[23, 24, 27] This finding clearly indicates the importance of GlcCer for virulence in alveolar spaces, cerebrospinal fluid or bloodstream of the host wherein the pH is neutral or alkaline, and thus makes GlcCer a promising target for drug discovery.[23, 24] As

shown in Figure 1A, aromatic acylhydrazone **1**, bearing a salicylaldehyde-hydrazone moiety, selectively inhibited the synthesis of fungal GlcCer in a dose-dependent manner without affecting the synthesis of mammalian GlcCer.[23, 24] When the efficacy of **1** and **2** was evaluated *in vivo*, using mice infected with *C. neoformans*, both compounds substantially improved the survival of mice, compared to the untreated ones (Figure 1B).[22] Moreover, **2** exhibited better antifungal activity than fluconazole which is a clinically used drug to treat fungal infections.

Furthermore, **2** was effective against *A. fumigatus in vivo*, and more efficacious than the clinically used antifungal drug, voriconazole, with very high "selectivity index" (SI = LD_{50}/MIC_{80}) (>500).[22] Although aromatic acylhydrazones are known to be pan assay interference compounds (PAINS), our initial hits possessed promising antifungal activity not only *in vitro*, but also *in vivo*.[22–24, 28] Moreover, the fact that these aromatic acylhydrazones, bearing a salicylaldehyde-hydrazone moiety, were also highly fungus-selective (*vide supra*), gave us confidence to further explore this class of compounds as next-generation antifungal agents.[29] We describe here the library synthesis, biological evaluations and SAR study of new aromatic acylhydrazones, bearing a salicylaldehyde-hydrazone moiety, against *C. neoformans* and six other pathogenic fungi. Our study has led to the identification of five highly potent and selective lead compounds, which have been further examined for their synergy/cooperativity with five antifungal drugs currently used in clinic against seven pathogenic fungal strains.

RESULTS AND DISCUSSION

Library synthesis.

For the synthesis of initial library of aromatic acylhydrazones **5.0**~**5.7**, commercially available benzoyl chlorides or benzoic acids (**1.0**~**1.3**) were converted to their methyl esters (**2.0**~**2.3**), which were reacted with excess hydrazine monohydrate under reflux to give the corresponding hydrazides **3.0**~**3.3**. Hydrazides **3**, thus obtained, were condensed with different salicylaldehydes **4a**-**g** in the presence of an arenesulfonic acid resin as a catalyst in DMSO. Excess unreacted aldehyde **4** was removed from the reaction mixture by treating with an aminomethylated resin. The reaction mixture was then filtered to afford the corresponding aromatic acylhydrazones **5.0**~**5.4** (Scheme 1, Table 1).

The same protocol was used for the synthesis of other aromatic acylhydrazones. In some cases (**2.4**~**2.7**, Scheme 1; **2.19**~**2.23**, Scheme 3; **2.24**~**2.26**, Scheme 5; **2.27**, Scheme 6), the methyl or ethyl esters of substituted benzoic acids were commercially available. A variety of hydrazides **3** were condensed with different salicylaldehydes **4** to give the corresponding acylhydrazones **5** (57 compounds) (Schemes 2~5). For the synthesis of **5.21** (Scheme 4), *p*-fluoromethylbenzoic acid was condensed with Boc-hydrazine in the presence of EDC.HCl and DMAP to give Boc-protected hydrazide **3.21**. Then, the Boc group was removed by TFA, followed by the condensation with salicylaldehyde **4** in the same pot to give **5.21**.

An oxadiazole mimic of acylhydrazone, **6a**, was synthesized by treating **1** with hypervalent iodine ($PhI(OCOCF_3)_2$ (Scheme 7a). Another diazole mimic of acylhydrazone, **6b**, was synthesized through diazole formation from 3,5-dibromo-1-benzyloxycinnamaldehyde (**4f.2**)

and tosylhydrazine, followed by deprotection, 4-bromobenzoylation and debenzylation (Scheme 7b). Cinnamaldehyde **4f.2** was prepared from 3,5-dibromosalicylaldehyde (**4f**) in two steps (Scheme 7b). *N*-Methylacylhydrazone **5.28** was synthesized through the condensation of 3,5-dibromosalicylaldehyde (**4f**) with *N*-methylhydrazide **3.29**, which prepared by reacting 4-bromobenzoyl chloride (**1.29**) with *N*-methylhydrazine at −78 °C (Scheme 7c). C-Methylacylhydrazones **5.2i**~**5.3h** were synthesized by condensing hydrazides **3.2** and **3.3** with 2-acetylphenols **4.4a** and **4.4b** (Scheme 7d). Bis-acylhydrazones **5.29** and **5.30** were synthesized through the condensation of salicylaldehydes with isophthaloyl bishydrazide (**3.28**), which was prepared from dimethyl isophthalate (**2.28**) with hydrazine monohydrate (Scheme 7e).

Evaluation of antifungal activities against *C. neoformans*.

All aromatic acylhydrazones **5** and their mimics **6** thus synthesized were evaluated for their antifungal activity against *C. neoformans*. The antifungal activities of the compounds are indicated by MIC_{80} values. Compounds that displayed MIC_{80} ≤1 μg/mL were further examined for their fungicidal activities by a time-kill assay *in vitro*, indicated by K100 values. Results are summarized in Tables 1~6.

Activities of diarylacylhydrazones 5.0~5.7 in the initial library.—Nineteen out of the 20 compounds shown in Table 1 are found to be fungicidal in the time-kill assay *in vitro*, except for compound **5.7**, which is fungistatic. It is worthy of note that compounds **5.2d**, **5.2e** and **5.6a** are highly potent and selective to fungus with a selectivity index (SI) (*vide supra*) of ≥500 based on their LD_{50} values for HepG2 and A549.

Modifications of ring A: Introduction of 2,3-, 3,4- and 3,5-diboromophenyl groups.—Since the majority of compounds with high potency from the initial library was bearing either a 2,4- or 2,5-dibromophenyl group as the ring A, other dibromobenzoylhydrazones bearing a 2,3-, 3,4- or 3,5-diboromophenyl group as ring A were synthesized (Scheme 2) and examined for their activities against *C. neoformans* (Table 2). Compounds **5.8** and **5.8a** were not only highly potent (MIC_{80} 0.03–0.06 μg/mL), but also showed very low toxicity in mammalian cell lines (SI >1,000). Most of acylhydazones bearing 2,3-dibromophenyl group as ring A (**5.10**, **5.10b** and **5.10c**) were not potent (MIC_{80} >16 μg/mL) against *C. neoformans* except **5.10a** (Table 2)

Modifications of ring A: Introduction of bioisosteres of bromine and fluorine-containing groups.—Since the vast majority of active compounds contained one or more bromine atoms on ring A, bromine appears to have a favorable size and polarity as substituent(s) in ring A. However, it is rather rare to have many bromines in a pharmaceutical drug candidate. Thus, we examined if bromine could be replaced with its bioisosters such as CN and CF_3 groups (Scheme 3), and found that indeed 4-cyanobenzoyl- and 4-trifluoromethylbenzoylhydrazones (**5.11** and **5.13**) exhibited good MIC_{80} values (Table 3). Accordingly, other acylhydrazones containing F, OCF_3 or $OCHF_2$ group, as well as 2-F-4-OCF_3 or 2-F-4-CF_3 groups in ring A, were synthesized (Schemes 3 and 4) and their biological activities examined. As Table 3 shows, all compounds, except **5.22** and **5.23c**, in this series exhibited ≤1 μg/mL MIC_{80} values against *C. neoformans*. Compounds **5.11**,

5.14a, **5.15**, **5.15b**, **5.17**, **5.18a** and **5.20** possess good selectivity index (SI >100). Except for compound **5.19**, all compounds with ≤1μg/mL MIC_{80} values are fungicidal.

Modifications of ring A: Introduction of 4-aminobenzoyl groups.—Several benzoylhydrazones bearing an amino-, *N,N*-dimethylamino or *N*-acetamido group were synthesized and examined for their biological activities (Scheme 5, Table 4). As Table 4 shows, the introduction of a 4-aminobenzoyl group as ring A reduced the potency (**5.24**, MIC_{80} 4 μg/mL) and that of a 4-acetamidobenzoyl group was detrimental to the potency (**5.26**, MIC_{80} 16 μg/mL). Compounds with a 4- *N,N*-dimethylaminobenzoyl group exhibited mixed results, i.e., **5.25** did not show appreciable potency, while **5.25a**, **5.25b** and **5.25c** exhibited good potency (MIC_{80} 0.25–0.5) with modest SI values. However, **5.25a** and **5.25b** were fungistatic. Compound **5.25c**, bearing a 2-hydroxynaphthyl group as ring B, was found to be fungicidal with a good time-kill profile.

Modifications of ring B. Introduction of bioisosteres of bromine and fluorine-containing groups.—In a manner similar to that performed on ring A, a bromine substituent on ring B was replaced with a CN, CF_3, F or OCF_3 group and examined for their activity against *C. neoformans* (Scheme 6, Table 5). As Table 5 shows, **5.2f**, **5.2h**, **5.4a** and **5.4b** exhibited good potency (MIC_{80} ≤1 μg/mL) and were fungicidal in the time-kill assay. Compound **5.2g** and **5.27a** were weakly active (MIC_{80} 8 and 4 μg/mL, respectively), while compound **5.3f** did not show appreciable activity (MIC_{80} >16 μg/mL).

Modifications of the central acylhydrazone moiety.—In order to explore structural variations, as well as examine the effects of conformational rigidification on antifungal activity, heterocyclic mimics of aromatic acylhydrazones, **6a** and **6b**, were synthesized (Scheme 7a and 7b) and their activity against *C. neoformans* was examined. Rather unexpectedly, these two mimics did not show appreciable activity (MIC_{80} >16 μg/mL, Table 6). The results indicate that the acylhydrazone structure appears to be essential for antifungal activity. It is noteworthy that *N*-methylated (**5.28**) and C-methylated (**5.2i**, **5.2j**, **5.3g** and **5.3h**) acylhydrazones also did not exhibit appreciable activity (MIC_{80} >16 μg/mL, Table 6). The results clearly indicate that the NH and CH groups in the acylhydrazone moiety are essential for their antifungal activity, which is also consistent with the lack of appreciable activity in the heterocyclic mimics, **6a** and **6b**, wherein both NH and CH groups are eliminated by the introduction of oxadiazole and diazole moieties. Bis-acylhydrazones, **5.29** and **5.30** (Scheme 7e) did not exhibit appreciable activity (MIC_{80} >16 μg/mL, Table 6). Thus, the double units of the acylhydrazone moieties did not enhance binding, but deteriorated the affinity. The result may indicate that the binding site of aromatic acylhydrazones **5**, especially around the ring A, is rather compact.

Structure-activity relationship (SAR) analysis.

A library of acylhydrazones (192 compounds) was synthesized and screened for activity against *C. neoformans* H99. Out of the 192 compounds, 42 of them exhibited strong potency (MIC_{80} ≤1 μg/mL) against *C. neoformans* and 20 of these hit compounds have not been reported in literature (Scheme 1, Table 1). The SAR of these early hit compounds shown in Table 1 indicated that bromine was well-tolerated on both rings A and B. 2-Hydroxyl group

on ring B was found to be essential for antifungal activity. Other halogens such as chlorine and iodine were tolerated on ring B, as well. Most of aromatic acylhydrazones with 2-hydroxyl-5-methylphenyl as ring B showed lower selectivity indices. Compounds **5.1** and **5.1a** with 2,3-difluorophenyl as ring A displayed low SI, as well. Compounds, bearing 2,4-dibromophenyl as ring A and 2-hydroxy-4-bromophenyl (**5.2d**) and 2-hydroxy-5-bromophenyl (**5.2e**) as ring B were not only highly selective (SI >1,000), but also fungicidal at a very low concentration (0.06 μg/mL). Compounds **5.2c** and **5.3d** exhibited an excellent time-kill profile by completely eradicating *C. neoformans* in 6 h at very low concentrations, i.e., 0.5 and 0.25 μg/mL, respectively. Compound **5.4** needed a much longer incubation time (96 h) to show fungicidal activity. Even though compound **5.7**, bearing a quinolin-3-yl group as ring A, displayed a low MIC_{80} value (0.25 μg/mL) and fairly good SI (>100), it was found to be fungistatic in the time-kill assay.

Compounds, bearing 3,4-dibromophenyl as ring A and 2-hydroxy-3,5-dibromophenyl (**5.8**) or 2-hydroxy-3,5-dichlorophenyl (**5.8a**) as ring B exhibited excellent antifungal activity (MIC_{80} 0.06 and 0.03 μg/mL, respectively) as well as very high selectivity to the fungus (SI >1,000) (Table 2). However, compounds, bearing 2-hydroxy-5-bromophenyl (**5.8d**) or 2-hydroxy-4-bromophenyl (**5.8f**, **5.9c**, and **5.10**) as ring B, were less selective to the fungus. Compound **5.9**, bearing 3,5-dibromophenyl as ring A and 2-hydroxy-5-bromophenyl as ring B displayed good selectivity (SI 266.6), but was found to be fungistatic. Compounds, bearing 2-hydroxy-4-bromophenyl (**5.9c**) and 2-hydroxy-3,5-dibromophenyl (**5.9e**) as ring B did not show good selectivity and appreciable activity, respectively. In contrast, compound, bearing 2-hydroxy-5-chlorophenyl (**5.9d**) or 2-hydroxy-3,5-dichlorophenyl (**5.9b**) as ring B was fungicidal with fairly good selectivity (SI >100). Accordingly, for compounds with 3,5-dibromophenyl as ring A, chlorine substitutions on ring B were better tolerated than the bromine counterparts. Having 2,3-dibromophenyl as ring A appears to impair aromatic acylhydrazone's antifungal activity, since 3 out of 5 compounds in this series (**5.10**, **5.10b** and **5.10c**) (Table 2) did not exhibit appreciable activity (MIC_{80} >16) and even the remaining 2 compounds (**5.10a** and **5.10d**, Table 2) exhibited low selectivity (SI = 32 and 16, respectively).

Replacement of 4-bromophenyl with 4-CN-phenyl in ring A was well tolerated (**5.11**, Table 3), showing activity equivalent to that of **2**.[22] Replacement of bromine with CF_3 in ring A resulted in acylhydrazones that displayed good MIC_{80} values and time-kill activities, but with low to modest selectivity. Replacement of 3- or 4-bromophenyl with 3- or 4-CHF_2O-phenyl was relatively well-tolerated. Use of 3-CF_3O-phenyl as ring A significantly reduced the antifungal activity (**5.22**, Table 3). Compounds with 2-F, 4-OCF_3 or 2-F, 4-CF_3 substitutions on the phenyl group as ring A and 2-hydroxy-3,5-dibromophenyl as ring B possessed better selectivity than that of the counterparts bearing 2-hydroxy-5-bromophenyl as ring B (Table 3). Compounds with a 3-fluorophenyl as ring A were either fungistatic or not selective. However, compound bearing a 4-fluorophenyl as ring A exhibited good antifungal activity, good selectivity and excellent time-kill activity at a very low concentration (**5.20**, Table 3). Replacement of 4-fluorophenyl with 4-FCH_2-phenyl resulted in drastic reduction of selectivity (**5.21**, Table 3).

The use of 4-amino- and 4-acetamidophenyl as ring A was found to be detrimental to antifungal activity (Table 4). Compounds with 4-dimethylaminophenyl as ring A were either fungistatic or lacking appreciable activity, except for a compound bearing 2-hydroxynaphthyl as ring B (**5.25c**, Table 4).

Ring B was found to be more sensitive to the replacement of bromine with its bioisosteres and fluorine-containing groups, as compared to ring A (Table 5). The replacement of bromine at the 5-poisiton of the phenyl group in ring B with CN, CF_3 or F drastically affected either the antifungal activity or selectivity, whereas replacing bromine with OCF_3 on ring B was well-tolerated.

As described above, tricyclic analogs of **1** or **2**, bearing either an oxadiazole or diazole moiety as the third ring, did not show appreciable antifungal activity (Table 6). Also, acylhydrazone derivatives with *N*-methylation of the hydrazone's NH moiety or *C*-methylation of the hydrazone's methylidene CH moiety did not exhibit appreciable activity, either (Table 6). Furthermore, the bis-acylhydrazone skeleton was detrimental to the antifungal activity (Table 6).

Based on the findings and analysis described above, a summary of the SAR of aromatic acylhydrazones in this study is illustrated in Figure 2.

Five most potent and selective acylhydrazones.

Among the 83 new aromatic acylhydrazones evaluated, 5 of them exhibited excellent potency and time-kill profile against *C. neoformans* with very high selectivity indices (SI >500). These 5 lead compounds are summarized in Table 7.

Also, the time-kill activities of the 5 compounds are shown in Figure 3. All 5 compounds were fungicidal at very low concentrations. Compounds **5.2d** and **5.2e** were fungicidal at extremely low concentrations (0.06 μg/mL). Compound **5.8** even though displayed low MIC_{80} and good selectivity, it behaved slightly erratic in the time-kill assay (Figure 3D). Compound **5.8a** showed fungicidal activity at a slightly higher concentration (1 μg/mL), but was able to completely eradicate the fungi in a short span of 6 h.

Evaluation of antifungal activities against other pathogenic fungal strains.

These 5 lead compounds were evaluated for their antifungal activities against other pathogenic strains of fungi such as ***C. albicans, C. auris, C. krusei, C. krusei*** R (resistant to a majority of current antifungal agents), *C. parapsilosis* and *A. fumigatus*. As Table 8 shows, compounds **5.6a** and **5.8a** exhibited broad spectrum antifungal activity. It should be noted that **5.6a** and **5.8a** were active against *C. auris*, an emerging pathogen that is resistant to all currently available antifungal agents.[30] Compound **5.2d** was only modestly active against *C. parapsilosis*, but did not show appreciable activity to other fungi. Compound **5.2e** showed modest activity against *C. krusei* and *C. parapsilosis*, while **5.8** exhibited moderate activity against the resistant strain of *C. krusei* and weak activity against *C. auris* and *C. parapsilosis*. It is worthy of note that each fungal strain appears to be very sensitive to rather small variations in the substitution patterns in ring A and ring B.

Drug combination studies.

Since the next-generation antifungal agents are likely to be used in combination with antifungal drugs that are currently available in clinic, it is important to examine possible drug-drug interactions or synergy/antagonism between our lead compounds and clinically used antifungal drugs. Accordingly, the 5 lead compounds, **5.2d**, **5.2e**, **5.6a**, **5.8** and **5.8a** (Tables 7 and 8) were examined for their potential synergism/antagonism with 5 clinical drugs, i.e., fluconazole (Flu), voriconazole (Vori), itraconazole (Itra), caspofungin (Caspo); amphotericin B (AB) against 7 fungal strains, i.e., *C. neoformans*, *C. albicans*, fluconazole-resistant *C. krusei* (Ck Flu R), *C. krusei* 6258 (Ck6258), *C. auris*, *C. parapsilosis* and *A. fumigatus*. Results are shown in Tables 9,10,11, 12, and 13, in which each Table summarizes the activity of each of 5 lead compounds against 7 fungal strains when combined with clinically used drugs. The level of cooperativity was estimated based on the fractional inhibitory concentration index (FICI). The FICI is defined as ($MIC_{combined}/MIC_{drugA}$) + ($MIC_{combined}/MIC_{drugB}$).[31] The level of cooperativity is categorized as follows: strongly synergistic: FICI <0.5; synergistic: FICI <1; autonomous: FICI = 1; additive: FICI 1 ~ 4; antagonistic: FICI >4.[31, 32]

All 5 lead compounds showed synergistic effect (i.e., FICI <1) with these clinical drugs against *C. neoformans*, while they were not tested in combination with caspofungin against *C. neoformans*, since caspofungin is known to be inactive against this fungal strain.[33] In all other fungal strains, drug combinations with amphotericin B were not studied since Amphotericin B is the first line treatment only for cryptococcosis clinically.[34] For *C. albicans*, **5.2d**, **5.8** and **5.8a** showed very to fairly strong synergistic effects (FICI = 0.077~0.625) with only exception of **5.8a**/Caspo combination, which was autonomous (FICI = 1). Compound **5.2e** exhibited strong synergistic effects with voriconazole, itraconazole and caspofungin, but the **5.2e**/Flu and **5.6**/Flu combinations were additive (FICI = 2). For fluconazole-resistant *C. krusei* (Ck Flu R), **5.6a** demonstrated exceptionally strong synergism with all 4 clinical drugs (FICI = 0.00016~0.018), especially with fluconazole, restoring and enhancing the drug sensitivity (Table 11). Compound **5.8** also exhibited strong synergism with all 4 drugs (FICI = 0.121~0.528) and **5.8a** showed synergistic and additive effects. For *C. krusei* 6258 (Ck6258), **5.8** and **5.8a** exhibited strong synergism (FICI <0.5) in most combinations, except for two combinations that were autonomous (FICI = 1). Compounds **5.6a** showed strong synergism with 2 clinical drugs, but became additive with fluconazole and caspofungin, depending on fungal strains. Other two compounds, **5.2d** and **5.2e** did not show synergistic effects in all combinations. For *C. auris*, **5.8a** and **5.8** exhibited strongly synergistic and autonomous effects in all drug combinations, while **5.6a** showed strong synergism with 3 drugs, but the combination with caspofungin was additive. For *C. parapsilosis*, only **5.8a** exhibited strong synergistic and additive effects with two drugs each. All other drug combinations resulted in mixed results, although the **5.2d**/Vori combination showed extremely strong synergism (FICI = 0.076). Finally, for *A. fumigatus*, combinations with caspofungin in addition to amphotericin B were not studied because caspofungin is not active against this fungal strain. For the combinations with remaining three drugs, only **5.2d**/Vori, **5.2d**/Itra and **5.6a**/Vori showed synergistic effects, i.e., all other combinations were additive.

CONCLUSIONS

The SAR study on the 83 new aromatic acylhydrazones, designed, synthesized and their antifungal activities examined against *C. neoformans*, resulted in several critical findings: (i) 2-Hydroxyl group in the phenyl or naphthyl moiety as ring B is essential for good antifungal activity; (ii) Bromine substituent in both ring A and ring B is found to have significantly positive effect on the antifungal activity, as well as selectivity index (SI); (iii) Bromine isosteres, such as CN and CF3, are well-tolerated in ring A, but mostly detrimental in ring B to potency and/or SI; (iv) Other fluorine-based bromine surrogates, F, CF_3O, CHF_2O are well-tolerated in ring A, while F was detrimental in ring B, especially to SI, but CF_3O was well-tolerated in ring B; (v) Ring B is much more sensitive to the replacement of bromine, compared to ring A; (vi) Free NH and CH in the hydrazine moiety are found to be essential to antifungal activity.

Our SAR study has led to the identification of 5 lead compounds, **5.2d**, **5.2e**, **5.6a**, **5.8** and **5.8a**, that exhibit excellent antifungal activity against *C. neoformans* with very low toxicity on mammalian cells, resulting in a very high SI. These 5 compounds were examined for their activities against a panel of 7 pathogenic fungal strains, i.e., *C. neoformans*, *C. albicans*, fluconazole-resistant *C. krusei*, *C. krusei* 6258, *C. auris*, *C. parapsilosis* and *A. fumigatus*. Then, **5.6a** and **5.8a** demonstrated broad spectrum antifungal activity against all 7 fungal strains. Finally, the 5 lead compounds were examined for their synergism/cooperativity with 5 clinical drugs, i.e., fluconazole, voriconazole, itraconazole, caspofungin and amphotericin B against the 7 fungal strains. Notable findings are (i) the combination of all 5 lead compounds with voriconazole exhibited either synergistic or additive effect to all 7 fungal strains and no antagonism was observed and (ii) the combinations of **5.2d**, **5.6a** and **5.8a** with itraconazole showed a very similar profile. These are noteworthy findings for drug discovery and development of the next-generation antifungal agents. Further studies on *in vivo* efficacy, PK/PD, ADME profiles of these lead compounds are actively ongoing in these laboratories.

EXPERIMENTAL SECTION

General Methods.

Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker Ascend 700 spectrometer operating at 700 MHz for ^{1}H and 175 MHz for ^{13}C, a Bruker 500 Advance spectrometer operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C,respectively, or a Bruker 400 Nanobay spectrometer operating at 400 MHz, 100 MHz, and 376 MHz for ^{1}H, ^{13}C, and ^{19}F, respectively or a Bruker 300 Nanobay spectrometer operating at 300 MHz of ^{1}H. Chemical shifts were referenced to the residual proton and carbon-13 peaks of solvents used for ^{1}H and ^{13}C NMRs, respectively (^{1}H: $CDCl_3$, δ7.26; ^{13}C: $CDCl_3$, δ77.23; ^{1}H: DMSO-d_6, δ 2.50; ^{13}C: DMSO-d_6, δ 39.51). Signals are listed in ppm, and multiplicity identified as s ¼ singlet, br ¼ broad, d ¼ doublet, dd ¼ doublet of doublets, t ¼ triplet, q ¼ quartet, m ¼ multiplet; J-coupling constants in Hz, and integration. High resolution mass spectrometry (HRMS) analysis was carried out on an Agilent LC-UV-TOF mass spectrometer at the Institute of Chemical Biology and Drug Discovery, Stony Brook. Purity of the synthesized

compounds was determined by Shimadzu LC-2010A HT series HPLC assembly. Three analytical conditions were used and noted as a part of the characterization data for synthesized compounds. HPLC (A): Kinetex PFP 2.6 μM, 100 × 2.1 mm, 100 Å column, acetonitrile and water, flow rate of 0.2 mL/min, t = 0–30 min, gradient of 40–95% acetonitrile. HPLC (B): Kinetex PFP 2.6 μM, 100 × 2.1 mm, 100 Å column, acetonitrile and water, flow rate of 0.2 mL/min, t = 0–30 min, gradient of 50–95% acetonitrile. HPLC (C): Kinetex PFP 2.6 μM, 100 × 2.1 mm, 100 A column, acetonitrile and water, flow rate of 0.2 mL/min, t = 0–30 min, gradient of 55–95% acetonitrile. Measurements were made at 220 and 254 nm.

Materials.

All air- and moisture-insensitive reactions were carried out under an ambient atmosphere, magnetically stirred, and monitored by thin layer chromatography (TLC) using Agilent Technologies TLC plates pre-coated with 250 μm thickness silica gel 60 F254 plates and visualized by fluorescence quenching under UV light. Flash chromatography was performed on SiliaFlash® Silica Gel 40–63 μm 60 Å particle size using a forced flow of eluent at 0.3–0.5 bar pressure. All air- and moisture-sensitive manipulations were performed using oven-dried glassware using the standard Schlenk and glovebox techniques under nitrogen. Diethyl ether and THF were distilled from deep purple sodium benzopheone ketyl. Dichloromethane, chloroform and acetonitrile were dried over calcium hydride and distilled. Dichloromethane was degassed via three freeze-pump-thaw cycles. All other chemicals were used as received. All deuterated solvents were purchased from Cambridge Isotope Laboratories. 3-Bromobenzohydrazide (**3.0**),[35] 4-Bromobenzohydrazide (**3.4**),[36] 2-Hydroxy-5-bromobenzohydrazide (**3.5**),[37] 2-Hydroxy-3,5-dibromobenzohydrazide (**3.6**),[37] 3-Quinolinecarbohydrazide (**3.7**),[38] 3,5-Dibromobenzohydrazide (**3.9**),[39] 4-Cyanobenzohydrazide (**3.11**),[40] 4-Trifluoromethylbenzohydrazide (**3.13**),[41] 4-Fluorobenzohydrazide (**3.20**),[42] 4-Aminobenzohydrazide (**3.24**),[43] 4-Acetamidobenzohydrazide (**3.26**),[44] 2-Methylbenzohydrazide (**3.27**),[45] 2-Benzyloxy-3,5-dibromobenzaldehyde (**4.1**),[46] were synthesized by the literature methods.

Synthesis and characterization of benzohydrazides (3): 2,3-Difluorobenzohydrazide (3.1)

To a solution of 2,3-Difluromehtylbenzoate in methanol hydrazine monohydrate (15 eq.) was added. The reaction mixture was refluxed overnight. After the completion of reaction, the reaction mixture was concentrated in a rotary evaporator under pressure, followed by the addition of ice cold water (30 mL). This resulted in the precipitation of the product, which was filtered and dried to yield the product as a white solid (67% yield); mp 139–141 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.56 (s, 2 H), 7.24 – 7.28 (m, 1 H), 7.32 – 7.35 (m, 1 H), 7.50 – 7.56 (m, 1 H), 9.65 (s, 1 H); ^{13}C NMR (100 MHz DMSO-d_6) δ 119.0, 119.2, 124.9, 124.9, 124.97, 125.04, 125.4, 125.5, 145.7, 145.9, 148.2, 148.36, 148.4, 148.5, 150.8, 151.0, 162.15, 162.17; HRMS (TOF) *m/z* calcd for $C_7H_6F_2N_2OH^+$: 173.0521, found: 173.0524 (Δ= −2.06 ppm).

All other benzohydrazides were prepared in the same manner. Characterization data for new benzohydrazides are shown below.

2,4-Dibromobenzohydrazide (3.2).

White solid (83 % yield); mp 176–177 °C; ^{1}H NMR (500 MHz, DMSO- d_6) δ 4.48 (s, 2 H), 7.29 (d, 1 H, J = 8.1 Hz), 7.63 d, 1 H, J = 8.1 Hz), 7.91 (s, 1 H), 9.57 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 120.6, 123.0, 130.55, 130.63, 134.7, 137.0, 165.7; HRMS (TOF) calcd for $C_7H_6Br_2N_2O_2H^+$: 292.89197, found 292.89284 (Δ = −2.98 ppm).

2,5-Dibromobenzohydrazide (3.5).

White solid (91 % yield); mp 186 – 188 °C; ^{1}H NMR (300 MHz, DMSO- d_6) δ 4.49 (s, 2 H), 7.28 (d, 1 H, J = 8.2 Hz), 7.63 (d, 1 H, J = 8.2 Hz), 7.92 (s, 1 H), 9.58 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 118.6, 120.4, 131.6, 133.7, 134.7, 139.6, 165.0; HRMS (TOF) calcd for $C_7H_6Br_2N_2OH^+$ 292.8920, found 292.8922 (Δ = −0.8 ppm).

3,4-Dibromobenzohydrazide (3.8).

White solid (91% yield); mp 160–162 °C; ^{1}H NMR (500 MHz, DMSO- d_6) δ 4.57 (s, 2 H), 7.72 (dd, 1 H, J = 8.3, 2.0 Hz), 7.84 (d, 1 H, J = 8.3 Hz), 8.13 (d, 1 H, J = 2.0 Hz), 9.94 (s, 1 H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 124.0, 126.9, 127.7, 131.9, 133.8, 134.1, 163.5; HRMS (TOF) *m/z* calcd for $C_7H_6Br_2N_2OH^+$: 292.89197, found 292.89211 (Δ = −0.49 ppm).

2,3-Dibromobenzohydrazide (3.10).

White solid (77% yield); mp 219–221 °C; ^{1}H NMR (400 MHz, DMSO- d_6) δ 4.30 (s, 2 H), 7.29 – 7.36 (m, 2 H), 7.80 (dd, 1 H, J = 9.5, 1.8 Hz), 9.57 (s, 1 H); ^{13}C NMR (100 MHz, DMSO- d6) δ 121.8, 125.2, 127.8, 129.2, 134.2, 140.7, 166.2, HRMS (TOF) *m/z* calcd for $C_7H_6Br_2N_2OH^+$: 292.8919, found: 292.8924(Δ= −1.57 ppm).

2-Trifluoromethylbenzohydrazide (3.12).

White solid (70% yield); mp 123–125 °C; ^{1}H NMR (500 MHz DMSO- d_6) δ 4.47 (s, 2 H), 7.46 (d, 1 H, J = 7.5 Hz), 7.62 – 7.65 (m, 1 H), 7.68 – 7.71 (m, 1 H), 7.77 (d, 1 H, J = 6.8 Hz), 9.59 (s, 1 H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 122.6, 124.8, 125.9, 126.16, 126.22, 126.26, 126.4, 128.9, 129.8, 132.4, 135.1, 166.4; HRMS (TOF) *m/z* calcd for $C_8H_7F_3N_2OH^+$: 205.0583, found: 205.0589 (Δ = −2.86 ppm).

3-Difluoromethoxybenzohydrazide (3.14).

White solid (89% yield); mp 98–99 °C; ^{1}H NMR (300 MHz DMSO- d_6) δ 4.61 (s, 2 H, 100%), 7.03 (s, 1 H, 25%), 7.27 – 7.32 (m, 2 H, 100%, 50%), 7.47 – 7.52 (m, 2 H, 100%, 25%), 7.58 (s, 1 H, 100%), 7.67 (d, 1 H, 100%, J = 7.5 Hz), 9.87 (s, 1 H, 100%); ^{13}C NMR (125 MHz, DMSO- d_6) δ 114.3, 116.3, 117.2, 118.4, 121.3, 123.6, 130.2, 135.2, 150.9, 164.7; HRMS (TOF) *m/z* calcd for $C_8H_8F_2N_2O_2H^+$: 203.0626, found: 203.0636 (Δ = −5.05 ppm).

4-Difluoromethoxybenzohydrazide (3.15).

White solid (85 % yield); mp 108–110 °C; ^{1}H NMR (400 MHz DMSO- d_6) δ 4.50 (s, 2 H), 7.14 (s, 1 H, 25%), 7.23 (d, 2 H, J = 8.8 Hz), 7.33 (s, 1 H, 50%), 7.51 (s, 1 H, 25%), 7.89 (d, 2 H, J = 8.8 Hz), 9.78 (s, 1 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 113.5, 116.1, 117.9,

118.6, 129.0, 129.9, 152.93, 152.96, 153.0, 164.9; HRMS (TOF) *m/z* calcd for $C_8H_8F_2N_2O_2H^+$: 203.0626, found: 203.0632 (Δ = −2.82 ppm).

4-Trifluoromethoxybenzohydrazide (3.16).

White solid (79% yield); mp 112 – 113 °C; ^{1}H NMR (500 MHz DMSO- d_6) δ 4.53 (s, 2 H), 7.43 (d, 2 H, J = 8.6 Hz), 7.93 (d, 2 H, J = 8.8 Hz), 9.87 (s, 1 H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 118.9, 120.6, 121.0, 129.2, 132.4, 150.1, 164.6; HRMS (TOF) *m/z* calcd for $C_8H_7F_3N_2O_2H^+$: 221.0534, found: 221.0540 (Δ = −3.54 ppm).

2-Fluoro-4-trifluoromethoxybenzohydrazide (3.17).

White solid (77% yield); mp 93–95 °C; ^{1}H NMR (400 MHz DMSO- d_6) δ 4.19 (s, 2 H), 7.0 (d, 1 H, J = 10.9 Hz), 7.13 (d, 1 H, J = 8.8 Hz), 7.93 (d, 1 H, J = 10 Hz), 8.15 (t, 1 H, J = 8.6 Hz); ^{13}C NMR (100 MHz, DMSO- d_6) δ 108.8, 109.0, 116.5, 117.0, 117.9, 118.1, 119.1, 121.6, 133.56, 133.60, 152.3, 152.5, 159.5, 161.9, 163.5, 163.6; HRMS (TOF) *m/z* calcd for $C_8H_6F_4N_2O_2H^+$: 239.0438, found: 239.0440 (Δ = −0.97 ppm).

2-Fluoro-4-trifluoromethylbenzohydrazide (3.18).

White solid (79% yield); mp 108–110 °C; ^{1}H NMR (400 MHz DMSO- d_6) δ 4.64 (s, 2 H), 7.66 (d, 1 H, J = 8.0 Hz), 7.75 – 7.81 (m, 2 H), 9.74 (s, 1 H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 113.59, 113.63, 113.85, 113.88, 121.4, 121.7, 124.4, 127.3, 127.5, 131.20, 131.23, 131.85, 131.93, 132.2, 132.3, 157.5, 160.0, 162.1; HRMS (TOF) *m/z* calcd for $C_8H_6F_4N_2OH^+$: 223.0489, found: 223.0493 (Δ = −1.83 ppm).

3-Fluorobenzohydrazide (3.19).

White solid (81% yield); mp 135 – 136 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 4.54 (s, 2H), 7.36 (m, 1H), 7.50 (m, 1H), 7.61 (m, 1H), 7.68 (m, 1H), 9.88 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 113.7, 113.8, 117.9, 118.0, 123.10, 123.11, 130.5, 130.6, 135.60, 135.64, 161.2, 162.6, 164.4; HRMS (TOF) calcd for $C_7H_7FN_2OH^+$: 155.0615, found 155.0617 (Δ = −1.0 ppm).

3-Trifluoromethoxybenzohydrazide (3.22).

White solid (89 % yield); mp 92 – 93 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 4.57 (s, 1H), 7.53 (br d, J = 8.1 Hz, 1H), 7.60 (t, J = 8.1 Hz, 1H), 7.77 (s, 1H), 7.86 (d, J = 8.1 Hz, 1H), 9.97 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 119.3, 119.5, 120.8, 123.6, 126.0, 130.6, 135.5, 148.3, 164.1; HRMS (TOF) calcd for $C_8H_7F_3N_2O_2H^+$: 221.0532, found 221.0537 (Δ = −2.0 ppm).

3-Trifluoromethylbenzohydrazide (3.23).

White solid (83 % yield); mp 109 – 110 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 4.64 (s, 1H), 7.71 (t, J = 7.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 7.8 Hz, 1H), 8.15 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 123.2, 123.56, 123.58, 124.7, 127.7, 129.1, 129.2, 129.7, 131.0, 134.2, 164.2; HRMS (TOF) calcd for $C_8H_7F_3N_2OH^+$ 205.0583, found 205.0586 (Δ = −1.1 ppm).

4-Bromo-*N*-methylbenzohydrazide (3.29).

White solid (83 % yield); mp 115 – 116 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.20 (s, 3 H), 4.62 (s, 2 H), 7.34 (s, 2 H), 7.54 (d, 2 H, *J* = 8.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.7, 124.6, 129.3, 130.2, 131.8, 134.0, 169.2; HRMS (TOF) *m/z* calcd for $C_8H_9BrN_2OH^+$: 228.9971, found: 228.9969 (Δ = 0.61 ppm).

tert-Butyl 2-(4-(fluoromethyl)benzoyl)hydrazine-1-carboxylate (3.21).

To a solution of 4-Fluoromethylbenzoic acid (0.65 mmol) in dichloromethane (5 mL) was added *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (1.1 eq.), 4-Dimethylaminopyridine (1.1 eq.) and *tert*- Butyl carbazate (1.1 eq.). The reaction mixture was stirred at room temperature overnight. After completion, the reaction mixture was washed with brine, water and extracted with dichloromethane. The organic layer was collected, dried over magnesium sulfate and concentrated using rotary evaporator. Recrystallization of the crude product using dichloromethane: hexanes (2:8) resulted in pure product as white crystals (86% yield); mp 143 – 144 °C; ^{1}H NMR (500 MHz DMSO- d_6) δ 1.4 (s, 9 H), 5.43 (s, 1 H), 5.53 (s, 1 H), 7.50 (d, 2 H, 8.1 Hz), 7.88 (d, 2 H, *J* = 7.8 Hz), 8.91 (s, 1 H), 10.22 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.1, 79.2, 82.9, 84.2, 127.2, 127.3, 132.6, 139.7, 139.9, 155.4, 165.6; HRMS (TOF) *m/z* calcd for $C_{13}H_{17}FN_2O_3H^+$: 269.1296, found: 269.1293 (Δ = 1.02 ppm).

Synthesis and characterization of acylhydrazones (5): 3-Bromo-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.0).

To a solution of 3-Bromobenzohydrazide (0.26 mmol) and 4-Bromosalicylaldehyde (1.1 eq.) in methanol (3 mL), 2 drops of glacial acetic acid were added. The reaction mixture was stirred at room temperature overnight. Addition of water to the reaction mixture resulted in precipitation of the product, which was filtered, washed with water and dried to give pure product as a white solid (98 % yield); mp 223–225 °C; ^{1}H NMR (500 MHz, DMSO- d_6) δ 7.11 (dd, 1 H, *J* = 8.3, 1.9 Hz), 7.14 (d, 1 H, *J* = 1.9 Hz), 7.51 (t, 1 H, *J* = 7.9 Hz), 7.58 (d, 1 H, *J* = 8.3 Hz), 7.81 (dd, 1 H, *J* = 8.0, 1.0 Hz), 7.93 (d, 1 H, *J* = 7.9 Hz), 8.11 (t, 1 H, *J* = 1.7 Hz), 8.62 (s, 1 H), 11.39 (s, 1 H), 12.17 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 118.6, 119.1, 121.8, 122.5, 124.0, 126.9, 130.2, 130.8, 134.7, 135.0, 146.7, 158.0, 161.3; HRMS (TOF) *m/z* calcd for $C_{14}H_{10}Br_2N_2O_2H^+$: 396.9181, found: 396.9188 (Δ = −1.77 ppm). HPLC (A): *t* = 12.56 min, purity >98%.

All other acylhydrazones were prepared in the same manner. Characterization data for new acylhydrazones are shown below.

2,3-Difluoro-*N*'-(5-chloro-2-hydroxybenzylidene)benzohydrazide (5.1).

Brown solid (56% yield); mp 171–173 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 6.85 (d, 1 H, 25%, *J* = 8.6 HZ), 6.94 (d, 1 H, 75%, *J* = 8.8HZ), 7.20 (d, 1 H, 15%, *J* = 2.6 Hz), 7.21 (s, 1 H, 40%), 7.30 – 7.37 (m, 4 H, 85%, 61%, 25%, 20%), 7.48 – 7.52 (m, 1 H, 75%), 7.55 – 7.64 (m, 1 H, 100%), 7.67 (d, 1 H, 80%, *J* = 2.6 Hz), 8.29 (s, 1 H, 25%), 8.51 (s, 1 H, 75%), 10.25 (s, 1 H, 25%), 11.01 (s, 1 H, 75%), 12.22 (s, 1 H, 100%); ^{13}C NMR (100 MHz DMSO-d_6) δ 118.1, 118.2, 118.9, 119.1, 119.9, 120.1, 121.4, 123.0, 123.1, 124.6, 124.8, 124.9, 125.3, 125.4, 127.2, 130.7, 131.1, 141.1, 146.1, 155.3, 156.0, 159.16, 159.18; ^{19}F

NMR (376 MHz DMSO-d_6) δ −137.95 (d, 1 F, J = 23 Hz), −138.73 (d, 1 F, J = 23 Hz), −139.07 (d, 1 F, J = 23 Hz), −139.92 (d, 1 F, J = 23 Hz); HRMS (TOF) m/z calcd for $C_{14}H_9OF_2N_2O_2H^+$: 311.0393, found: 311.0387 (Δ= 1.75 ppm). HPLC (B): t = 5.33 min, purity >95%.

2,3-Difluoro-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.2).

White solid (87% yield); mp 219–221 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 6.97 (d, 1 H, 25%, J = 8.3 Hz), 7.03 (s, 1 H, 25%), 7.10 (d, 1 H, 75%, 8.3 Hz), 7.13 (s, 1 H, 75%), 7.19 (d, 1 H, 25%, J = 8.4 Hz), 7.30 – 7.37 (m, 3 H, 75%, 25%, 22%), 7.48 – 7.51 (m, 1 H, 75%), 7.58 (d, 1 H, 100%, J = 8.3 Hz), 7.60 – 7.66 (m, 1 H, 75%), 8.30 (s, 1 H, 26%), 8.52 (s, 1 H, 74%), 10.41 (s, 1 H, 25%), 11.22 (s, 1 H, 75%), 12.16 (s, 1 H, 100%); ^{13}C NMR (100 MHz DMSO-d_6) δ 118.6, 118.8, 118.9, 119.1, 119.9, 120.0, 122.5, 123.7, 124.2, 124.6, 124.1, 124.9, 125.27, 125.31, 127.9, 129.9, 141.8, 146.7, 148.4, 157.2, 158.0, 159.1; ^{19}F NMR (376 MHz DMSO-d_6) δ −137.95(d, 1 F, J = 23 Hz), −138.74 (d, 1 F, J = 23 Hz), −139.00 (d, 1 F, 23 Hz), −139.94 (d, 1 F, J = 23 Hz); HRMS (TOF) m/z calcd for $C_{14}H_9BrF_2N_2O_2H^+$: 354.9888, found: 354.9887 (Δ= 0.26 ppm). HPLC (A): t = 7.35 min, purity >95%.

2,4-Dibromo-*N*'-(2-hydroxy-5-methylbenzylidene)benzohydrazide (5.3).

White solid (84% yield); mp 181 – 183 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (s, 1H, 33%), 2.24 (s, 1H, 67%), 6.70 (d, 1H, 33%, J = 8.4 Hz), 6.83 (d, 1H, 67%, J = 8.4 Hz), 7.02 (dd, 1H, 33%, J = 8.4 Hz, J = 2.0 Hz), 7.07 (d, 1H, 33%, J = 1.6 Hz), 7.11 (dd, 1H, 67%, J = 8.4 Hz, J = 2.0 Hz), 7.39 (d, 1H, 67%, J = 1.8 Hz), 7.41 (d, 1H, 33%, J = 8.2 Hz), 7.54 (d, 1H, 67%, 8.2 Hz), 7.72 (m, 1H), 8.00 (d, 1H, 33%, J = 1.8 Hz), 8.02 (d, 1H, 67%, J = 1.8 Hz), 8.25 (s, 1H, 33%), 8.44 (s, 1H, 67%), 9.61 (s, 1H, 33%), 10.69 (s, 1H, 67%), 12.10 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.9, 116.0, 116.2, 118.30, 118.34, 119.7, 120.7, 122.7, 123.7, 127.9, 128.0, 128.4, 128.9, 130.0, 130.8, 130.9, 132.0, 132.4, 133.5, 134.2, 134.8, 136.2, 137.1, 144.7, 148.1, 154.5, 155.2, 162.3, 168.0; HRMS (TOF) calcd for $C_{15}H_{12}Br_2N_2O_2H^+$: 410.9338, found 410.9338 (Δ = −1.0 ppm). HPLC (B): t = 4.0 min, purity >95%.

2,4-Dibromo-*N*'-(5-chloro-2-hydroxybenzylidene)benzohydrazide (5.2a).

White solid (72% yield); mp 187 – 190 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.94 (d, 1H, 75%, J = 8.8 Hz), 6.98 (d, 1H, 25%, J = 8.8 Hz), 7.30 (dd, 1H, 75%, J = 8.8 Hz, J = 2.7 Hz), 7.40 (dd, 1H, 25%, J = 8.8 Hz, J = 2.7 Hz), 7.66 (d, 1H, 78%, J = 2.7 Hz), 7.75 (d, 1H, 22%, J = 2.7 Hz),7.71 (m, 2H, 83%), 8.00 (d, 1H, 35%, J = 1.8 Hz), 8.02 (d, 1H, 65%, 1.8 Hz), 8.27 (s, 1H, 35%), 8.47 (s, 1H, 65%),10.20 (br s, 1H, 35%), 11.01 (br s, 1H, 65%), 12.19 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 118.0, 118.2, 119.8, 120.67, 120.68, 121.0, 122.7, 123.0, 123.1, 123.8, 126.0, 127.1, 130.2, 130.69, 130.71, 130.8, 130.9, 131.1, 134.2, 134.9, 136.1, 137.2, 141.6, 145.7, 155.3, 155.9, 162.5, 168.3; HRMS (TOF) calcd for $C_{14}H_9Br_2ClN_2O_2H^+$: 430.8792, found 430.8794 (Δ = −0.5 ppm). HPLC (B): t_1 = 3.2, t_2 = 4.6 min, purity >95%.

2,4-Dibromo-*N*'-((2-hydroxynaphthalen-1-yl)methylene)benzohydrazide (5.2b).

White solid (68% yield); mp > 220 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.12 (d, 1H, 40%, J = 8.9 Hz), 7.28 (m, 2H, 70%), 7.41 (t, 1H, 60%, J = 7.3 Hz), 7.47 (d, 1H, 40%, J = 8.2 Hz), 7.60 (m, 2H, 60%), 7.80 (m, 3H, 60%), 8.06 (m, 1H), 8.14 (d, 1H, 40%, 8.5 Hz), 8.31 (d, 1H, 60%, 8.5 Hz), 8.95 (s, 1H, 40%), 9.28 (s, 1H, 60%), 10.66 (s, 1H, 40%), 12.11 (s, 1H, 40%), 12.24 (s, 1H, 60%), 12.36 (s, 1H, 60%); ^{13}C NMR (125 MHz, DMSO-d_6) δ 108.5, 109.6, 118.2, 118.8, 119.7, 120.8, 121.1, 122.5, 122.8, 123.4, 123.6, 123.9, 127.3, 127.88, 127.92, 128.0, 128.7, 129.0, 129.8, 130.95, 131.03, 131.5, 132.6, 133.1, 134.3, 135.0, 135.9, 137.7, 143.3, 147.2, 157.0, 158.1, 162.1, 168.1; HRMS (TOF) calcd for $C_{18}H_{13}Br_2N_2O_2H^+$: 446.9338, found 446.9344 (Δ = −1.3 ppm). HPLC (B): t_1 = 3.0, t_2 = 3.5 min, purity >96%.

2,4-Dibromo-*N*'-(3,5-dichloro-2-hydroxybenzylidene)benzohydrazide (5.2c).

White solid (68% yield); 197 – 199 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41 (d, 1H, 30%, J = 2.5 Hz), 7.45 (d, 1H, 30%, ***J*** = 8.2 Hz), 7.55 (d, 1H, 30%, J = 2.5 Hz), 7.58 (d, 1H, 70%, J = 8.2 Hz), 7.65 (d, 1H, 70%, 2.5 Hz), 7.69 (d, 1H, 70%, J = 2.5 Hz), 7.75 (m, 1H), 8.05 (d, 1H, J = 1.6 Hz), 8.27 (s, 1H, 30%), 8.44 (s, 1H, 70%), 10.34 (s, 1H, 30%), 12.12 (s, 1H, 70%), 12.45 (s, 1H, 30%), 12.55 (s, 1H, 70%); ^{13}C NMR (125 MHz, DMSO-d_6) δ 119.5, 120.6, 120.8, 121.5, 121.6, 121.7, 123.06, 123.10, 123.4, 124.1, 127.2, 128.3, 130.0, 130.3, 130.6, 130.8, 130.9, 131.0, 134.4, 134.9, 135.5, 136.6, 143.4, 147.3, 151.0, 152.1, 162.6, 168.2; HRMS (TOF) calcd for $C_{14}H_8Br_2Cl_2N_2O_2H^+$: 464.8402, found 464.8407 (Δ = −1.1 ppm). HPLC (B): t = 3.4 min, purity >96%.

2,4-Dibromo-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.2d).

White solid (83% yield); mp 187 – 188 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 6.99 (dd, 1H, 37%, J = 8.3 Hz, J = 1.8 Hz), 7.03 (d, 1H, 37%, J = 1.8 Hz), 7.12 (dd, 1H, 63%, J = 8.3 Hz, J = 1.8 Hz), 7.14 (d, 1H, 63%, J = 1.8 Hz), 7.18 (d, 1H, 37%, J = 8.4 Hz), 7.40 (d, 1H, 37%, J = 8.2 Hz), 7.54 (d, 1H, 63%, J = 8.2 Hz), 7.59 (d, 1H, 63%, J = 8.4 Hz), 7.69 (dd, 1H, 37%, J = 8.1 Hz, J = 1.8 Hz), 7.74 (dd, 1H, 63%, J = 8.1 Hz, J = 1.8 Hz), 7.98 (d, 1H, 37%, J = 1.8 Hz), 8.02 (d, 1H, 63%, J = 1.8 Hz), 8.28 (s, 1H, 37%), 8.46 (s, 1H, 63%), 10.36 (s, 1H, 37%), 11.20 (s, 1H, 63%), 12.14 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 118.6, 118.8, 118.97, 119.05, 119.8, 120.7, 122.6, 122.7, 123.7, 123.8, 124.2, 128.5, 129.9, 130.2, 130.7, 130.8, 130.9, 134.2, 134.9, 136.1, 137.2, 142.1, 146.3, 157.2, 157.9, 162.4, 168.2; HRMS (TOF) calcd for $C_{14}H_9Br_3N_2O_2H^+$: 474.8287, found 474.8286 (Δ = 0.2 ppm). HPLC (B): t = 3.8 min, purity >96%.

2,4-Dibromo-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.2e).

White solid (91% yield); mp : > 220 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.80 (d, 1H, 35%, J = 8.7 Hz), 6.90 (d, 1H, 65%, 8.7 Hz), 7.35 (m, 1H, 65%), 7.43 (m, 1H), 7.54 (d, 1H, 65%, J =8.2 Hz), 7.71 (dd, 1H, 35%, J = 8.2 Hz, J = 1.8 Hz), 7.73 (dd, 1H, 65%, J = 8.2 Hz, J = 1.8 Hz), 7.81 (d, 1H, 65%, J = 2.5 Hz), 8.26 (s, 1H, 35%), 8.46 (s, 1H, 65%), 10.22 (br s, 1H, 35%), 11.00 (br s, 1H, 65%), 12.18 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 110.51, 110.52, 118.5, 118.7, 119.8, 120.6, 121.3, 121.6, 122.7, 123.8, 129.0, 130.0, 130.2, 130.7, 130.8, 130.9, 133.5, 133.9, 134.1, 134.8, 136.1, 137.2, 141.5, 145.6, 155.7, 156.4, 162.5,

168.3; HRMS (TOF) calcd for $C_{14}H_9Br_3N_2O_2H^+$: 474.8287, found 474.8292 (Δ = −1.1 ppm). HPLC (A): *t* = 7.8 min, purity >98%.

2,5-Dibromo-*N*'-(2-hydroxy-5-methylbenzylidene)benzohydrazide (5.3).

White solid (71% yield); mp 190 – 192 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 2.14 (s, 3H, 33%), 2.24 (s, 3H, 67%), 6.70 (d, 1H, 33%, *J* = 8.3 Hz), 6.83 (d, 1H, 67%, *J* = 8.3 Hz), 7.02 (d, 1H, 33%, *J* = 8.4 Hz), 7.07 (s, 1H, 33%), 7.12 (d, 1H, 67%, *J* = 8.4 Hz), 7.40 (s, 1H, 67%), 7.60 (dd, 1H, 33%, *J* = 8.5 Hz, *J* = 2.2 Hz), 7.67 (m, 2H), 7.84 (d, 1H, 67%, *J* = 2.2 Hz), 8.25 (s, 1H, 33%), 8.44 (s, 1H, 67%), 9.60 (s, 1H, 33%), 10.67 (s, 1H, 67%), 12.13 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 20.0, 116.1, 116.3, 117.8, 118.3, 118.4, 118.8, 120.67, 120.73, 127.9, 128.0, 128.3, 128.8, 131.0, 131.9, 132.0, 132.4, 133.5, 134.2, 134.3, 134.9, 138.8, 139.9, 144.6, 148.1, 154.5, 155.2, 161.6, 167.2; HRMS (TOF) calcd for $C_{15}H_{12}Br_2N_2O_2H^+$: 410.9338, found 410.9330 (Δ = 1.9 ppm). HPLC (B): *t* = 4.8 min, purity >95%.

2,5-Dibromo-*N*'-(5-chloro-2-hydroxybenzylidene)benzohydrazide (5.3a).

White solid (72% yield); mp 185 – 187 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 6.85 (m, 1H, 35%), 6.96 (d, 1H, 65%, *J* = 8.8 Hz), 7.23 (m, 1H, 65%), 7.33 (dd, 1H, 65%, *J* = 8.8 Hz, *J* = 2.2 Hz), 7.64 (m, 2H, 68%), 7.69 (m, 2H, 83%), 7.84 (d, 1H, 65%, *J* = 1.5 Hz), 8.27 (s, 1H, 35%), 8.46 (s, 1H, 65%), 10.19 (br s, 1H, 35%), 10.99 (br s, 1H, 65%), 12.22 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 117.9, 118.0, 118.2, 118.7, 120.6, 120.69, 120.73, 121.1, 123.0, 123.1, 126.0, 127.0, 130.7, 131.09, 131.12, 131.9, 133.5, 134.2, 134.4, 134.9, 138.7, 139.8, 141.5, 145.8, 155.3, 156.0, 161.8, 167.5; HRMS (TOF) calcd for $C_{14}H_9Br_2ClN_2O_2H^+$: 430.8792, found 430.8789 (Δ = 0.7 ppm). HPLC (B): *t* = 4.2 min, purity >95%.

2,5-Dibromo-*N*'-((2-hydroxynaphthalen-1-yl)methylene)benzohydrazide (5.3b).

White solid (68% yield); mp > 220 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.13 (d, 1H, 40%, 8.9 Hz), 7.26 (d, 1H, 60%, 8.9 Hz), 7.30 (m, 2H, 40%), 7.42 (t, 1H, 60%, *J* = 7.4 Hz), 7.61 (t, 1H, 60%, *J* = 7.6 Hz), 7.66 (td, 1H, 60%, *J* = 10.4 Hz, *J* = 1.8 Hz), 7.69 (d, 1H, 40%, *J* = 1.8 Hz), 7.72 (t, 1H), 7.79 (m, 2H, 40%), 7.83 (d, 1H, 40%, *J* = 8.9 Hz), 7.90 (d, 1H, 60%, *J* = 8.1 Hz), 7.92 (d, 1H, 60%, *J* = 1.6 Hz), 7.96 (d, 1H, 60%, 8.9 Hz), 8.20 (d, 1H, 40%, *J* = 8.3 Hz), 8.33 (d, 1H, 60%, *J* = 8.6 Hz), 8.94 (s, 1H, 40%), 9.28 (s, 1H, 60%), 10.65 (s, 1H, 40%), 12.15 (s, 1H, 40%), 12.28 (s, 1H, 60%), 12.35 (s, 1H, 60%); ^{13}C NMR (175 MHz, DMSO-d_6) δ 108.5, 109.6, 117.7, 118.1, 118.76, 118.84, 120.78, 120.80, 121.1, 123.0, 123.3, 123.6, 127.4, 127.88, 127.94, 128.0, 128.6, 129.0, 130.7, 131.0, 131.5, 132.0, 132.6, 133.2, 133.4, 134.3, 134.5, 135.0, 138.5, 140.4, 143.3, 147.4, 157.0, 158.1, 161.3, 167.3; HRMS (TOF) calcd for $C_{18}H_{12}Br_2N_2O_2H^+$: 446.9338, found 446.9333 (Δ = 1.1 ppm). HPLC (B): *t* = 3.6 min, purity >97%.

2,5-Dibromo-*N*'-(3,5-dichloro-2-hydroxybenzylidene)benzohydrazide (5.3c).

White solid (49% yield); mp 196 – 198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (d, 1H, 28%, *J* = 2.5 Hz) 7.55 (d, 1H, 28%, *J* = 2.5 Hz), 7.64 (m, 1H), 7.69 (m, 3H, 72%), 7.75 (d, 1H, 28%, *J* = 2.5 Hz), 7.88 (d, 1H, 72%, *J* = 2.2 Hz), 8.27 (s, 1H, 28%), 8.44 (s, 1H, 72%), 10.33 (br s, 1H, 28%), 12.10 (br s, 1H, 72%), 12.48 (s, 1H, 28%), 12.58 (s, 1H, 72%); ^{13}C

NMR (100 MHz, DMSO-d_6) δ 118.7, 120.7, 120.8, 120.9, 121.6, 121.7, 121.8, 123.1, 127.2, 128.3, 130.6, 131.9, 134.4, 134.6, 134.9, 143.4, 147.4, 151.1, 152.2, 161.8; HRMS (TOF) calcd for $C_{14}H_8Br_2Cl_2N_2O_2H^+$: 464.8402, found 464.8394 (Δ = 1.7 ppm). HPLC (B): t = 4.5 min, purity >95%.

2,5-Dibromo-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.3d).

White solid (87% yield); mp 186 – 187 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.98 (dd, 1H, 33%, J = 8.4 Hz, J = 1.6 Hz), 7.03 (d, 1H, 33%, J = 1.7 Hz), 7.11 (dd, 1H, 67%, J = 8.4 Hz, J = 1.6 Hz), 7.14 (d, 1H, 67%, J = 1.7 Hz), 7.18 (d, 1H, 33%, J = 8.4 Hz), 7.59 (m, 1H), 7.66 (m, 2H), 7.83 (d, 1H, 67%, J = 2.3 Hz), 8.28 (s, 1H, 33%), 8.47 (s, 1H, 67%), 10.34 (s, 1H, 33%), 11.19 (s, 1H, 67%), 12.17 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 117.8, 118.6, 118.7, 118.8, 118.9, 119.0, 120.6, 120.7, 122.5, 123.7, 124.2, 128.5, 129.8, 131.0, 131.8, 133.5, 134.2, 134.4, 134.8, 138.7, 139.9, 142.3, 146.5, 157.2, 157.9, 161.6, 167.4; HRMS (TOF) calcd for $C_{14}H_9Br_3N_2O_2H^+$: 474.8287, found 474.8279 (Δ = 1.7 ppm). HPLC (B): t = 4.2 min, purity >95%.

2,5-Dibromo-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.3e).

White solid (87% yield); mp >220 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 6.80 (d, 1H, 35%, J = 8.7 Hz), 6.91 (d, 1H, 65%, J = 8.7 Hz), 7.34 (dd, 1H, 35%, J = 8.7 Hz, J = 2.3 Hz), 7.36 (d, 1H, 35%, J = 2.3 Hz), 7.44 (dd, 1H, 65%, J = 8.7 Hz, J = 2.3 Hz), 7.60 (dd, 1H, 35%, J = 8.7 Hz, J = 2.3 Hz), 7.66 (m, 3H, 55%), 7.70 (d, 1H, 35%, J = 2.0 Hz), 7.82 (d, 1H, 65%, J = 2.3 Hz), 7.84 (d, IH, 65%, J = 2.0 Hz), 8.26 (s, 1H, 35%), 8.46 (s, 1H, 65%), 10.20 (s, 1H, 35%), 10.99 (s, 1H, 65%), 12.22 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 110.56, 110.59, 117.9, 118.5, 118.68, 118.72, 120.6, 120.7, 121.3, 121.6, 129.0, 139.9, 131.1, 131.9, 133.50, 133.53, 133.9, 134.2, 134.4, 134.9, 138.7, 139.8, 141.5, 145.6, 155.7, 156.3, 161.8, 167.5; HRMS (TOF) calcd for $C_{14}H_9Br_3N_2O_2H^+$ 474.8287, found 474.8281 (Δ = 1.3 ppm). HPLC (B): t_1 = 3.9, t_2 = 4.9 min, purity >95%.

4-Bromo-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.4).

White solid (88 % yield); mp >230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.11 (d, 1 H, J = 8.3 Hz), 7.14 (s, 1 H), 7.57 (d, 1 H, J = 8.3 Hz), 7.76 (d, 2 H, J = 8.5 Hz), 7.88 (d, 2 H, J = 8.5 Hz), 8.62 (s, 1 H), 11.44 (s, 1 H), 12.17 (s, 1 H); ^{13}C NMR (100 MHz DMSO-d_6) δ 118.6, 119.1, 122.4, 124.0, 125.8, 129.7, 130.2, 131.6, 131.9, 146.7, 158.0, 161.9; HRMS (TOF) *m/z* calcd for $C_{14}H_{10}Br_2N_2OH^+$: 396.9181, found: 396.9188 (Δ = −1.56 ppm). HPLC (A): t = 9.69 min, purity >97%.

3-Bromo-6-hydroxy-*N*'-(2-hydroxy-5-methylbenzylidene)benzohydrazide (5.5).

Light yellow solid (89 % yield); mp >230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.25 (s, 3 H), 6.84 (d, 1 H, J = 8.2 Hz), 6.96 (d, 1 H, J = 8.8 Hz), 7.12 (d, 1 H, J = 7.8 Hz), 7.38 (s, 1 H), 7.59 (d, 1 H, J = 8.5 Hz), 8.03 (s, 1 H), 8.62 (s, 1 H), 10.82 (s, 1 H), 11.88 (s, 1 H), 12.01 (s, 1 H); ^{13}C NMR (100 MHz DMSO-d_6) δ 19.9, 110.0, 116.3, 118.0, 118.3, 119.6, 128.0, 129.0, 130.7, 132.5, 136.2, 149.0, 155.3, 158.0, 163.0; HRMS (TOF) *m/z* calcd for $C_{15}H_{13}BrN_2O_3H^+$: 349.0182, found: 349.0187 (Δ = −1.35 ppm). HPLC (B): t = 5.74 min, purity >95%.

3,5-Dibromo-2-hydroxy-*N*'-(5-methyl-2-hydroxyphenylmethylidene)benzohydrazide (5.6).

Yellow solid (88% Yield); mp 223 – 224 °C; ^{1}H NMR (700 MHz DMSO-d_6) δ 2.23 (s, 3 H), 6.83 (d, 1 H, J = 8.3 Hz), 7.12 (dd, 1 H, J = 8.3, 1.9 Hz), 7.43 (s, 1 H), 8.01 (d, 1 H, J = 2.2 Hz), 8.19, (d, 1 H, J = 2.2 Hz), 8.67 (s, 1 H), 10.63 (s, 1 H), 12.37 (s, 1 H), 13.09 (s, 1 H); ^{13}C NMR (175 MHz DMSO-d_6) δ 20.0, 109.8, 112.4, 116.3, 116.6, 118.4, 128.1, 128.5, 129.2, 132.8, 138.7, 149.5, 155.4, 156.8, 164.2; HRMS (TOF) *m/z* calcd for $C_{15}H_{12}Br_2N_2O_3H^+$: 426.9287, found: 426.9290 (Δ = −0.64 ppm). HPLC (C): t = 4.9 min, purity >96%.

3,5-Dibromo-2-hydroxy-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.6a).

Yellow solid (95% Yield); mp >230 °C; ^{1}H NMR (700 MHz DMSO-d_6) δ 7.10 (dd, 1 H, J = 8.3, 1.8 Hz), 7.12 (s, 1 H), 7.62 (d, 1 H, J = 8.3 Hz), 8.00 (d, 1 H, J = 2.2 Hz), 8.17 (d, 1 H, J = 2.2 Hz), 8.68 (s, 1 H), 11.17 (s, 1 H), 12.41 (s, 1 H); ^{13}C NMR (175 MHz DMSO-d_6) δ 109.8, 112.5, 116.6, 118.6, 119.1, 122.6, 124.6, 129.2, 129.5, 138.8, 147.8, 156.8, 158.1, 164.2; HRMS (TOF) *m/z* calcd for $C_{14}H_9Br_3N_2O_3H^+$: 490.8236, found: 490.8234 (Δ = 0.42 ppm). HPLC (A): t = 7.8 min, purity >95%.

***N*'-(3,5-Dibromo-2-hydroxybenzylidene)quinolinylhydrazide (5.7).**

Beige solid (99 % yield); mp >215 °C; ^{1}H NMR (700 MHz DMSO-d_6) δ 7.73 (t, 1 H, J = 7.9 Hz), 7.85 (dd, 2 H, J = 13.4, 2.4 Hz), 7.91 (t, 1 H, J = 7.7 Hz), 8.12 (d, 1 H, J = 8.5 Hz), 8.16 (d, 1 H, J = 7.8 Hz), 8.57 (s, 1 H), 8.95 (d, 1 H, J = 2.0 Hz), 9.34 (d, 1 H, J = 2.2 Hz), 12.63 (s, 1 H), 12.85 (s, 1 H); ^{13}C NMR (175 MHz DMSO-d_6) δ 110.5, 111.3, 121.0, 125.1, 126.4, 127.7, 128.9, 129.3, 131.8, 132.2, 135.8, 136.5, 147.5, 148.8, 153.7, 161.8; HRMS (TOF) *m/z* calcd for $C_{17}H_{11}Br_2N_3O_2H^+$: 447.9290, found: 447.9036 (Δ = −3.47 ppm). HPLC (A): t = 8.3 min, purity >95%.

3,4-Dibromo-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.8).

Yellow solid (78 % yield); mp >230 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.81 – 7.86 (m, 3 H), 7.95 (d, 1 H, 8.3 Hz), 8.27 (d, 1 H, J = 1.8 Hz), 8.50 (s, 1 H), 12.61 (d, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 110.5, 111.3, 120.9, 124.3, 128.4, 128.6, 132.1, 132.5, 132.9, 134.1, 135.8, 147.6, 153.6, 160.8; HRMS (TOF) m/z calcd for $C_{14}H_8Br_4N_2O_2H^+$: 552.7391, found: 552.7392 (Δ= 0.18 ppm). HPLC (A): t = 9.1 min, purity >98%.

3,4-Dibromo-*N*'-(3,5-dichloro-2-hydroxybenzylidene)benzohydrazide (5.8a).

Yellow solid (64 % yield); mp 212–214 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (d, 1 H, J = 2.3 Hz), 7.67 (d, 1 H, J = 2.4 Hz), 7.84 (dd, 1 H, J = 8.1, 1.5 Hz), 7.95 (d, 1 H), 8.27 (s, 1 H), 8.54 (s, 1 H), 12.31 (s, 1 H), 12.59 (s, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 120.8, 121.6, 123.0, 124.3, 128.36, 128.39, 128.6, 130.5, 132.5, 133.0, 134.1, 147.5, 152.2, 160.8; HRMS (TOF) *m/z* calcd for $C_{l4}H_8Br_2C_{l2}N_2O_2H^+$: 464.8404, found: 464.8402 (Δ= −0.32 ppm). HPLC (B): t = 4.1 min, purity >98%.

3,4-Dibromo-*N*'-(5-chloro-2-hydroxybenzylidene)benzohydrazide (5.8b).

Light yellow solid (35% yield); mp >230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.94 (d, 1 H, 75%, J = 8.8Hz), 6.98 (d, 1 H, 25%, J = 8.8 Hz), 7.30 (dd, 1 H, 75%, J = 8.8, 2.7 Hz),

7.40 (dd, 1 H, 25%, *J* = 8.8, 2.6 Hz), 7.66 (d, 1 H, 78%, *J* = 2.7 Hz), 7.75 (d, 1 H, 22%, *J* = 2.7 Hz), 7.84 (dd, 1 H, 80%, *J* = 8.3, 2.0 Hz), 7.93 (d, 1 H, 78%, *J* = 8.3 Hz), 8.28 (d, 1 H, 76%, *J* = 2.0 Hz), 8.60 (s, 1 H, 77%), 8.93 (s, 1 H, 23%), 11.10 (s, 1 H, 25%), 11.12 (s, 1 H, 75%), 12.24 (s, 1 H, 73%); ^{13}C NMR (125 MHz, DMSO-d_6) δ 118.2, 118.5, 119.9, 120.7, 123.0, 123.1, 124.2, 127.2, 128.0, 128.5, 128.6, 131.0, 132.4, 132.7, 133.6, 134.0, 146.1, 156.0, 157.2, 160.7, 160.9; HRMS (TOF) m/z calcd for $C_{l4}H_9Br_2ClN_2O_2H^+$: 430.8812, found: 430.8792 (Δ= −4.51 ppm). HPLC (B): *t* = 8.1 min, purity >95%.

3,4-Dibromo-*N*'-(2-hydroxy-1-naphthylidene)benzohydrazide (5.8c).

Yellow solid (66 % yield); mp >230 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.23 (d, 1 H, ***J*** = 8.9 Hz), 7.40 (t, 1 H, *J* = 7.2 Hz), 7.60 (t, 1 H, *J* = 7.3 Hz), 7.87 – 7.99 (m, 4 H), 8.27 – 8.32 (m, 2 H), 9.44 (s, 1 H), 12.30 (s, 1 H), 12.57 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 108.6, 118.9, 120.91, 123.6, 124.3, 127.86, 127.88, 128.1, 128.5, 129.0, 131.6, 132.4, 133.1, 133.5, 134.2, 147.6, 158.1, 160.4; HRMS (TOF) m/z calcd for $C_{18}H_{12}Br_2N_2O_2H^+$: 446.9340, found: 446.9338 (Δ= −0.46 ppm). HPLC (B): *t* = 4.2 min, purity >96%.

3,4-Dibromo-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.8d).

Light yellow solid (53 % yield); mp >230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.89 (d, 1 H, *J* = 8.8 Hz), 7.42 (dd, 1 H, *J* = 8.7, 2.5 Hz), 7.79 (d, 1 H, *J* = 2.5 Hz), 7.84 (dd, 1 H, *J* = 8.3, 2.0 Hz), 7.93 (d, 1 H, *J* = 8.4 Hz), 8.28 (d, 1 H, *J* = 1.9 Hz), 8.60 (s, 1 H), 11.13 (s, 1 H), 12.24 (s, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 110.5, 118.7, 121.3, 124.2, 128.0, 128.5, 130.0, 132.5, 133.6, 133.8, 134.0, 145.9, 156.4, 160.7; HRMS (TOF) *m/z* calcd for $C_{14}H_9Br_3N_2O_2H^+$: 474.8288, found: 474.8287 (Δ= −0.19 ppm). HPLC (B): *t* = 7.5 min, purity >95%.

3,4-Dibromo-*N*'-(2-hydroxy-5-methylbenzylidene)benzohydrazide (5.8e).

White solid (94 % yield); mp 214–216 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 2.23 (s, 3 H, 78%), 2.24 (s, 3 H, 22%), 6.82 (d, 1 H, 77%, *J* = 8.3 Hz), 6.86 (d, 1 H, 23%, *J* = 8.3 Hz), 7.09 (dd, 1 H, 77%, *J* = 8.3, 1.6 Hz), 7.18 (dd, 1 H, 23%, *J* = 8.3, 1.7 Hz), 7.36 (s, 1 H, 78%), 7.45 (s, 1 H, 22%), 7.83 (dd, 1 H, 78%, *J* = 8.3, 1.9 Hz), 7.93 (d, 1 H, 78%, *J* = 8.3 Hz), 8.27 (d, 1 H, 77%, *J* = 1.9 Hz), 8.58 (s, 1 H, 78%), 8.90 (s, 1 H, 22%), 10.85 (s, 1 H, 75%), 10.87 (s, 1 H, 25%), 12.15 (s, 1 H, 77%); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.9, 116.3, 116.4, 117.9, 118.4, 124.2, 127.90, 127.94, 128.1, 128.5, 129.0, 130.5, 132.3, 132.4, 133.7, 133.9, 134.0, 148.5, 155.3, 156.5, 160.5, 162.5; HRMS (TOF) *m/z* calcd for $C_{15}H_{12}Br_2N_2O_2H^+$: 410.9343, found: 410.9338 (Δ= −1.25 ppm). HPLC (A): *t* = 9.5 min, purity >97%.

3,4-Dibromo-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.8f).

Off-white solid (63 % yield); m.p. >230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.09 (dd, 1 H, *J* = 8.3, 1.8 Hz), 7.12 (d, 1 H, *J* = 1.8 Hz), 7.57 (d, 1 H, *J* = 8.4 Hz), 7.83 (dd, 1 H, *J* = 8.4, 2.0 Hz), 7.93 (d, 1 H, *J* = 8.4 Hz), 8.27 (d, 1 H, *J* = 2 Hz), 8.60 (s, 1 H), 11.33 (s, 1 H), 12.18 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 118.6, 119.0, 122.5, 124.1, 124.2, 128.0, 128.5, 130.0, 132.4, 133.6, 134.0, 146.8, 158.0, 160.6; HRMS (TOF) *m/z* calcd for

$C_{14}H_9Br_3N_2O_2H^+$: 474.8286, found: 474.8287 (Δ= 0.16 ppm). HPLC (A): *t* = 7.6 min, purity >95%.

3,5-Dibromo-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.9).

White solid (58 % yield); m.p. >230 °C; 6.90 (d, 1 H, *J* = 8.8 Hz), 7.43 (dd, 1 H, *J* = 8.8, 2.6 Hz), 7.82 (d, 1 H, *J* = 2.5 Hz), 8.11 (s, 3 H), 8.61 (s, 1 H), 11.10 (s, 1 H), 12.25 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.5, 118.7, 121.3, 122.7, 129.6, 130.0, 133.9, 136.4, 136.6, 146.0, 156.4, 160.1; HRMS (TOF) *m/z* calcd for $C_{14}H_9Br_3N_2O_2H^+$: 474.8288, found: 474.8287 (Δ= −0.32 ppm). HPLC (A): *t* = 15.1 min, purity >95%.

3,5-Dibromo-*N*'-(2-hydroxy-1-naphthylidene)benzohydrazide (5.9a).

Yellow solid (42 % yield); m.p. >230 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.24 (d, 1 H, *J* = 8.9 Hz), 7.42 (t, 1 H, *J* = 7.4 Hz), 7.62 (t, 1 H, *J* = 7.6 Hz), 7.90 (d, 1 H, *J* = 8 Hz), 7.95 (d, 1 H, *J* = 8.9 Hz), 8.14 (t, 1 H, *J* = 1.6 Hz), 8.16 (d, 2 H, *J* = 1.6 Hz), 8.32 (d, 1 H, *J* = 8.6 Hz), 9.44 (s, 1 H), 12.31 (s, 1 H), 12.50 (s, 1 H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 108.5, 118.8, 121.0, 122.9, 123.6, 127.86, 127.90, 129.0, 129.6, 131.6, 133.1, 136.3, 136.7, 147.8, 158.2, 159.7; HRMS (TOF) *m/z* calcd for $C_{18}H_{12}Br_2N_2O_2H^+$: 446.9340, found: 446.9338 (Δ= −0.34 ppm). HPLC (B): *t* = 8.1 min, purity >97%.

3,5-Dibromo-*N*'-(3,5-dichloro-2-hydroxybenzylidene)benzohydrazide (5.9b).

Tan solid (99% yield); mp >230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.63 (d, 1 H, *J* = 2.4 Hz), 7.69 (d, 1 H, *J* = 2.4 Hz), 8.11 (s, 2 H), 8.12 (d, 1 H, *J* = 1.4 Hz), 8.55 (s, 1 H), 12.24 (s, 1 H), 12.610 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 120.8, 121.6, 122.8, 123.1, 128.3, 129.7, 130.5, 135.8, 136.9, 147.7, 152.2, 160.2; HRMS (TOF) *m/z* calcd for $C_{14}H_8Br_2Cl_2N_2O_2H^+$: 464.8402, found: 464.8427 (Δ = −5.33 ppm). HPLC (A): *t* = 9.6 min, purity >95%.

3,5-Dibromo-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.9c).

White solid (84% yield); mp >230 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.15 (d, 1 H, *J* = 8.3 Hz), 7.14 (s, 1 H), 7.59 (d, 1 H, *J* = 8.3 Hz), 8.10 (s, 3 H), 8.62 (s, 1 H), 11.30 (s, 1 H), 12.20 (s, 1 H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 118.6, 119.1, 122.5, 122.8, 124.2, 129.6, 129.9, 136.5, 136.6, 146.8, 158.0, 160.0; HRMS (TOF) *m/z* calcd for $C_{14}H_9Br_3N_2O_2H^+$: 474.8289, found: 474.8287 (Δ= −0.42 ppm). HPLC (C): *t* = 5.2 min, purity >96%.

3,5-Dibromo-*N*'-(5-chloro-2-hydroxybenzylidene)benzohydrazide (5.9d).

White solid (92% yield); mp >230 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 6.95 (d, 1 H, *J* = 8.8Hz), 7.33 (dd, 1 H, *J* = 8.8, 2.7 Hz), 7.69 (s, 1 H), 8.11 (s, 3 H), 8.62 (s, 1 H), 11.09 (s, 1 H), 12.25 (s, 1 H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 118.3, 120.7, 122.8, 123.1, 127.1, 129.6, 131.1, 136.4, 136.6, 146.2, 156.0, 160.1; HRMS (TOF) *m/z* calcd for $C_{14}H_9Br_2ClN_2O_2H^+$: 430.8802, found: 430.8792 (Δ= −2.34ppm). HPLC (B): *t* = 5.3 min, purity >95%.

3,5-Dibromo-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.9e).

Yellow solid (69% yield); mp >230 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.84 (d, 2 H, J = 3.9 Hz), 8.11 (d, 2 H, J = 1.6 Hz), 8.13 (s, 1 H), 8.51 (s, 1 H), 12.49 (s, 1 H), 12.64 (s, 1 H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 110.5, 111.4, 120.9, 122.8, 129.7, 132.2, 135.8, 135.9, 136.9, 147.9, 153.7, 160.3; HRMS (TOF) *m/z* calcd for $C_{14}H_8Br_4N_2O_2H^+$: 552.7392, found: 552.7389 (Δ = 0.43 ppm). HPLC (A): t = 8.7 min, purity >95%.

3,5-Dibromo-*N*'-(2-hydroxy-5-methylbenzylidene)benzohydrazide (5.9f).

White solid (92% yield); mp >230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.25 (s, 3 H), 6.82 (d, 1 H, J = 8.2 Hz), 7.10 (dd, 1 H, J = 8.3, 1.8 Hz), 7.38 (s, 1 H), 8.09 (s, 3 H), 8.91 (s, 1 H), 10.78 (s, 1 H), 12.15 (s, 1 H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 19.9, 116.2, 118.4, 122.7, 127.9, 128.8, 129.5, 132.4, 136.5, 148.5, 155.3, 159.9; HRMS (TOF) *m/z* calcd for $C_{15}H_{12}Br_2N_2O_2H^+$: 410.9345, found: 410.9338 (Δ = −1.65 ppm). HPLC (A): t = 8.8 min, purity >99%.

2,3-Dibromo-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.10).

White solid (99% yield); mp >230 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.96 (dd, 1 H, 36%, J = 8.4, 1.7 Hz), 7.01 (d, 1 H, 35%, J = 1.8 Hz), 7.09 (dd, 1 H, 64%, J = 8.4, 1.7 Hz), 7.12 (s, 1 H, 70%), 7.14 (s, 1 H, 30%), 7.37 – 7.44 (m, 3 H, 65%, 35%, 35%), 7.54 (dd, 1 H, 65%, J = 7.6, 1.4 Hz), 7.58 (d, 1 H, 65%, J = 8.4 Hz), 7.81 – 7.84 (m, 1 H, 36%), 7.88 (dd, 1 H, 64%, J = 8.0, 1.4 Hz), 8.26 (s, 1 H, 35%), 8.44 (s, 1 H, 65%), 10.33 (s, 1 H, 30%), 11.19 (s, 1 H, 70%), 12.13 (s, 1 H, 100%); ^{13}C NMR (100 MHz, DMSO-d_6) δ 118.6, 118.8, 119.0, 120.9, 121.8, 122.5, 123.7, 124.2, 124.8, 125.4, 127.2, 128.0, 128.6, 129.3, 129.4, 129.8, 133.9, 134.8, 139.7, 140.5, 142.3, 146.4, 157.2, 157.9, 162.8, 168.4; HRMS (TOF) *m/z* calcd for $C_{14}H_9Br_3N_2O_2H^+$: 474.8286, found: 474.8285 (Δ = 0.26 ppm). HPLC (A): t = 11.7 min, purity >96%.

2,3-Dibromo-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.10a).

White solid (98% yield); m.p. > 230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 – 7.46 (m, 2 H, 30%, 100%), 7.56 – 7.58 (m, 2 H, 70%, 30%), 7.74 (s, 1 H, 25%), 7.82 (s, 1 H, 75%), 7.84 (s, 1 H, 70%), 7.88 (d, 1 H, 20%), 7.90 (dd, 1 H, 80%, J = 7.9, 1.4 Hz), 8.21 (s, 1 H, 25%), 8.38 (s, 1 H, 75%), 10.30 (s, 1 H, 25%), 12.32 (s, 1 H, 75%), 12.47 (s, 1 H, 35%), 12.60 (s, 1 H, 65%); ^{13}C NMR (175 MHz, DMSO-d_6) δ 110.6, 111.5, 120.8, 120.9, 121.5, 121.8, 125.2, 125.5, 127.2, 128.1, 129.5, 129.7, 131.3, 132.2, 134.5, 135.2, 135.7, 135.9, 139.2, 139.7, 144.0, 147.8, 152.6, 153.6, 163.1, 168.3; HRMS (TOF) *m/z* calcd for $C_{14}H_8Br_4N_2O_2H^+$: 552.7393, found: 552.7392 (Δ= −0.19 ppm). HPLC (A): t_1 = 10.3, t_2 = 13.1 min, purity >97%.

2,3-Dibromo-*N*'-(2-hydroxy-5-methylbenzylidene)benzohydrazide (5.10b).

White solid (41% yield); mp 202–203 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.12(s, 1 H, 33%), 2.23(s, 1 H, 66%), 6.67 (d, 1 H, 33%, J = 8.3 Hz), 6.82 (d, 1 H, 67%, J = 8.3 Hz), 7.0 (dd, 1 H, 34%, J = 8.4, 1.9 Hz), 7.03 (s, 1 H, 32%), 7.10 (dd, 1 H, 66%, J = 8.4, 1.9 Hz), 7.38 (s, 1 H, 68%), 7.40 – 7.44 (m, 2 H, 100%, 30%), 7.54 (dd, 1 H, 70%, J = 7.5, 1.5 Hz), 7.83 – 7.85 (m, 1 H, 34%), 7.88 (dd, 1 H, J = 8.0, 1.5 Hz), 8.22 (s, 1 H, 32%), 8.41 (s, 1 H,

68%), 9.54 (s, 1 H, 33%), 10.66 (s, 1 H, 67%), 12.10 (s, 1 H, 100%); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.9, 116.1, 116.3, 118.3, 118.4, 120.9, 121.8, 124.8, 125.4, 127.1, 127.9, 128.0, 128.1, 128.4, 128.9, 129.4, 129.5, 132.0, 132.4, 133.9, 134.8, 139.8, 140.5, 144.5, 148.1, 154.5, 155.2, 162.7, 168.2; HRMS (TOF) *m/z* calcd for $C_{15}H_{12}Br_2N_2O_2H^+$: 410.9338, found: 410.9346 (Δ = −1.97 ppm). HPLC (A): *t* = 10.5 min, purity >97%.

2,3-Dibromo-*N*'-(2-hydroxy-1-naphthylidene)benzohydrazide (5.10c).

Light yellow solid (67% yield); mp >230 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 7.10 (d, 1 H, 45%, *J* = 8.9 Hz), 7.22 – 7.30 (m, 2 H, 55%, 95%), 7.38 – 7.41 (m, 1 H, 63%), 7.45 – 7.48 (m, 2 H, 100%, 37%), 7.56 – 7.62 (m, 2 H, 100%, 10%), 7.75 – 7.80 (m, 1 H, 90%), 7.84 – 7.94 (m, 2 H, 100%, 100%), 8.15 (d, 1 H, 45%, *J* = 8.3 Hz), 8.30 (d, 1 H, 55%, *J* = 8.6 Hz), 8.91 (s, 1 H, 45%), 9.24 (s, 1 H, 55%), 10.58 (s, 1 H, 40%), 12.10 (s, 1 H, 52%), 12.32 (s, 1 H, 100%); ^{13}C NMR (125 MHz DMSO-d_6) δ 108.5, 109.7, 118.1, 118.7, 120.9, 121.1, 121.9, 123.0, 123.3, 123.6, 124.9, 125.5, 126.9, 127.3, 127.87, 127.91, 128.0, 128.1, 128.6, 129.0, 129.48, 129.51, 131.0, 131.5, 132.6, 133.1, 133.8, 134.9, 139.6, 141.0, 143.2, 147.2, 157.0, 158.1, 162.5, 168.4; HRMS (TOF) *m/z* calcd for $C_{18}H_{12}Br_2N_2O_2H^+$: 446.9338, found: 446.9350 (Δ = −2.79 ppm). HPLC (A): *t* = 9.5 min, purity >95%.

2,3-Dibromo-*N*'-(3,5-dichloro-2-hydroxybenzylidene)benzohydrazide (5.10d).

Product washed with water and ~1 mL ethyl acetate, filtered, and washed with DCM and hexanes. Tan solid (43% yield); mp 229–230 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 7.37 (d, 1 H, 30%, 2.6 Hz), 7.42 – 7.46 (m, 3 H, 30%, 70%, 30%), 7.53 (d, 1 H, 30%, *J* = 2.6 Hz), 7.57 (dd, 1 H, 70%, *J* = 7.6, 1.5 Hz), 7.64 (d, 1 H, *J* = 2.6 Hz), 7.68 (d, 1 H, 70%, *J* = 2.6 Hz), 7.89 (dd, 1 H, 40%, *J* = 6.7, 2.9 Hz), 7.91 (dd, 1 H, 60%, *J* = 8. 1.5 Hz), 8.25 (s, 1 H, 30%), 8.42 (s, 1 H, 70%), 10.27 (s, 1 H, 30%), 12.08 (s, 1 H, 70%), 12.45 (s, 1 H, 30%), 12.56 (s, 1 H, 70%); ^{13}C NMR (125 MHz, DMSO-d_6) δ 120.79, 120.84, 121.57, 121.66, 121.74, 121.8, 123.2, 123.4, 125.0, 125.5, 127.1, 127.3, 128.1, 128.3, 129.5, 129.6, 130.3, 130.6, 134.3, 135.1, 139.2, 139.9, 143.4, 147.4, 151.1, 152.2, 163.0, 168.4; HRMS (TOF) *m/z* calcd for $C_{14}H_8Br_2Cl_2N_2O_2H^+$: 464.8402, found: 464.8398 (Δ= 0.76 ppm). HPLC (A): *t* = 9.6 min, purity >95%.

4-Cyano-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.11).

White solid (47 % yield); mp >215 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 7.83 (s, 2 H), 8.05 (d, 2 H, *J* = 8.6 Hz), 8.09 (d, 2 H, *J* = 8.6 Hz), 8.53 (s, 1 H), 12.64 (s, 2 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 110.5, 111.3, 114.5, 118.2, 120.9, 128.6, 132.2, 132.7, 135.8, 136.2, 147.9, 153.7, 161.8; HRMS (TOF) *m/z* calcd for $C_{15}H_9Br_2N_3O_2H^+$: 421.9134, found: 421.9150 (Δ = −3.67 ppm). HPLC (A): *t* = 8.0 min, purity >99%.

2-Trifluoromethyl-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.12).

Beige solid (91 % yield); m.p. 98–100 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 6.75 (d, 1 H, 35%, *J* = 8.6 Hz), 6.89 (d, 1 H, 65%, *J* = 8.8 Hz), 7.27 – 7.30 (m, 2 H, 38%, 30%), 7.43 (dd, 1 H, 62%, *J* = 8.8, 2.6 Hz), 7.54 (d, 1 H, 35%, *J* = 7.6 Hz), 7.68 – 7.86 (m, 5 H, 100%, 100%, 85%, 70%, 65%), 8.22 (s, 1 H, 35%), 8.45 (s, 1 H, 65%), 10.12 (s, 1 H, 35%), 11.00 (s, 1 H, 65 %), 12.15 (s, 1 H, 35%), 12.22 (s, 1 H, 63%); ^{13}C NMR (125 MHz DMSO-d_6) δ

110.47, 110.54, 118.4, 118.7, 121.3, 121.6, 126.07, 126.11, 126.14, 126.3, 126.48, 126.52, 126.6, 128.1, 128.9, 129.0, 129.6, 130.0, 130.6,132.4, 132.6, 133.8, 134.0, 134.5, 140.9, 145.4, 155.6, 156.3, 163.0, 169.1;^{19}F NMR (376 MHz DMSO-D6) δ −57.85, −58.58; HRMS (TOF) *m/z* calcd for $C_{15}H_{10}BrF_3N_2O_2H^+$: 386.9951, found: 386.9957 (Δ = −1.69 ppm). HPLC (A): *t* = 11.2 min, purity >96%.

4-Trifluoromethyl-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.13).

Yellow solid (98 % yield); mp >215 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 7.83 (s, 2 H); 7.93 (d, 2 H, *J* = 8.3 Hz), 8.14 (d, 2 H, *J* = 8.1 Hz), 8.55 (s, 1 H), 12.61 (s, 1 H), 12.68 (s, 1 H); ^{13}C NMR (100 MHz DMSO-d_6) δ 110.5, 111.3, 120.9, 125.59, 125.62, 128.7, 132.2, 135.8, 136.0, 147.8, 153.7, 161.9; ^{19}F NMR (376 MHz DMSO-d_6) δ −61.44 (s, 3 F); HRMS (TOF) *m/z* calcd for $C_{15}H_9Br_2F_3N_2O_2H^+$: 464.9056, found: 464.9059 (Δ = −0.73 ppm). HPLC (C): *t* = 6.7 min, purity >96%.

3-Difluoromethoxy-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.14).

Yellow solid (90 % yield); mp 166–168 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 6.91 (d, 1 H, 90%, *J* = 8.8 Hz), 6.95 (d, 1 H, 10%, *J* = 8.8 Hz), 7.18 (s, 1 H, 25%), 7.33 (s, 1 H, 50%), 7.42 – 7.44 (m, 2 H, 100%, 80%), 7.48 (s, 1 H, 25%), 7.54 – 7.56 (m, 2 H, 20%), 7.61 (t, 1 H, 100%, *J* = 8 Hz), 7.72 (s, 1 H, 90%), 7.81 – 7.83 (m, 2 H, 100%, 80%), 7.90 (s, 1 H, 10%), 8.63 (s, 1 H, 90%), 8.93 (s, 1 H, 10%), 11.13 (s, 1 H, 10%), 11.20 (s, 1 H, 90%), 12.22 (s, 1 H, 87%); ^{13}C NMR (125 MHz DMSO-d_6) δ 110.5, 110.6, 114.3, 117.9, 118.4, 118.7, 118.9, 120.6, 121.3, 122.3, 122.5, 124.5, 130.2, 130.4, 131.6, 133.7, 134.7, 135.5, 145.8, 150.9, 151.0, 156.4, 157.7, 160.8, 161.8; ^{19}F NMR (376 MHz DMSO-d_6) δ −82.16 (s, 2 F); HRMS (TOF) *m/z* calcd for $C_{15}H_{11}Br_2F_2N_2O_3H^+$: 384.9994, found: 384.9996 (Δ = −0.49 ppm). HPLC (B): *t* = 3.5 min, purity >95%.

3-Difluoromethoxy-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.14a).

Yellow solid (99 % yield); m.p. 187–188 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 7.19 (s, 1 H, 25%), 7.34 (s, 1 H, 50%), 7.46(d, 1 H, 100%, *J* = 8 Hz), 7.49 (s, 1 H, 25%), 7.63 (t, 1 H, 100%, *J* = 8 Hz), 7.73 (s, 1H, 100%), 7.83 −7.96 (m, 3 H, 100%, 100%, 90%), 7.96 (d, 1 H, 10%, *J* = 2.3 Hz), 8.54 (s, 1 H, 90%), 9.06 (s, 1 H, 10%), 10.04 (s, 1 H, 3%), 11.99 (s, 1 H, 10%), 12.60 (s, 1 H, 90%), 12.64 (s, 1 H, 100%); ^{13}C NMR (125 MHz DMSO-d_6) δ 110.4, 110.8, 111.3, 111.6, 114.2, 116.3, 118.1, 118.3, 120.4, 120.9, 122.6, 124.5, 130.5, 132.1, 133.5, 134.0, 135.7, 137.8, 147.5, 150.98, 151.00, 153.7, 154.7, 161.9, 164.0; ^{19}F NMR (376 MHz DMSO-d_6) δ −82.22 (s, 2 F); HRMS (TOF) *m/z* calcd for $C_{15}H_{10}Br_2F_2N_2O_3H^+$: 462.9099, found: 462.9100 (Δ = −0.2 ppm). HPLC (B): *t* = 7.6 min, purity >95%.

4-Difluoromethoxy-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.15).

Yellow solid (96% yield); mp 208–209 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 7.10 – 7.14 (m, 2 H, 100%), 7.20 (s, 1 H, 30%), 7.33 (d, 2 H, 100%, *J* = 8.5 Hz), 7.39, (s, 1 H, 50%), 7.56 (d, 2 H, 20%, 100%, *J* = 7.4 Hz), 8.01 (d, 2 H, 100% *J* = 8.5 Hz), 8.62 (s, 1 H, 100%), 11.49 (s, 1 H, 100%), 12.14 (s, 1 H, 100%); ^{13}C NMR (100 MHz, DMSO-d_6) δ 113.5, 116.0, 118.1, 118.56, 118.59, 119.1, 122.4, 123.9, 129.3, 129.9, 130.3, 146.5, 153.7, 158.0, 161.8; ^{19}F NMR (376 MHz DMSO-d_6) δ −82.94 (s, 1 F); HRMS (TOF) *m/z* calcd for

$C_{15}H_{11}BrF_2N_2O_3H^+$: 384.9994, found: 385.0007 (Δ = −3.49 ppm). HPLC (C): *t* = 3.6 min, purity >95%.

4-Difluoromethoxy-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.15a).

Beige solid (94% yield); mp 194–196 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 6.90 (d, 1 H, 100%, *J* = 8.8 Hz), 7.20 (s, 1 H, 25%), 7.33 (d, 2 H, 100%, *J* = 8.6), 7.39 (s, 1 H, 50%), 7.43 (dd, 1 H, 100%, *J* = 8.8, 2.2 Hz), 7.57 (s, 1 H, 25%), 7.80 (s, 1 H, 100%), 8.01 (d, 2 H, 100%, *J* = 8.6 Hz), 8.61 (s, 1 H, 100%), 11.26 (s, 1 H, 100%), 12.19 (s, 1 H, 100%); ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.5, 113.5, 116.0, 118.1, 118.6, 118.7, 121.3, 129.3, 129.9, 130.4, 133.6, 145.6, 153.7, 156.4, 161.9; ^{19}F NMR (376 MHz DMSO-d_6) δ −82.94 (s, 1 F); HRMS (TOF) *m/z* calcd for $C_{15}H_{11}BrF_2N_2O_3H^+$: 384.9993, found: 385.0011 (Δ = −4.62 ppm). HPLC (B): *t* = 8.9 min, purity >96%.

4-Difluoromethoxy-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.15b).

Beige solid (89% yield); mp >220 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 7.21 (s, 1 H, 25%), 7.35 (d, 2 H, 100%, *J* = 8.6 Hz), 7.40 (s, 1 H, 50%), 7.58 (s, 1H, 25%), 7.83 (s, 2 H, 100%), 8.03 (d, 2 H, 100% *J* = 8.7 Hz), 8.53 (s, 1 H, 100%), 12.56 (s, 1 H, 100%), 12.70 (s, 1 H, 100%); ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.4, 111.2, 113.4, 116.0, 118.1, 121.0, 128.7, 130.1, 132.1, 135.6, 147.1, 153.7, 162.0; ^{19}F NMR (376 MHz DMSO-d_6) δ −83.03 (s, 1 F); HRMS (TOF) *m/z* calcd for $C_{15}H_{10}Br_2F_2N_2O_3H^+$: 462.9099, found: 462.9103 (Δ = −0.92 ppm). HPLC (A): *t* = 11.3 min, purity >95%.

4-Trifluoromethoxy-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.16).

Beige solid (88 % yield); mp 199–201 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 6.91 (d, 1 H, *J* = 8.8 Hz), 7.43 (dd, 1 H, *J* = 8.7, 2.5 Hz), 7.54 (d, 2 H, *J* = 8.3 Hz), 7.81 (s, 1 H), 8.07 (d, 2 H, *J* = 8.8 Hz), 8.61 (s, 1 H), 11.21 (s, 1 H), 12.25 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 110.5, 116.9, 118.7, 118.9, 120.8, 121.0, 121.31, 123.0, 130.1, 130.3, 131.9, 133.7, 145.8, 150.7, 156.4, 161.8; ^{19}F NMR (376 MHz DMSO-d_6) δ −56.64; HRMS (TOF) *m/z* calcd for $C_{15}H_{10}BrF_3N_2O_3H^+$: 402.9900, found: 402.9902 (Δ = −0.63 ppm). HPLC (B): *t* = 6.6 min, purity >95%.

4-Trifluoromethoxy-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.16a).

Beige solid (79 % yield); mp >215 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 7.55 (d, 2 H, *J* = 8.3 Hz), 7.82 (s, 2 H), 8.05 (d, 2 H, *J* = 8.7 Hz), 8.52 (s, 1 H), 12.63 (s, 2 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 110.4, 111.2, 118.9, 120.86, 120.91, 130.2, 131.2, 132.2, 135.7, 147.4, 150.9, 153.7, 161.9; ^{19}F NMR (376 MHz DMSO-d_6) δ −56.65 (s, 3 F); HRMS (TOF) *m/z* calcd for $C_{15}H_9Br_2F_3N_2O_3H^+$:480.9005, found: 480.9000 (Δ = 0.9 ppm). HPLC (A): *t* = 12.9 min, purity >95%.

2-Fluoro-4-trifluoromethoxy-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.17).

Yellow solid (62 % yield); mp 206–207 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 6.80 (dd, 1H, 30%, *J* = 7.4, 1.8 Hz), 6.89 (d, 1 H, 73%, *J* = 8.8 Hz), 7.31 – 7.33 (m, 2 H, 27%, 23%), 7.35 (d, 1 H, 31%, *J* = 8.8 Hz), 7.39 (d, 1 H, 73%, *J* = 8.6 Hz), 7.43 (dd, 1 H, 70%, *J* = 8.8, 2.6 Hz), 7.53 (d, 1 H, 29%, *J* = 10.0 Hz), 7.58 (d, 1 H, 71%, *J* = 10.3 Hz), 7.67 (t, 1 H, 31%, *J* =

8.0 Hz), 7.80 (d, 1 H, 77%, *J* = 2.6 Hz), 7.84 (t, 1 H, 69%, *J* = 8.2 Hz), 8.27 (s, 1 H, 28%), 8.49, (s, 1 H, 72%), 10.27 (s, 1 H, 26%), 11.03 (s, 1 H, 74%), 12.15 (s, 1 H, 34%), 12.15 (s, 1 H, 66%); ^{13}C NMR (125 MHz DMSO-d_6) δ 109.9, 110.1, 110.5, 111.1, 111.3, 111.8, 117.2, 117.4, 118.8, 120.9, 121.3, 121.4, 122.2, 122.9, 130.6, 130.9, 132.07, 132.10, 132.2, 135.6, 135.9, 143.3, 147.8, 150.6, 150.7, 153.6, 158.6, 159.4, 160.6; 165.6; ^{19}F NMR (376 MHz DMSO-d_6) δ −56.94 (s, 3 F), −57.02 (s, 3 F), −109.09 (s, 1 F), −109.94 (s, 1 F); HRMS (TOF) *m/z* calcd for $C_{15}H_8Br_2F_4N_2O_3H^+$: 498.8911, found: 498.8923 (Δ = −2.57 ppm). HPLC (A): *t* = 12.7 min, purity >95%.

2-Fluoro-4-trifluoromethoxy-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.17a).

Yellow solid (57 % yield); m.p. 151–152 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 7.40 (d, 1 H, 100%, *J* = 8.5 Hz), 7.54 (d, 1 H, 15%, *J* = 2.3 Hz), 7.60 (d, 1 H, 100%, *J* = 8.9 Hz), 7.70 (t, 1 H, 15%, *J* = 8.0 Hz), 7.74 (d, 1 H, 15%, *J* = 2.3 Hz), 7.82 (d, 1 H, 85%, *J* = 2.3 Hz), 7.83 (d, 1 H, 85%, *J* = 2.3 Hz), 7.87 (t, 1 H, 85%, *J* = 8.2 Hz), 8.25 (s, 1 H, 15%), 8.45 (s, 1 H, 85 %), 12.40 (s, 1 H, 100%), 12.56 (s, 1 H, 100%); ^{13}C NMR (125 MHz DMSO-d_6) δ 109.9, 110.1,110.5, 111.3, 117.2, 118.8, 120.9, 121.3, 121.4, 132.07, 132.1, 132.2, 135.9, 147.8, 150.6, 150.7, 153.6, 158.6, 159.4, 160.6; ^{19}F NMR (376 MHz DMSO-d_6) δ −56.95 (s, 3 F), −57.04 (s, 3 F), −109.09 (s, 1 F), 109.47 (s, 1 F); HRMS (TOF) *m/z* calcd for $C_{15}H_9BrF_4N_2O_3H^+$: 420.9805, found: 420.9820 (Δ = −3.47 ppm). HPLC (A): *t* = 10.9 min, purity >97%.

2-Fluoro-4-trifluoromethyl-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.18).

Yellow solid (61 % yield); mp 180–182 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 6.80 (d, 1 H, 30%, *J* = 8.5 Hz), 6.90 (d, 1 H, 70% *J* = 8.8 Hz), 7.30 – 7.34 (m, 2 H, 26%, 31%), 7.44 (dd, 1 H, 69%, *J* = 8.8, 2.5 Hz), 7.70 – 7.94 (m, 4 H, 100%, 100%, 100%, 74%), 8.30 (s, 1 H, 30%), 8.52 (s, 1 H, 70%), 10.27 (s, 1 H, 30%), 11.01 (s, 1 H, 70%), 12.28 (s, 1 H, 100%); ^{13}C NMR (125 MHz DMSO-d_6) δ 110.6, 113.87, 113.9, 114.0, 114.1, 118.5, 118.7, 121.2, 121.62, 121.65, 121.7, 121.9, 126.7, 126.8, 128.2, 130.0, 130.7, 131.55, 131.58, 133.5, 134.0, 141.0, 146.0, 155.6, 156.4, 158.0, 159.2, 160.0, 165.6; ^{19}F NMR (376 MHz DMSO-d_6) δ −61.33 (s, 3 F), −61.46 (s, 3 F), −111.26 (s, 1 F), −111.49 (s, 1 F); HRMS (TOF) *m/z* calcd for $C_{15}H_9BrF_4N_2O_2H^+$: 404.9856, found: 404.9864 (Δ = −1.84 ppm). HPLC (B): *t* = 6.5 min, purity >95%.

2-Fluoro-4-trifluoromethyl-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.18a).

Yellow solid (74 % yield); mp 193–195 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 7.52 (d, 1 H, 17%), 7.74 – 7.97 (m, 5 H, 100%, 100%, 100%, 100%, 69%), 8.27 (s, 1 H, 18%), 8.46 (s, 1 H, 82%), 12.34 (s, 1 H, 81%), 12.53 (s, 1 H, 19%), 12.67 (s, 1 H, 81%); ^{13}C NMR (100 MHz DMSO-d_6) δ 110.6, 111.4, 114.0, 114.2, 120.9, 121.69, 121.73, 126.0, 126.2, 131.66, 131.69, 132.2, 136.0, 143.6, 148.1, 152.3, 153.6, 157.8, 159.4, 160.3; ^{19}F NMR (376 MHz DMSO-d_6) δ −61.34 (s, 3 F), −61.50 (s, 3 F), −111.16 (s, 1 F), −111–93 (s, 1 F); HRMS (TOF) *m/z* calcd for $C_{15}H_8Br_2F_4N_2O_2H^+$: 482.8961, found: 482.8958 (Δ = 0.69 ppm). HPLC (A): *t* = 9.4 min, purity >95%.

3-Fluoro-*N*'-(3-bromo-2-hydroxybenzylidene)benzohydrazide (5.19).

White solid (68% yield); mp 154 – 155 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 6.92 (t, J = 7.8 Hz, 1H, 75%), 6.99 (t, J = 7.8 Hz, 1H, 25%), 7.50 (td, J = 8.4 Hz, J = 2.2 Hz, 1H, 75%), 7.54 (d, J = 7.6 Hz, 1H, 75%), 7.63 (m, 2H, 88%), 7.76 (m, 1H), 7.81 (d, J = 7.8 Hz, 1H, 75%), 8.58 (s, 1H, 75%), 9.13 (s, 1H, 25%), 12.08 (m, 2H, 25%), 12.48 (m, 2H, 75%); ^{13}C NMR (175 MHz, DMSO-d_6) δ 110.0, 110.1, 114.5, 114.6, 118.8, 119.1, 119.27, 119.33, 120.6, 121.1, 123.96, 123.97, 130.5, 130.9, 130.95, 132.2, 134.5, 134.6, 134.7, 136.4, 149.1, 154.2, 155.3, 161.3, 161.6, 162.7, 165.1; ^{19}F NMR (376 MHz, DMSO-d_6) δ −112.26 (m, 1F); HRMS (TOF) calcd for $C_{14}H_{10}BrFN_2O_2H^+$ 336.9983, found 336.9988 (Δ = 1.2 ppm). HPLC (B): t = 5.2 min, purity >95%.

3-Fluoro-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.19a).

White solid (72% yield); mp 171 – 172 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.12 (d, J = 8.3 Hz, 1H, 80%), 7.16 (m, 1H), 7.19 (s, 1H, 20%), 7.47 (m, 1H, 80%), 7.60 (m, 2H, 80%), 7.67 (d, J = 8.3 Hz, 1H, 20%), 7.74 (d, J = 9.7 Hz, 1H, 80%), 7.79 (d, J = 7.7 Hz, 1H, 80%), 8.63 (s, 1H, 80%), 8.96 (s, 1H, 20%), 11.42 (s, 1H), 12.17 (s, 1H, 80%); ^{13}C NMR (175 MHz, DMSO-d_6) δ 114.4, 114.5, 118.0, 118.6, 118.9, 119.0, 119.1, 119.3, 122.5, 122.8, 123.89, 123.90, 124.1, 126.1, 130.2, 130.79, 130.84, 131.5, 135.1, 135.2, 146.7, 158.0, 159.2, 161.26, 161.33, 161.6, 162.6; ^{19}F NMR (376 MHz, DMSO-d_6) δ −112.41 (m, 1F); HRMS (TOF) calcd for $C_{14}H_{10}BrFN_2O_2H^+$ 336.9983, found 336.9985 (Δ = −0.8 ppm). HPLC (A): t = 9.4 min, purity >95%.

4-Fluoro-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.20).

Yellow solid (97 % yield); mp 216–217 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 7.40 (t, 2 H, J = 8.8 Hz), 7.82 (s, 2 H), 8.02 – 8.04 (m, 2 H), 8.52 (s, 1 H), 12.55 (s, 1 H), 12.70 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 110.4, 111.2, 115.6, 115.8, 120.9, 128.60, 128.62, 130.5, 130.6, 132.1, 135.6, 147.1, 153.6, 162.0, 163.5, 165.5; ^{19}F NMR NMR (376 MHz DMSO-d_6) δ −107.36 (s, 1 F); HRMS (TOF) m/z calcd for $C_{14}H_9Br_2FN_2O_2H^+$: 414.9087, found: 414.9123 (Δ = −1.57 ppm). HPLC (A): t = 10.1 min, purity >97%.

3-Trifluoromethoxy-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.22).

White solid (65% yield); mp 158 – 160 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.66 (d, J = 8.1 Hz, 1H), 7.72 (t, J = 8.1 Hz, 1H), 7.84 (s, 2H), 7.91 (s, 1H), 8.00 (d, J = 8.1 Hz, 1H), 8.55 (s, 1H), 12.63 (s, 2H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 110.4, 111.3, 119.3, 120.3, 120.8, 120.9, 124.8, 127.0, 130.9, 132.2, 134.4, 135.8, 147.7, 148.4, 153.7, 161.5; ^{19}F NMR (376 MHz, DMSO-d_6) δ - 56.79 (s, 3F); HRMS (TOF) calcd for $C_{15}H_9Br_2F_3N_2O_3H^+$ 480.9005, found 480.9008 (Δ = −0.7 ppm). HPLC (A): t = 7.7 min, purity >97%.

3-Trifluoromethyl-*N*'-(3-chloro-2-hydroxybenzylidene)benzohydrazide (5.23).

White solid (71% yield); mp 168 – 170 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.98 (t, J = 7.8 Hz, 1H), 7.51 (m, 2H), 7.82 (t, J = 7.8 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 8.26 (d, J = 7.8 Hz, 1H), 8.29 (s, 1H), 8.64 (s, 1H), 12.28 (s, 1H), 12.55 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 119.6, 120.1, 120.4, 124.2, 128.7, 129.2, 129.5, 130.0, 131.5, 131.9, 133.3, 149.2, 153.3, 161.5. ^{19}F NMR (376 MHz, DMSO-d_6) δ −61.14 (s, 3F); HRMS (TOF) calcd

for $C_{15}H_{10}ClF_3N_2O_2H^+$ 343.0456, found 343.0457(Δ = −0.3 ppm). HPLC (B): *t* = 5.9 min, purity >95%.

3-Trifluoromethyl-*N*'-(3-bromo-2-hydroxybenzylidene)benzohydrazide (5.23a).

White solid (86% yield); mp 173 – 175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.93 (t, *J* = 7.8 Hz, 1H), 7.55 (dd, *J* = 7.8 Hz, *J* = 1.4 Hz, 1H), 7.65 (dd, *J* = 7.8 Hz, *J* = 1.4 Hz, 1H), 7.82 (t, 7.8 Hz, 1H), 8.02 (d, *J* = 7.8 Hz, 1H), 8.26 (d, *J* = 7.8 Hz, 1H), 8.29 (s, 1H), 8.60 (s, 1H), 12.49 (br s, 1H), 12.55 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 110.0, 119.3, 120.6, 128.8, 130.0, 130.4, 131.9, 133.3, 134.5, 149.3, 154.2, 161.5; ^{19}F NMR (376 MHz, DMSO-d_6) δ −61.14 (s, 3F); HRMS (TOF) *m/z* calcd for $C_{15}H_{10}BrF_3N_2O_2H^+$: 386.9951, found 386.9955 (Δ = −1.1 ppm). HPLC (B): *t* = 5.2 min, purity >95%.

3-Trifluoromethyl-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.23b).

White solid (69% yield); mp 178 – 180 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.13 (m, 2H), 7.60 (d, *J* = 8.1 Hz, 1H), 7.80 (t, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 8.24 (d, *J* = 8.1 Hz, 1H), 8.28 (s, 1H), 8.66 (s, 1H), 11.37 (s, 1H), 12.28 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 118.6, 119.1, 122.5, 124.1, 129.9, 130.0, 131.9, 133.8, 146.8, 158.0, 161.4; ^{19}F NMR (376 MHz, DMSO-d_6) δ −61.12 (s, 3F); HRMS (TOF) *m/z* calcd for $C_{15}H_{10}BrF_3N_2O_2H^+$: 386.9951, found 386.9954 (Δ = −0.9 ppm). HPLC (B): *t* = 5.0 min, purity >95%.

3-Trifluoromethyl-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.23c).

White solid (63% yield); mp 136 – 137 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.82 (m, 3H), 8.02 (d, *J* = 7.3 Hz, 1H), 8.26 (d, *J* = 7.3 Hz, 1H), 8.28 (s, 1H), 8.55 (s, 1H), 12.60 (s, 1H), 12.73 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) δ 110.5, 111.3, 120.9, 123.1, 124.3, 124.6, 128.9, 129.2, 129.4, 130.0, 132.0, 132.2, 133.1, 135.8, 147.7, 153.7, 161.7; ^{19}F NMR (376 MHz, DMSO-d_6) δ −61.14 (s, 3F); HRMS (TOF) $C_{15}H_9Br_2F_3N_2O_2H^+$: 464.9056, found 464.9062 (Δ = −1.4 ppm). HPLC (A): *t* = 9.4 min, purity >95%.

4-Amino-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.24).

Yellow solid (85 % yield); mp 226–227 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 5.83 (s, 2 H), 6.59 (2 H, *J* = 8.7 Hz), 7.08 (dd, 1 H, *J* = 8.3, 1.9 Hz), 7.12 (d, 1 H, *J* = 1.9 Hz), 7.46 (d, 1 H, *J* = 8.3 Hz), 7.66 (d, 2 H, *J* = 8.7 Hz), 8.52 (s, 1 H), 11.77 (s, 2 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 112.6, 118.55, 118.59, 119.1, 122.3, 123.3, 129.4, 130.7, 145.3, 152.6, 158.0, 162.7; HRMS (TOF) *m/z* calcd for $C_{14}H_{12}BrN_3O_2H^+$: 334.0817, found: 334.01945 (Δ = −2.63 ppm). HPLC (B): *t* = 7.9 min, purity >96%.

4-Dimethylamino-*N*'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5.25).

Yellow solid (99 % yield); mp >230 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 2.99 (s, 6 H), 6.75 (d, 2 H, *J* = 9 Hz), 6.88 (d, 1 H, *J* = 8.8 Hz), 7.39 (dd, 1 H, *J* = 8.72, 2.52 Hz), 7.73 (d, 1 H, *J* = 2.5 Hz), 7.82 (d, 2 H, *J* = 8.9 Hz), 8.54 (s, 1 H), 11.51 (s, 1 H), 11.90 (s, 1 H)); ^{13}C NMR (100 MHz DMSO-d_6) δ 110.3, 110.8, 118.5, 118.6, 121.3, 129.2, 130.7, 133.1, 144.5, 152.6, 156.4, 162.7; HRMS (TOF) *m/z* calcd for $C_{16}H_{16}BrN_3O_2H^+$: 362.0487, found: 362.0500 (Δ = −0.43 ppm). HPLC (C): *t* = 3.7 min, purity >95%.sssssss

4-Dimethylamino-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.25a).

Yellow solid (99% yield); mp >215 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 3.0 (s, 6 H), 6.76 (d, 2 H, J = 9 Hz), 7.76 (dd, 2 H, J = 13.1, 2.3 Hz), 7.83 (d, 2 H, J = 9 Hz), 8.46 (s, 1 H), 12.21 (s, 1 H), 12.96 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 110.2, 110.9, 111.0, 117.9, 121.1, 129.3, 131.9, 135.1, 145.3, 152.8, 153.6; HRMS (TOF) *m/z* calcd for $C_{16}H_{15}Br_2N_3O_2H^+$: 439.9604, found: 439.9603 (Δ = −0.19 ppm). HPLC (B): t = 6.5 min, purity >95%.

4-Dimethylamino-*N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5.25b).

Yellow solid (93 % yield); m.p. 195–196 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 2.99 (s, 6 H), 6.75 (d, 2 H, J = 9 Hz), 7.09 (dd, 1 H, J = 8.24, 1.8 Hz), 7.12 (s, 1 H), 7.47 (d, 1 H, J = 8.3 Hz), 7.81 (d, 2 H, J = 9 Hz), 8.55 (s, 1 H), 11.76 (s, 1 H), 11.85 (s, 1 H); ^{13}C NMR (100 MHz DMSO-d_6) δ 110.8, 118.6, 119.0, 122.3, 123.4, 129.1, 130.7, 145.4, 152.6, 158.0, 162.6; HRMS (TOF) *m/z* calcd for $C_{16}H_{16}BrN_3O_2H^+$: 362.0499, found: 362.0504 (Δ = −1.37 ppm). HPLC (B): t = 6.9 min, purity >95%.

4-Dimethylamino-*N*'-(2-hydroxynaphthylidene)benzohydrazide (5.25c).

Yellow solid (90 % yield); mp >215 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 3.00 (s, 6 H), 6.79 (d, 2 H, J = 8.9 Hz), 7.22 (d, 1 H, J = 9 Hz), 7.39 (t, 1 H, J = 7.5 Hz), 7.59 (t, 1 H, J = 7.5 Hz), 7.84 – 7.91 (m, 4 H), 8.15 (d, 1 H, J = 8.5 Hz), 9.46 (s, 1 H), 11.89 (s, 1 H), 12.99 (s, 1 H); ^{13}C NMR (100 MHz DMSO-d_6) δ 108.6, 110.9, 118.5, 119.0, 120.4, 123.4, 127.6, 127.8, 128.95, 129.04, 131.5, 132.2, 145.1, 152.7, 157.7, 162.3; HRMS (TOF) *m/z* calcd for $C_{20}H_{19}N_3O_2H^+$: 334.1550, found: 334.1555 (Δ = −1.39 ppm). HPLC (C): t = 4.5 min, purity >95%.

4-Acetamido-*N*'-(4-dibromo-2-hydroxybenzylidene)benzohydrazide (5.26).

Beige solid (85 % yield); mp >230 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 2.07 (s, 3 H), 7.08 (d, 1 H, J = 9.7 Hz), 7.13 (d, 1 H, J = 1.3 Hz), 7.52 (d, 1 H, J = 8.3 Hz), 7.71 (d, 2 H, J = 8.7 Hz), 7.88 (d, 2 H, J = 8.6 Hz), 8.59 (s, 1 H), 10.22 (s, 1 H), 11.57 (s, 1 H), 12.03 (s, 1 H); ^{13}C NMR (100 MHz DMSO-d_6) δ 24.1, 118.2, 118.5, 119.1, 122.4, 123.7, 126.8, 128.6, 130.5, 142.6, 146.2, 158.0, 162.3, 168.8; HRMS (TOF) *m/z* calcd for $C_{16}H_{14}BrN_3O_3H^+$: 376.0291, found: 376.0301 (Δ = −2.51 ppm). HPLC (B): t = 7.5 min, purity >99%.

3-Bromo-*N*'-(2-hydroxy-5-trifluoromethylbenzylidene)benzohydrazide (5a).

White solid (92 % yield); mp 175–176 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 7.10 (d, 1 H, 88%, J = 8.6 Hz), 7.15 (d, 1 H, 12%, J = 8.5 Hz), 7.51 (t, 1 H, 100%, J = 7.9 Hz), 7.62 (dd, 1 H, 86%, J = 8.6, 1.5 Hz), 7.74 (dd, 1 H, 14%, J = 7.9, 2.4 Hz), 7.81 (d, 1 H, 100%, J = 7.8 Hz), 7.93 (d, 1 H, 100%, J = 7.9 Hz), 8.00 (s, 1 H, 93%), 8.09 (s, 1 H, 7%), 8.12 (s, 1 H, 100%), 8.71 (s, 1 H, 90%), 9.06 (s, 1 H, 10%), 10.69 (s, 1 H, 6%), 11.64 (s, 1 H, 13%), 11.71 (s, 1 H, 87%), 12.27 (s, 1 H, 92%); ^{13}C NMR (100 MHz DMSO-d_6) δ 117.2, 119.7, 119.9, 120.2, 120.6, 121.8, 123.1, 125.2, 125.8, 126.9, 128.0, 130.2, 130.8, 134.7, 134.9, 145.7, 160.0, 161.5; ^{19}F NMR (376 MHz DMSO-d_6) δ −56.92 (s, 3 F), −60.12 (s, 3 F); HRMS (TOF) *m/z* calcd for $C_{15}H_{10}BrF_3N_2O_2H^+$:386.9951, found: 386.9957 (Δ = −1.78 ppm). HPLC (B): t = 6.9 min, purity >95%.

2,4-Dibromo-*N*'-(5-fluoro-2-hydroxybenzylidene)benzohydrazide (5.2f).

White solid (96 % yield); m.p. 182–183 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 6.83 (dd, 1 H, 39%, J = 9, 4.7 Hz), 6.94 (dd, 1 H, 61%, J = 9, 4.7 Hz), 6.98 (dd, 1 H, 39%, J = 9.4, 3.2 Hz), 7.06 (td, 1 H, 39%, J = 8.5, 3.3 Hz), 7.16 (td, 1 H, 61%, J = 8.5, 3.3 Hz), 7.41 (d, 1 H, 39%, J = 8.2 Hz), 7.44 (dd, 1 H, 60%, J = 9.4, 3.2), 7.54 (d, 1 H, 61%, J = 8.2 Hz), 7.70 (dd, 1 H, 40%, J = 8.2, 1.9 Hz), 7.74 (dd, 1 H, 60%, J = 8.2, 1.9 Hz), 8.00 (d, 1 H, 40%, J = 1.9 Hz), 8.02 (d, 1 H, 60%, J = 1.9 Hz), 8.27 (s, 1 H, 40%), 8.47 (s, 1 H, 60%), 9.90 (s, 1 H, 39%), 10.71 (s, 1 H, 61%), 12.15 (s, 1 H, 66%), 12.17 (s, 1 H, 34%); ^{13}C NMR (125 MHz DMSO-d_6) δ 112.0, 112.2, 113.2, 113.4, 117.4, 117.5, 117.6, 117.7, 117.7, 118.0, 118.3, 118.4, 119.75, 119.8, 120.18, 120.24, 120.6, 122.7, 123.8, 130.2, 130.7, 130.8, 130.9, 134.1, 134.8, 136.1, 137.2, 141.8, 146.1, 152.8, 153.5, 154.3, 154.4, 156.2, 156.3, 162.5, 168.3; ^{19}F NMR (376 MHz DMSO-d_6) δ −124.76 (s, 1 F), −124.91 (s, 1 F); HRMS (TOF) m/z calcd for $C_{14}H_9Br_2FN_2O_2H^+$: 414.9088, found: 414.9095 (Δ = −1.7 ppm). HPLC (B): t = 4.6 min, purity >99%.

2,4-Dibromo-*N*'-(5-cyano-2-hydroxybenzylidene)benzohydrazide (5.2g).

Off white solid (99% yield); mp >230 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 6.98 (d, 1 H, J = 5 Hz, 35%), 7.08 (d, 1 H, J = 5 Hz, 65%), 7.41 (d, 1 H, J = 4.6 Hz, 35%), 7.54 (d, 1 H, J = 4.6 Hz, 65%), 7.61 (d, 1 H, 1.2 Hz, 33%), 7.62 – 7.64 (m, 1 H, 35%), 7.70 – 7.71 (m, 1 H, 37%), 7.72 – 7.74 (m, 2 H, 100%, 37%), 8.0 (d, 1 H, J = 1 Hz, 35%), 8.02 (d, 1 H, J = 1 Hz, 65%), 8.08 (d, 1 H, J = 1.2 Hz, 65%), 8.28 (s, 1 H, 35%), 8.48 (s, 1 H, 65%), 11.03 (s, 1 H, 35%), 11.84 (s, 1 H, 65%), 12.26 (s, 1 H, 38%), 12.28 (s, 1 H, 62%); ^{13}C NMR (100 MHz DMSO-d_6) δ 48.6, 101.9, 117.5, 117.7, 118.8, 118.9, 119.8, 120.2, 120.6, 122.8, 123.9, 130.2, 130.7, 130.8, 130.9, 131.3, 132.7, 134.2, 134.7, 134.9, 135.0, 135.9, 137.0, 141.0, 145.2, 160.1, 160.7, 162.6, 168.4; HRMS (TOF) m/z calcd for $C_{15}H_9Br_2N_3O_2H^+$: 421.9134, found: 421.9130 (Δ = 0.94 ppm). HPLC (B): t = 3.2 min, purity >95%.

2,4-Dibromo-*N*'-(2-hydroxy-5-trifluoromethoxybenzylidene)benzohydrazide (5.2h).

White solid (99 % yield); mp 170–172 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 6.91 (d, 1 H, 36%, J = 8.8Hz), 7.01 (d, 1 H, 64%, J = 8.9 Hz), 7.16 – 7.19 (m, 2 H, 38 %, 35%), 7.30 (dd, 1 H, 65%, J = 9, 2.8 Hz), 7.40 (d, 1 H, 37%, J = 8.2 Hz), 7.54 (d, 1 H, 63%, J = 8.2 Hz), 7.64 (d, 1 H, 62%, J = 2.7 Hz), 7.69 (dd, 1 H, 37%, J = 8.2, 1.8 Hz), 7.74 (dd, 1 H, 63%, J = 8.2, 1.8 Hz), 7.97 (d, 1 H, 39%, J = 1.8 Hz), 8.02 (d, 1 H, 61%, J = 1.8 Hz), 8.28 (s, 1 H, 37%), 8.50 (s, 1 H, 63%), 10.28 (s, 1 H, 36%), 11.03 (s, 1 H, 64%), 12.18 (s, 1 H, 63%), 12.20 (s, 1 H, 37%); ^{13}C NMR (125 MHz DMSO-d_6) δ 117.5, 117.7, 118.5, 119.1, 119.2, 119.86, 119.94, 120.2, 120.61, 120.63, 121.1, 121.2, 122.6, 123.8, 123.9, 124.6, 130.2, 130.6, 130.8, 130.9, 134.0, 134.8, 136.1, 137.2, 140.69, 140.70, 140.9, 145.4, 155.2, 156.0, 162.5, 168.5; ^{19}F NMR (376 MHz DMSO-d_6) δ −57.31, −57.40; HRMS (TOF) m/z calcd for $C_{15}H_9Br_2F_3N_2O_3H^+$: 480.9005, found: 480.9013 (Δ = −1.74 ppm). HPLC (A): t = 10.6 min, purity >98%.

2,5-Dibromo-*N*'-(5-fluoro-2-hydroxybenzylidene)benzohydrazide (5.3f).

White solid (86% yield), mp >220 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 6.82 – 7.14 (m, 3 H, 100%, 100%, 39%), 7.43 (d, 1 H, 70%, J = 6.3 Hz), 7.59 – 7.68 (m, 3 H, 100%, 100%,

30%), 7.82 (s, 1 H, 61%), 8.27 (s, 1 H, 36%), 8.47 (s, 1 H, 64%), 9.89 (s, 1 H, 37%), 10.69 (s, 1 H, 63%), 12.18 (s, 1 H, 100%); ^{13}C NMR (125 MHz DMSO-d_6) δ 112.0, 112.2, 113.2, 113.4, 117.47, 117.53, 117.6, 117.7, 117.8, 117.9, 118.0, 118.3, 118.5, 118.7, 119.77, 119.83, 120.2, 120.3, 120.6, 120.7, 131.0, 131.9, 133.5, 134.1, 134.4, 134.8, 138.7, 139.9, 141.9, 146.2, 152.8, 153.5, 154.3, 154.4, 156.2, 156.3, 161.7, 167.5; ^{19}F NMR (376 MHz DMSO-d_6) δ −124.87 (s, 1 F), −124.77 (s, 1 F); HRMS (TOF) *m/z* calcd for $C_{14}H_9Br_2FN_2O_2H^+$: 414.9013, found: 414.9088 (Δ = −3.7 ppm). HPLC (A): t_1 = 6.9, t_2 = 7.4 min, purity >97%.

4-Bromo-*N*'-(2-hydroxy-5-trifluoromethoxybenzylidene)benzohydrazide (5.4a).

White solid (93 % yield); mp 213–214 °C; 1H NMR (500 MHz DMSO-d_6) δ 7.02 (d, 1 H, *J* = 9 Hz), 7.30 (dd, 1 H, *J* = 8.9, 2.6 Hz), 7.64 (d, 1 H, *J* = 2.5 Hz), 7.77 (d, 2 H, *J* = 8.5 Hz), 7.89 (d, 2 H, *J* = 8.5 Hz), 8.67 (s, 1 H), 11.25 (s, 1 H), 12.23 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 117.7, 119.2, 120.2, 120.4, 121.2, 123.3, 124.3, 125.8, 129.8, 131.6, 131.9, 140.7, 145.6, 156.1, 162.1; ^{19}F NMR (376 MHz DMSO-d_6) δ −57.30 (s, 3 F); HRMS (TOF) *m/z* calcd for $C_{15}H_{10}BrF_3N_2O_3H^+$: 402.9900, found: 402.9910 (Δ = −2.47 ppm). HPLC (A): *t* = 10.0 min, purity >95%.

4-Bromo-*N*'-(5-fluoro-2-hydroxybenzylidene)benzohydrazide (5.4b).

White solid (88 % yield); mp >220 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 6.92 – 6.94 (dd, 1 H, *J* = 9, 4.7 Hz), 7.15 (td, 1 H, *J* = 8.6, 3.2 Hz), 7.44 (dd, 1 H, *J* = 9.4 3.1 Hz), 7.76 (d, 2 H, *J* = 8.5 Hz), 7.89 (d, 2 H, *J* = 8.5 Hz), 8.63 (s, 1 H), 10.94 (s, 1 H), 12.20 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 113.6, 113. 8, 117.58, 117.64, 118.0, 118.2, 119.7, 119.8, 125.8, 129.7, 131.6, 131.9, 146.3, 153.6, 154.4, 156.3, 162.0; ^{19}F NMR (376 MHz DMSO-d_6) δ −125.06 (s, 1 F); HRMS (TOF) *m/z* calcd for $C_{14}H_{10}BrFN_2O_2H^+$: 336.9982, found: 336.9996 (Δ = −3.95 ppm). HPLC (B): *t* = 5.2 min, purity >95%.

2-Methyl-*N*'-(2-hydroxy-5-trifluoromethylbenzylidene)benzohydrazide (5.27).

White solid (94% yield); mp 194–196 °C; ^{1}H NMR (500 MHz DMSO- d_6) δ 2.23 (s, 3 H, 17%), 2.38 (s, 3 H, 83%), 6.96 (d, 1 H, *J* = 8.6 Hz, 17%), 7.10 (d, 1 H, *J* = 8.6 Hz, 83%), 7.23 – 7.42 (m, 4 H, 100%, 100%, 15%, 20%), 7.40 (t, 1 H, *J* = 7.4 Hz, 85%), 7.47 – 7.50 (m, 1 H, 90%, 10%), 7.59 (s, 1 H, 18%), 7.61 (d, 1 H, *J* = 8.6 Hz), 7.98 (s, 1 H, 82%), 8.32 (s, 1 H, 17%), 8.57 (s, 1 H, 83%), 10.67 (s, 1 H, 17%), 11.72 (s, 1 H, 83%), 12.00 (s, 1 H, 18%), 12.10 (s, 1 H, 82%); ^{13}C NMR (125 MHz, DMSO- d_6) δ 19.0, 19.4, 116.9, 117.2, 119.7, 119.9, 120.2, 123.3, 125.3, 125.42, 125.45, 125.50, 125.7, 126.9, 127.6, 127.91, 129.2, 129.8, 130.2, 130.7, 134.2, 134.5, 136.1, 141.1, 145.1, 159.2, 160.0, 165.1; ^{19}F NMR (376 MHz DMSO-d_6) δ −60.22 (s, 3 F), −59.94 (s, 3 F); HRMS (TOF) *m/z* calcd for $C_{16}H_{13}F_3N_2O_2H^+$: 323.1002, found: 323.1001 (Δ = 0.23 ppm). HPLC (A): *t* = 8.0 min, purity >96%.

2-Methyl-*N*'-(5-cyano-2-hydroxybenzylidene)benzohydrazide (5.27a).

Beige solid (99% yield); mp 208–209 °C; ^{1}H NMR (700 MHz DMSO-d_6) δ 2.23 (s, 3 H, 17%), 2.38 (s, 3 H, 83%), 6.94 (d, 1 H, *J* = 8.6 Hz, 19%), 7.08 (d, 1 H, *J* = 8.6 Hz, 81%), 7.26 – 7.32 (m, 3 H, 100%, 100%, 18%), 7.35 – 7.37 (m, 1 H, 19%), 7.40 – 7.42 (m, 1 H,

81%), 7.48 (d, 1 H, *J* = 7.4 Hz, 82%), 7.60 – 7.64 (m, 2 H, 20%, 20%), 7.71 (dd, 1 H, *J* = 8.5, 2.1 Hz, 80%), 8.07 (d, 1 H, *J* = 2.0 Hz, 80%), 8.26 (s, 1 H, 18%), 8.50 (s, 1 H, 82%), 10.93 (s, 1 H, 18%), 12.02 (s, 1 H, 19%), 12.07 (s, 1 H, 82%), 12.16 (s, 1 H, 81%); ^{13}C NMR (175 MHz DMSO-d_6) δ 19.0, 19.4, 101.8, 117.5, 117.7, 118.9, 120.2, 120.5, 125.4, 125.7, 126.9, 127.6, 129.3, 129.9, 130.3, 130.8, 131.8, 133.2, 134.2, 134.4, 134.5, 134.8, 135.5, 136.2, 140.7, 145.0, 160.0, 160.8, 165.1, 171.2; HRMS (TOF) *m/z* calcd for $C_{16}H_{13}N_3O_2H^+$: 280.1081, found: 280.1086 (Δ = −2.08 ppm). HPLC (A): *t* = 8.8 min, purity >97%.

4-Fluromethyl-*N*'-(3,5-dibromo-2-hydroxybenzylidene)benzohydrazide (5.21).

To a solution of *tert*-butyl 2-(4-(fluoromethyl)benzoyl)hydrazine-1-carboxylate (0.5 mmol) in dichloromethane (5 mL), trifluoroacetic acid (0.5 mL) was added and the reaction mixture was stirred at room temperature for 5 hours. This was followed by the addition of 3,5-dibromosalicylaldedhyde (1.1 ea.), to the reaction mixture and stirred at room temperature overnight. Ice cold water (15 mL) was added to the reaction mixture and filtered to give the product as a white solid (46 % yield); mp >230 °C; ^{1}H NMR (400 MHz DMSO-d_6) δ 5.46 (s, 1 H), 5.58 (s, 1 H), 7.57 (d, 2 H, *J* = 7.6 Hz), 7.80 – 7.82 (m, 2 H), 7.99 (d, 2 H, *J* = 7.9 Hz), 8.53 (s, 1 H), 12.56 (s, 1 H), 12.71 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 82.7, 84.3, 110.4, 111.2, 121.0, 127.27, 127.33, 128.1, 132.1, 135.6, 140.4, 140.6, 147.2, 153.7, 162.6; ^{19}F NMR (376 MHz DMSO-d_6) δ −209.61 (t, 1 F, *J* = 47.2 Hz); HRMS (TOF) *m/z* calcd for $C_{15}H_{11}Br_2FN_2O_2H^+$: 428.9244, found: 428.9247 (Δ = −0.69 ppm). HPLC (B): *t* = 6.2 min, purity >95%.

4-Bromo-2-(5-(2-tolyl)-1,3,4-oxadiazol-2-yl)phenol (6a).

To a solution of **1** (0.23 mmol) in DMF (2 mL) was added bis(trifluoroacetoxy)iodobenzene (1.1 eq.). The reaction mixture was heated at 100 °C for 7 h. After the completion of reaction, the reaction mixture was cooled to room temperature followed by the addition of water (15 mL) which resulted in precipitation of brown solid. The precipitate was filtered, dried and recrystallized in dichloromethane: hexanes (40:60) to give the product as white solid (53% yield); mp 152–153 °C; ^{1}H NMR (500 MHz DMSO-d_6) δ 2.67 (s, 3 H), 7.06(d, 1 H, J = 8.8 Hz), 7.40 – 7.45 (m, 2 H), 7.51 (t, 1 H, *J* = 7.4 Hz), 7.61 (dd, 1 H, *J* = 8.8, 2.5 Hz), 8.01 (d, 1 H, *J* = 2.5 Hz), 8.04 (d, 1 H, *J* = 7.7 Hz), 10.65 (s, 1 H); ^{13}C NMR (125 MHz DMSO-d_6) δ 21.5, 110.4, 111.9, 119.4, 122.3, 126.5, 129.0, 130.9, 131.6, 131.8, 135.8, 137.7, 155.7, 161.8, 164.0; HRMS (TOF) *m/z* calcd for $C_{15}H_{11}BrN_2O_2H^+$: 331.0076, found: (Δ = −3.6 ppm). HPLC (B): *t* = 6.0 min, purity >95%.

3-(2-(Benzyloxy)-3,5-dibromophenyl)acrylaldehyde (4f.2).

To a solution of **4f.1** (0.68 mmol) in THF (7 mL), was added (formylmethylene)triphenylposphorane (1.1 eq.). The reaction mixture was refluxed overnight. After completion, the reaction was quenched by the addition of water. The reaction mixture was concentrated under vacuum in a rotary evaporator, followed by the addition of ethyl acetate (40 mL). The organic layer was washed with brine (3×30 mL) and water (3×30 mL). The organic layer was collected, dried with magnesium sulfate, filtered and concentrated under vacuum in a rotary evaporator. The crude product was purified by

column chromatography using silica gel and a mixture of hexanes: ethyl acetate (2:1), to give the pure product as beige solid (87% yield); mp 104–105 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.00 (s, 2 H), 6.49 – 6.53 (m, 1 H), 7.37 – 7.42 (m, 6 H), 7.61 (d, 1 H, *J* = 2.3 Hz), 7.81 (d, 1 H, *J* = 2.3 Hz), 9.45 (d, 1 H, *J* = 7.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 118.1, 119.7, 128.9, 129.1, 129.2, 129.5, 130.7, 132.0, 135.4, 138.2, 145.0, 153.9, 193.3; MS (ESI) *m/z* calcd for $C_{16}H_{12}Br_2O_2H^+$: 394.9, found: 394.9.

(3-(2-(Benzyloxy)-5-bromophenyl)-1H-pyrazol-1-yl)(3,5-dibromophenyl)methanone (4f.3).

To a solution of **4.2** (1.2 mmol) in acetonitrile was added *p*-toluenesulfonylhydrazide (1.1 eq.). The reaction mixture was stirred at room temperature for one hour. This was followed by the addition of sodium hydroxide (1.5 eq.), and the reaction mixture was refluxed overnight. The mass peak corresponding to the cyclized product was seen on mass spectrometer. Then, 4-bromobenzoyl chloride (1.5 eq.) was added to the reaction mixture and stirred at room temperature for 3 hours. The reaction mixture was concentrated under vacuum in a rotary evaporator to give the crude product. Recrystallization of the crude product using hexanes: ethyl acetated gave the pure product as white solid (62% yield); mp 140–142 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.82 (s, 2 H), 7.03 (d, 1 H, *J* = 2.9 Hz), 7.34 – 7.41 (m, 5 H), 7.65 (d, 2 H, *J* = 8.8 Hz), 7.78 (d, 1 H, *J* = 2.4 Hz), 7.96 (d, 1 H, *J* = 2.4 Hz), 8.10 (d, 2 H, *J* = 8.7 Hz), 8.43 (d, 1 H, *J* = 2.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 75.7, 111.1, 118.0, 119.9, 128.5, 128.63, 128.64, 128.8, 129.3, 130.0, 131.6, 131.7, 132.0, 132.5, 133.4, 136.0, 136.5, 151.9, 153.3, 165.2; MS (ESI) *m/z* calcd for $C_{23}H_{15}Br_3N_2O_2H^+$: 588.8, found: 588.8.

(3-(3,5-Dibromo-2-hydroxyphenyl)-1*H*-pyrazol-1-yl)(3-bromophenyl)methanone (6b).

To a solution of **4f.3** (0.15 mmol) in dichloromethane (3 mL) at 0 °C, BBr_3 (1 eq.) was added dropwise. The reaction mixture was stirred at 0 °C for 30 min. This was followed by the addition of dichloromethane (30 mL). The organic layer was washed with brine (3×30 mL) and water (3×30 mL). The organic layer was collected, dried with magnesium sulfate, filtered and concentrated under vacuum in a rotary evaporator. The crude product was purified by column chromatography using silica gel and a mixture of hexanes: ethyl acetate (8:2), to give the pure product as white solid (50 % yield); mp 215–218 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (d, 1 H, *J* = 3.0 HZ), 7.69 (s, 2 H), 7.73 (d, 2 H, *J* = 8.7 Hz), 7.90 (d, 2 H, *J* = 8.7 Hz), 8.52 (d, 1 H, *J* = 3.0 Hz), 10.81 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 107.4, 111.7, 112.5, 117.6, 129.3, 129.4, 129.7, 131.9, 132.1, 132.3, 136.6, 152.4, 154.3, 164.9; HRMS (TOF) *m/z* calcd for $C_{16}H_9Br_3N_2O_2H^+$: 498.8287, found: 498.8286 (Δ = 0.24 ppm). HPLC (B): t_1 = 9.0, t_2 = 9.3 min, purity >95%.

4-Bromo-*N*'-(3,5-dibromo-2-hydroxybenzylidene)-*N*-methylbenzohydrazide (5.28).

White solid (89% yield); mp 222 – 223 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.47 (s, 3 H), 7.51 (d, 2 H, *J* = 8.4 Hz), 7.64 (d, 1 H, *J* = 2.1 Hz), 7.70 (d, 2 H, *J* = 8.4 Hz), 7.73 (d, 1 H, *J* = 2.3 Hz), 8.20 (s, 1 H), 10.42 (s, 1 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.8, 110.7, 111.4, 121.9, 123.8, 130.1, 131.3, 131.7, 134.1, 135.3, 140.9, 152.2, 168.9; HRMS (TOF) *m/z* calcd for $C_{l5}H_{ll}Br_3N_2O_2H^+$: 488.8443, found: 488.8449 (Δ = −1.33 ppm). HPLC (A): *t* = 13.6 min, purity >95%.

For compounds **5.2i, 5.2 j**, **5.3g** and **5.3h**, the same procedure was used as the rest of the acylhydrazones, except for the use of substituted acetophenones instead of salicylaldehydes.

2,4-Dibromo-*N*'-(1-(3,5-dibromo-2-hydroxyphenyl)ethylidene)benzohydrazide (5.2i).

White solid (88% yield); mp > 220 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.43 (s, 3H), 7.58 (d, J = 8.2 Hz, 1H), 7.75 (dd, J = 1.8 Hz, J = 8.2 Hz, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.85 (d, J = 2.3 Hz, 1H), 8.04 (d, J = 1.8 Hz, 1H), 11.88 (s, 1H), 14.36 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ14.2, 109.5, 111.9, 120.8, 121.7, 124.0, 130.3, 130.7, 131.2, 134.7, 135.6, 135.9, 154.5, 155.7, 163.6; HRMS (TOF) *m/z* calcd for $C_{15}H_{10}Br_4N_2O_2H^+$: 566.7549, found 566.7540 (Δ = 1.6 ppm). HPLC (C): t = 4.8 min, purity >98%.

2,4-Dibromo-*N*'-(1-(5-bromo-2-hydroxyphenyl)ethylidene)benzohydrazide (5.2j).

White solid (83% yield); mp > 220 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 2.36 (s, 1H, 20%), 2.40 (s, 1H, 80%), 6.70 (d, 1H, 20%, J = 8.8 Hz), 6.90 (d, 1H, 80%, J = 8.8 Hz), 7.34 (dd, 1H, 20%, J = 8.8 Hz, J = 2.4 Hz), 7.43 (d, 1H, 20%, J = 8.2 Hz), 7.46 (dd, 1H, 80%, J = 8.8 Hz, J = 2.4 Hz), 7.57 (d, 1H, 80%, J = 8.2 Hz), 7.59 (d, 1H, 20%, J = 2.4 Hz), 7.71 (dd, 1H, 20%, J = 8.2 Hz, J = 1.7 Hz), 7.74 (m, 2H, 80%), 8.01 (d, 1H, 20%, J = 1.6 Hz), 8.03 (d, 1H, 80%, J = 1.6 Hz), 10.81 (s, 1H, 20%), 11.71 (s, 1H), 13.22 (s, 1H, 80%); ^{13}C NMR (175 MHz, DMSO-d_6) δ 14.2, 109.7, 110.0, 119.0, 119.3, 119.6, 120.8, 121.3, 122.1, 123.8, 129.7, 130.7, 130.8, 131.15, 131.20, 133.4, 133.8, 134.5, 134.7, 135.9, 137.0, 152.8, 156.3, 156.4, 157.7, 163.4, 168.8; HRMS (TOF) *m/z* calcd for $C_{15}H_{11}Br_3N_2O_2H^+$: 488.8443, found 488.8449 (Δ = −1.2 ppm). HPLC (C): t = 3.3 min, purity >97%.

2,5-Dibromo-*N*'-(1-(3,5-dibromo-2-hydroxyphenyl)ethylidene)benzohydrazide (5.3g).

White solid (68% yield); mp > 220 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 2.44 (s, 3H), 7.67 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.80(d, J = 2.4 Hz, 1H), 7.85 (d, J = 2.2 Hz, 1H), 7.87 (d, J = 2.2 Hz, 1H), 11.92 (s, 1H), 14.36 (s, 1H); ^{13}C NMR (175 MHz, DMSO-d_6) 14.2, 109.6, 111.9, 118.8, 120.6, 121.7, 130.4, 132.1, 134.5, 134.7, 136.0, 138.2, 154.6, 155.8, 162.8; HRMS (TOF) *m/z* calcd for $C_{15}H_{10}Br_4N_2O_2H^+$: 566.7549, found 566.7553 (Δ = −0.7 ppm). HPLC (B): t_1 = 9.0, t_2 = 9.3 min, purity >95%.

2,5-Dibromo-*N*'-(1-(5-bromo-2-hydroxyphenyl)ethylidene)benzohydrazide (5.3h).

White solid (83% yield); mp > 220 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 2.37 (s, 3H, 20%), 2.41 (s, 3H, 80%), 6.70 (d, 1H, 20%, J = 8.8 Hz), 6.90 (d, 1H, 80%, J = 8.8 Hz), 7.34 (dd, 1H, 20%, J = 8.7 Hz, J = 1.8 Hz), 7.46 (dd, 1H, 80%, J = 8.7 Hz, J = 1.8 Hz), 7.59 (d, 1H, 20%, J = 1.8 Hz), 7.66 (m, 2H), 7.74 (d, 1H, J = 2.1 Hz), 7.86 (d, 1H, 80%, J = 1.8 Hz), 10.77 (s, 1H, 20%), 11.75 (s, 1H), 13.22 (s, 1H, 80%); ^{13}C NMR (175 MHz, DMSO-d_6) δ14.2, 109.7, 110.0, 117.3, 118.8, 119.0, 119.6, 120.6, 121.0, 121.3, 122.2, 130.6, 130.7, 130.8, 132.1, 133.4, 133.8, 134.4, 134.5, 134.7, 138.5, 139.7, 152.9, 156.4, 156.5, 157.7, 162.7, 168.0; HRMS (TOF) *m/z* calcd for $C_{15}H_{11}Br_3N_2O_2H^+$: 488.8443, found 488.8451 (Δ = −1.6 ppm). HPLC (B): t = 6.7 min, purity >97%.

N'$_1$,*N*'$_4$-Bis((*E*)-5-bromo-2-hydroxybenzylidene)isophthalohydrazide (5.29).

To a solution of isophthalohydrazide (0.170 mmol), 5-bromo-2-hydroxybenzaldehyde (0.374 mmol) in methanol (5 ml) was added 2 drops of glacial acetic acid. The reaction mixture was allowed to stir at room temperature overnight. Addition of water to the reaction mixture resulted in precipitation of the product. The product was filtered, washed with water and dried over vacuum to give the pure product as a white solid in 83% yield; mp > 220 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 6.92 (d, J = 8.8 Hz, 2H), 7.44 (dd, J = 2.4 Hz, J = 8.7 Hz, 2H), 7.72 (t, J = 7.7 Hz, 1H), 7.83 (d, J = 2.2 Hz, 2H), 8.16 (d, J = 7.6 Hz, 2H), 8.52 (s, 1H), 8.66 (s, 2H), 11.24 (s, 2H), 12.35 (s, 2H). ^{13}C NMR (175 MHz, DMSO-d_6) δ 110.5, 118.7, 118.9, 121.4, 127.1, 129.0, 130.3, 131.1, 133.2, 133.7, 145.8, 156.4, 162.4; HRMS (TOF) *m/z* calcd for $C_{22}H_{16}Br_2N_4O_4H^+$: 558.9611, found: 558.9601 (Δ = 1.79 ppm). This compound was not UV active, for some reason, and thus HPLC analysis for purity assessment was not possible. Nevertheless, we believe that the purity is >95% based on its ^{1}H NMR spectrum (see the Supporting Information).

The same procedure was used for the synthesis of **5.32.**

N'$_1$,*N*'$_4$-Bis((*E*)-3,5-dibromo-2-hydroxybenzylidene)isophthalohydrazide (5.32).

White solid (83% yield); mp > 220 °C; ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.77 (t, J = 7.8 Hz, 1H), 7.85 (d, J = 2.0 Hz, 4H), 8.20 (d, J = 7.7 Hz, 2H), 8.55 (s, 1H), 8.58 (s, 2H), 12.69 (s, 2H), 12.75 (s, 2H). ^{13}C NMR (175 MHz, DMSO-d_6) δ 110.5, 111.3, 121.0, 127.3, 129.1, 131.5, 132.2, 132.6, 135.7, 147.5, 153.7, 162.4; HRMS (TOF) *m/z* calcd for $C_{22}H_{14}Br_4N_4O_4H^+$: 714.7821, Found: 714.7827 (Δ = −0.83 ppm). This compound was not UV active, for some reason, and thus HPLC analysis for purity assessment was not possible. Nevertheless, we believe that the purity is >95% based on its ^{1}H NMR spectrum (see the Supporting Information).

Antifungal activity assay.

MICs were determined following the methods of the Clinical and Laboratory Standards Institutes (CLSI) with modifications. Yeast nitrogen base (YNB) medium without amino acid (pH 7.0, 2% glucose) buffered with (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES) was used for MIC studies in *C. neoformans*. YNB medium without ammonium sulfate, without amino acid and 1% asparagine (pH 7.0, 2% glucose) was used for MIC studies in *Candida* strains. RPMI medium (pH 7.0, 2% glucose) was used for MIC studies in *A. fumigatus*. HEPES was used instead of morpholinepropanesulfonic acid (MOPS), because MOPS was found to inhibit the activity of this kind of compounds. The compounds were serially diluted from 32 to 0.03 μg/mL, in a 96-well plate. The inoculum was prepared as described in the CLSI protocol M27A3 guidelines.[47] The plates were incubated at 37°C with 5% CO_2 for 24 to 72 h and the optical density was measure at 450 nm. The MICs were determined as the lowest concentration of the compound that inhibited 80% of growth compared to the control.

Cytotoxicity assay.

The human cancer cell lines A549 and HepG2 were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal bovine serum (FBS) and 1% penicillin-streptomycin. At passage 7, 10^5 cells/well in DMEM containing 10% FBS were transferred into 96-well plates and cultured for 14 h for the cells to adhere to the wells. The compounds were added to the cells at concentrations ranging from 0.03 to 128 μg/mL. The wells without the compound served as controls. The plate was incubated at 37 °C with 5% CO_2. After 24 or 48 h, the supernatant was removed, and 50 μl of 5-mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) solution in Phosphate-buffered saline (PBS) was added to each well. The plates were incubated for an additional 4h. The formazan crystal formed inside the cell was dissolved by adding 50 μL dimethyl sulfoxide (DMSO). The optical density was measured at 570 nm.

Time-kill assay.

C. neoformans cells from a culture grown overnight were washed in PBS and resuspended in YNB buffered with HEPES at pH 7.4. The cells were counted, and 2×10^4 cells were incubated with different concentration of the drugs in a final volume of 10 mL with a final concentration of 0.5% DMSO. The tubes were then incubated at 37 °C with 5% CO_2 on a rotary shaker at 200 rpm. Aliquots were taken at time points and diluted, and 100-l portions were plated onto yeast extract-peptone-dextrose (YPD) plates. YPD plates were incubated in a 30 °C incubator and after 48 h, the numbers of colony forming units (CFU) were counted and recorded.

Evaluation of synergism in drug combination.

Synergism/cooperativity in the combination of 5 lead compounds with clinical drugs was evaluated by calculating the fractional inhibitory concentration index (FICI). Briefly, in a 96-well plate, drug A (**5.2d**, **5.2c**, **5.6a**, **5.8** and **5.8a**) was serially diluted from 16 to 0.015 μg/mL (11 dilutions), whereas drug B (either fluconazole, amphotericin B, caspofungin, itraconazole, or voriconazole) was serially diluted from 12 to 0.19 μg/mL for fluconazole, 5 to 0.078 μg/mL for amphotericin B, or 8 to 0.007 μg/mL for caspofungin, itraconazole, or voriconazole (seven dilutions). The FICI is defined as ($MIC_{combined}/MIC_{drugA}$) + ($MIC_{combined}/MIC_{drugB}$).[31] The level of synergism/cooperativity was determined by using the following scale: strongly synergistic: FIC <0.5; synergistic: FIC <1; autonomous: FIC = 1; additive: FIC = 1~4; antagonistic: FIC >4.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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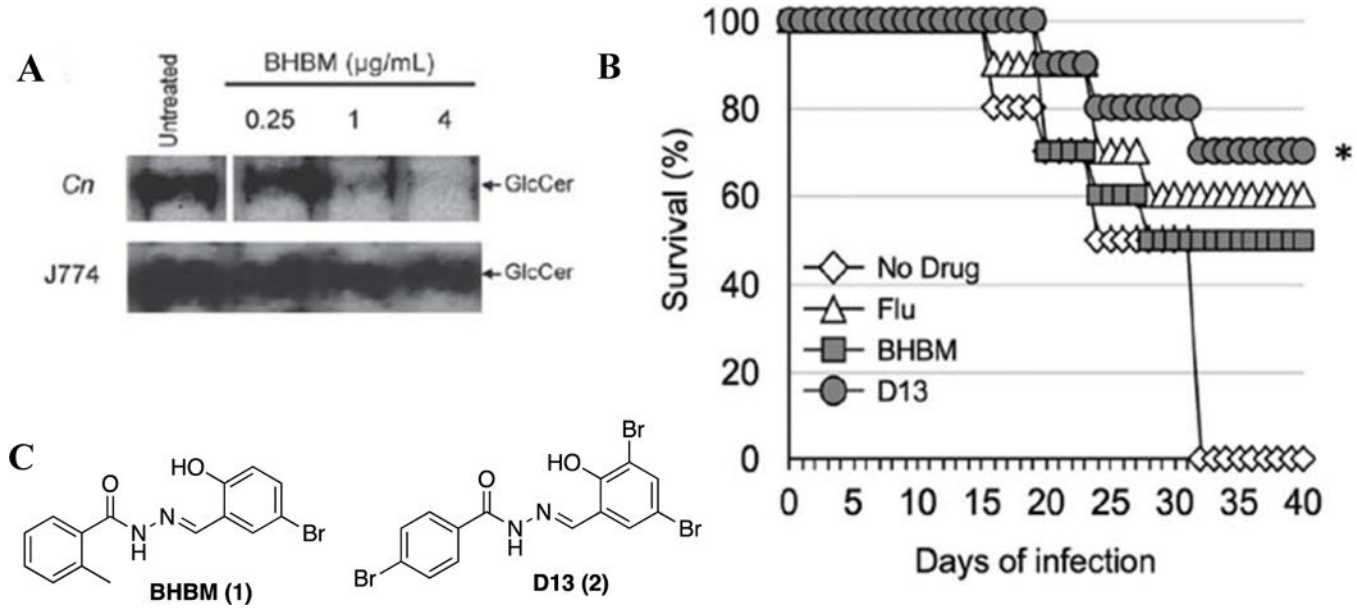


Figure 1.

(A) Analysis of the synthesis of glucosylceramide (GlcCer) in *C. neoformans* or J774 cells labelled with [^{3}H] palmitate and treated with **1** at the indicated concentrations; (B) Survival studies of mice infected intranasally with 5×10^5 *C. neoformans* cells and treated via i.p. injection on the day of infection with 1.2 mg/kg/day of fluconazole (Flu), **1** or **2**. *Compound **2** versus no drug, *P* value of 0.0018; (C) Structures of **1** (BHBM) and **2** (D13).

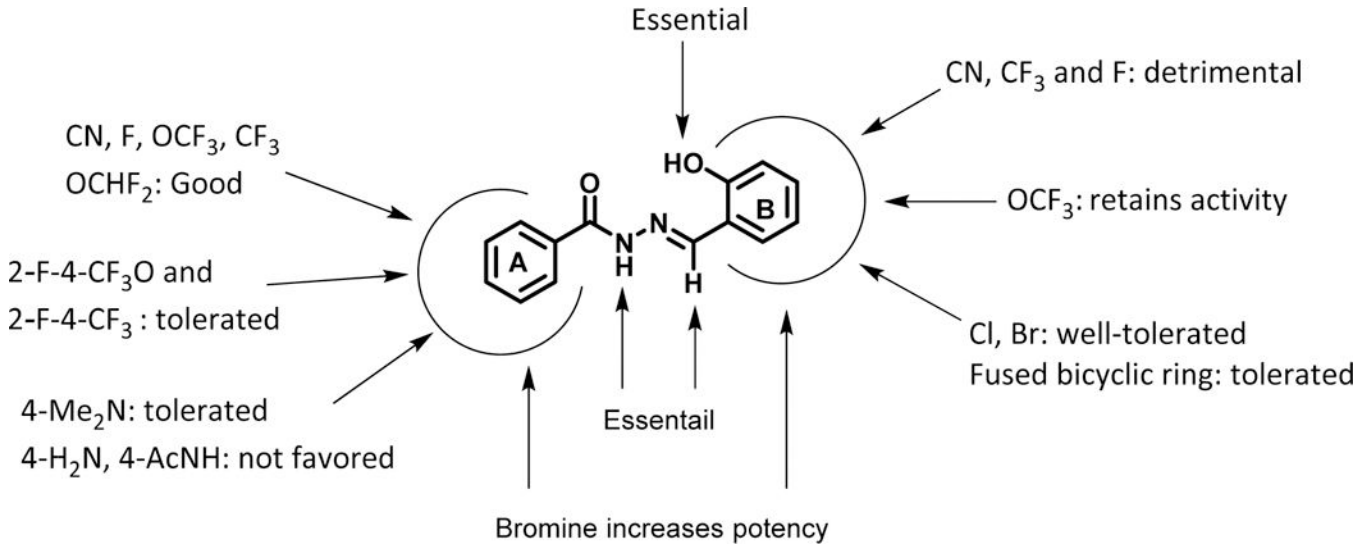


Figure 2.
SAR of aromatic acylhydrazones

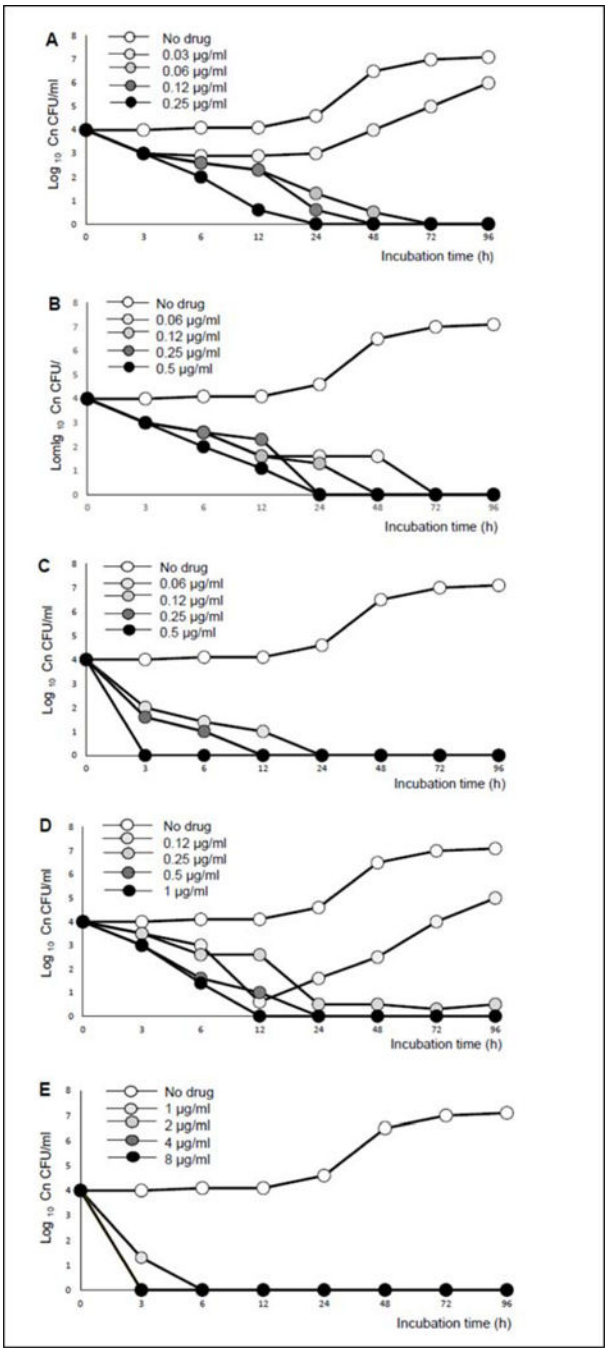
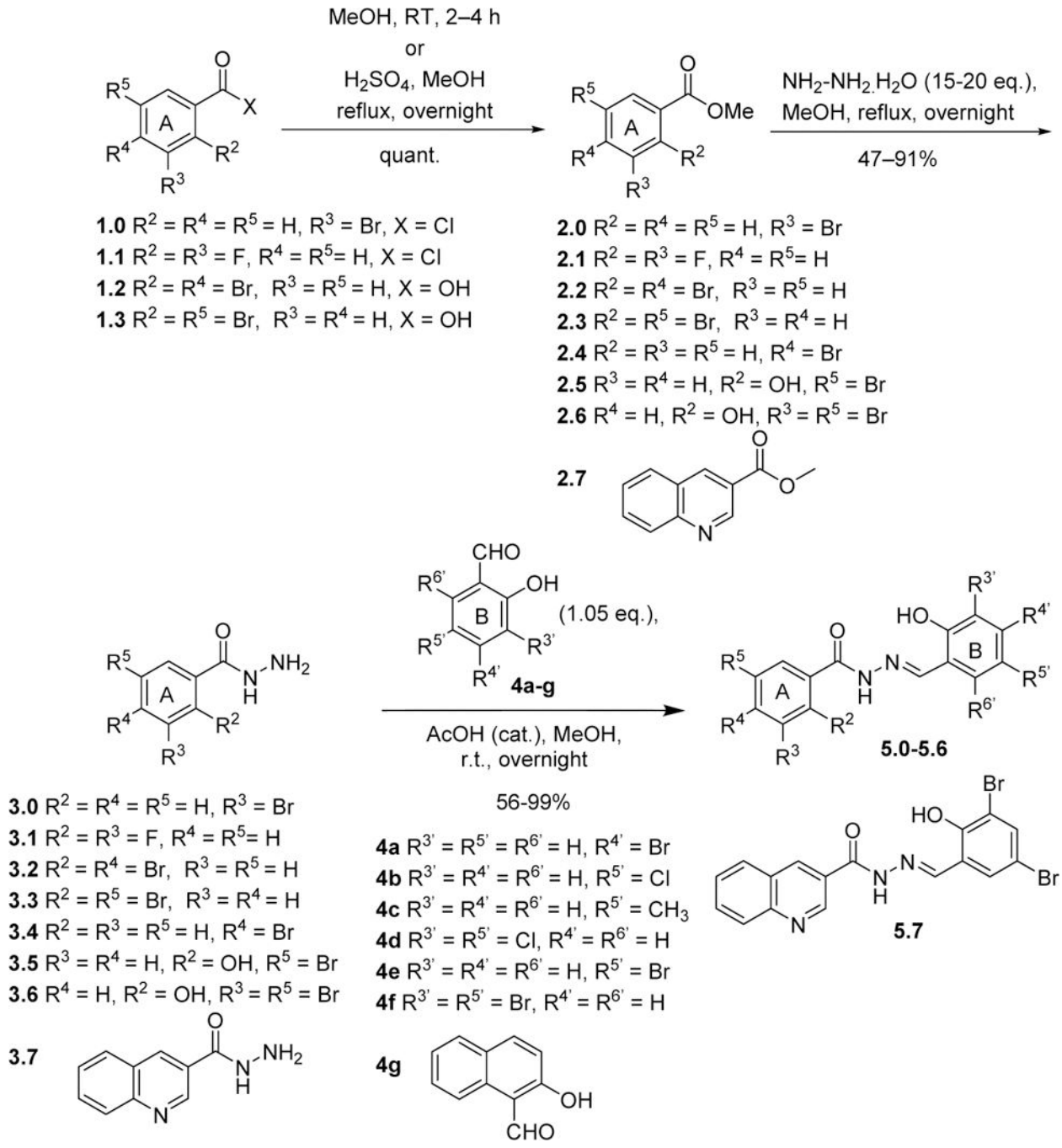


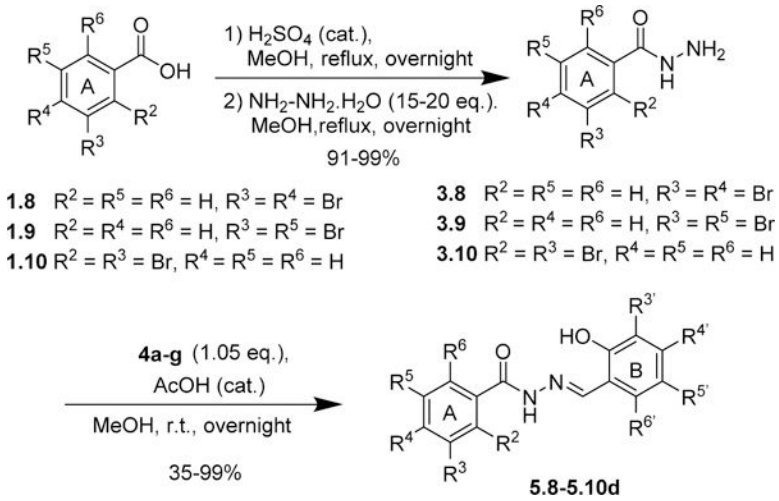
Figure 3.

In vitro time-kill activities of (A) **5.2d**, (B) **5.2e**, (C) **5.6a**, (D) **5.8** and (E) **5.8a**. Time-kill activity was determined using an in vitro time-kill kinetics assay in which the compounds were co-incubated with *C. neoformans* cells at 37°C, 5% CO_2, pH 7.4. The number of CFU is counted during 96 h of incubation. All of the compounds displayed antifungal activity in a dose-dependent manner.

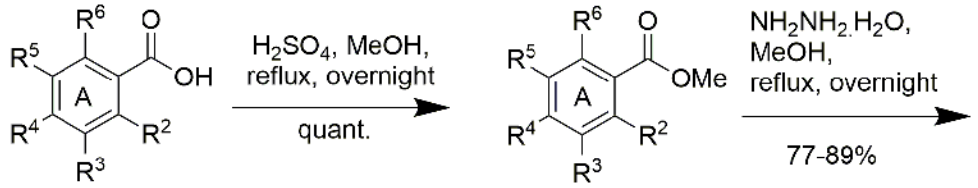


Scheme 1.

Synthesis of the initial library of acylhydrazones **5.0**~**5.7**

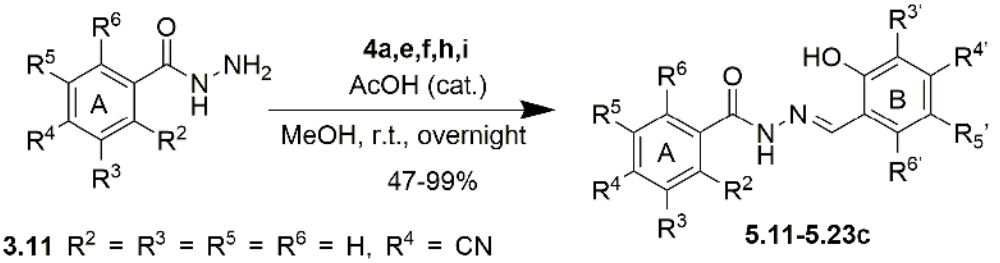


Scheme 2.



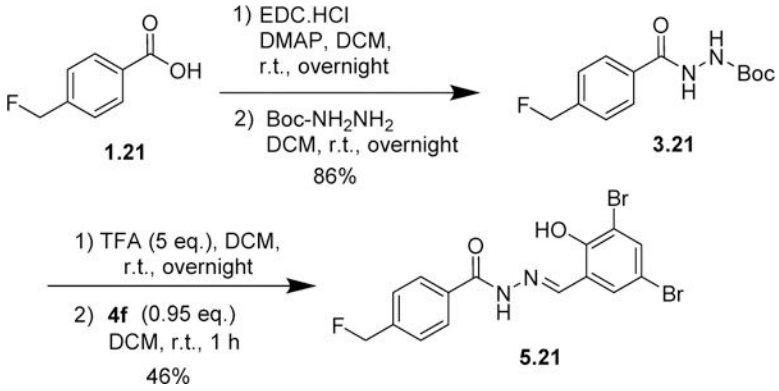
1.11 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = CN$
1.14 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = OCHF_2$
1.15 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = OCHF_2$
1.16 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = OCF_3$
1.17 $R^2 = F$, $R^4 = OCF_3$, $R^3 = R^5 = R^6 = H$
1.18 $R^2 = F$, $R^4 = CF_3$, $R^3 = R^5 = R^6 = H$
1.19 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = F$
1.20 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = F$

2.11 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = CN$
2.12 $R^3 = R^4 = R^5 = R^6 = H$, $R^2 = CF_3$
2.13 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = CF_3$
2.14 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = OCHF_2$
2.15 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = OCHF_2$
2.16 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = OCF_3$
2.17 $R^2 = F$, $R^4 = OCF_3$, $R^3 = R^5 = R^6 = H$
2.18 $R^2 = F$, $R^4 = CF_3$, $R^3 = R^5 = R^6 = H$
2.19 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = F$
2.20 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = F$
2.22 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = OCF_3$
2.23 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = CF_3$

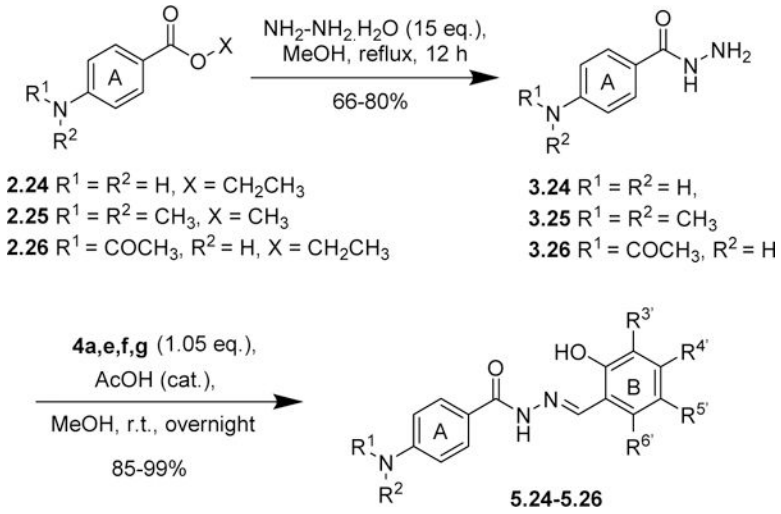


3.11 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = CN$
3.12 $R^3 = R^4 = R^5 = R^6 = H$, $R^2 = CF_3$
3.13 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = CF_3$
3.14 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = OCHF_2$
3.15 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = OCHF_2$
3.16 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = OCF_3$
3.17 $R^2 = F$, $R^4 = OCF_3$, $R^3 = R^5 = R^6 = H$
3.18 $R^2 = F$, $R^4 = CF_3$, $R^3 = R^5 = R^6 = H$
3.19 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = F$
3.20 $R^2 = R^3 = R^5 = R^6 = H$, $R^4 = F$
3.22 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = OCF_3$
3.23 $R^2 = R^4 = R^5 = R^6 = H$, $R^3 = CF_3$

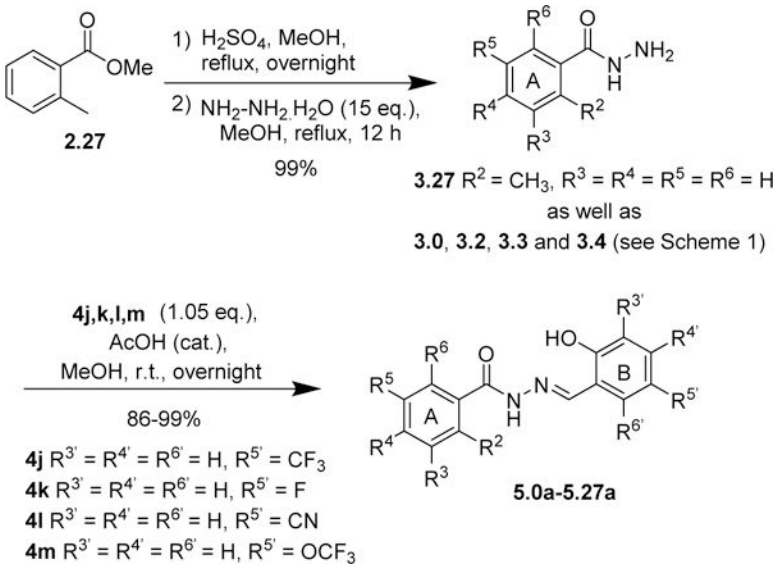
Scheme 3.



Scheme 4.

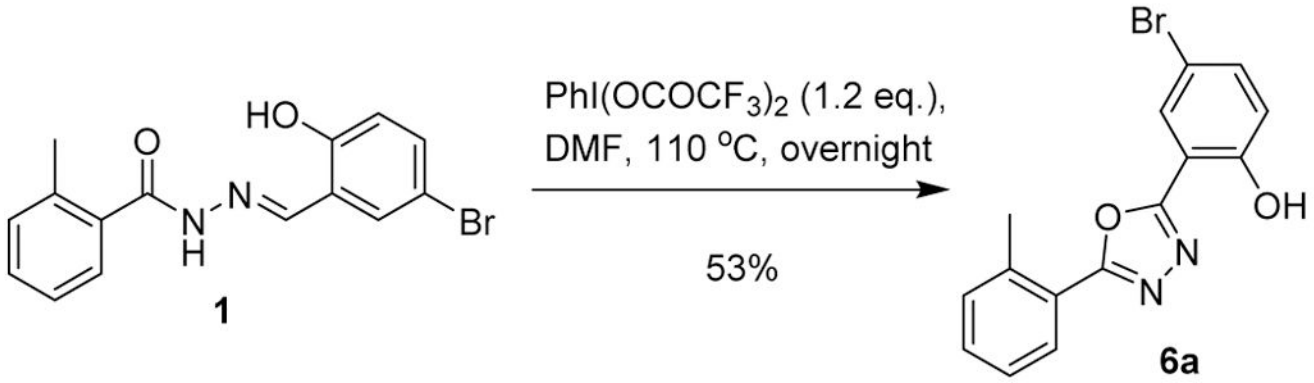


Scheme 5.

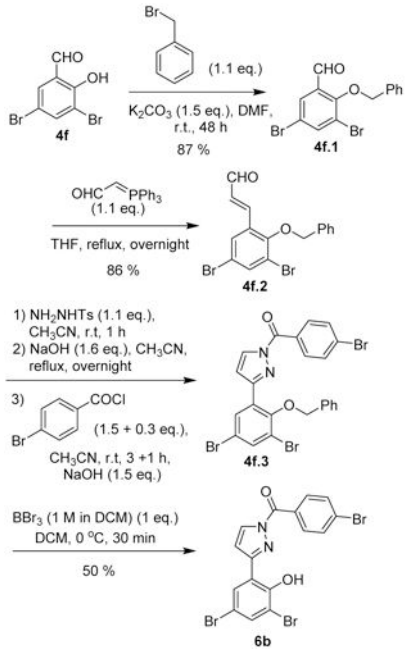


Scheme 6.

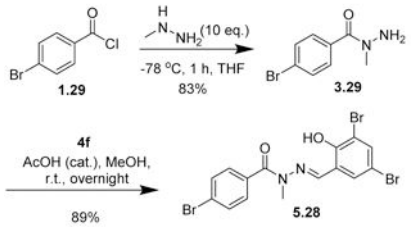
(a) Synthesis of oxadiazole mimicking acylhydrazone (**6a**)



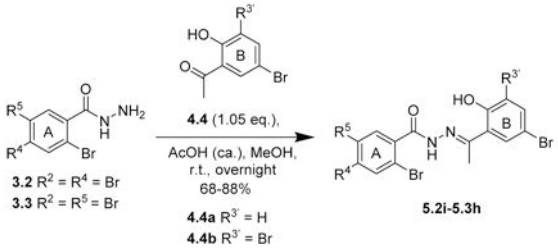
(b) Synthesis of acyldiazole mimicking acylhydrazone (**6b**)



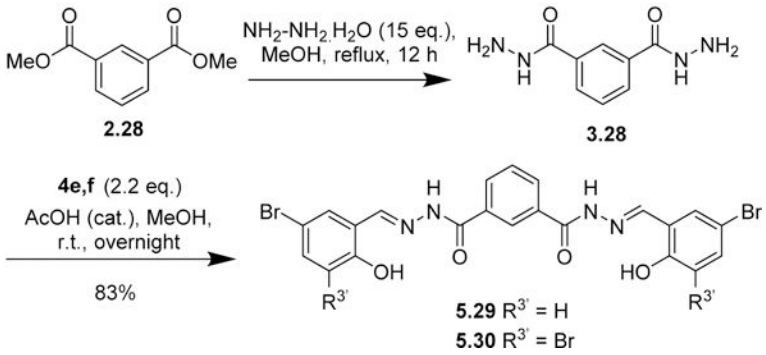
(c) Synthesis of *N*-methylacylhydrazone (**5.28**)



(d) Synthesis of *C*-methylacylhydrazones (**5.2i-5.3h**)



(e) Synthesis of bis-acylhydrazones (**5.29**, **5.30**)



Scheme 7.

Table 1.

Antifungal activity, time-kill activity (K100) and cytotoxicity of acylhydrazones 5.0~5.7 in the initial 20 compounds library (μg/mL)

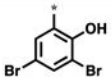
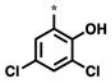
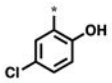
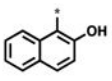
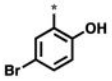
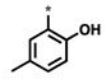
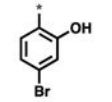
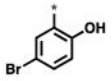
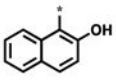
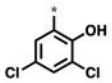
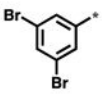
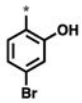
Compound	Ring A	Ring B	MIC_{80}[a]	K100[b]	A549[c] (LD_{50})	HepG2[c] (LD_{50})	SI[d] A549	SI[d] HepG2
5.0			0.5	0.25 (48 h)	64	8	128	16
5.1			1	0.5 (48)	72	32	72	32
5.1a			0.5	0.25 (48 h)	16	16	32	32

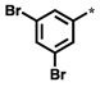
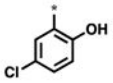
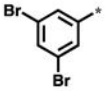
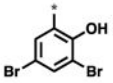
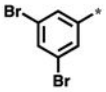
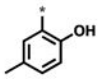
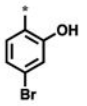
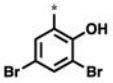
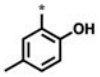
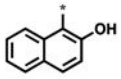
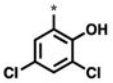
5.2			1	2 (24 h)	32	16	32	16
5.2a			0.25	1 (12 h)	64	64	256	256
5.2b			0.12	0.5 (48 h)	16	64	133.3	533.3
5.2c			1	0.5 (6 h)	128	64	128	64
5.2d			0.06	0.06 (72 h)	64	128	1066.6	2133.3
5.2e			0.06	0.06 (72 h)	64	64	1066.6	1066.6
5.3			1	0.5 (48 h)	32	32	32	32
5.3a			0.25	0.25 (24 h)	59	32	236	128
5.3b			0.25	0.25 (24 h)	32	16	128	64
5.3c			0.5	0.25 (24 h)	32	32	64	64
5.3d			0.12	0.25 (6 h)	32	16	266.6	133.3
5.3e			0.25	1 (48 h)	16	32	64	128
5.4			0.06	0.25 (96 h)	16	32	266.6	533.3
5.5			0.25	0.5 (24 h)	16	16	64	64
5.6			0.25	0.25 (24 h)	4	8	16	32
5.6a			0.06	0.25 (24 h)	32	32	533.3	533.3
5.7			0.25	Fungistatic	32	32	128	128

[a] Minimum concentration of the compound required to inhibit growth of 80% of *C. neoformans* cells.
[b] Minimum concentration of the compound required to show 100% killing of *C. neoformans.*
[c] Concentration of the compound required to kill 50% of mammalian cells.
[d] Ratio of LD_{50} value against mammalian cell line and MIC_{80} value against *C. neoformans*, constituting the "selectivity index".

Table 2.

Acylhydrazones 5.8~5.10 bearing dibromophenyl groups in ring A and their biological activities (μg/mL)

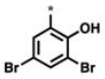
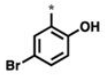
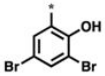
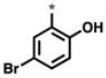
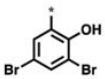
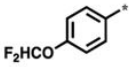
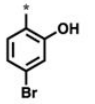
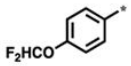
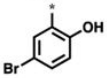
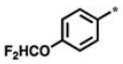
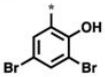
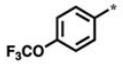
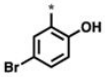
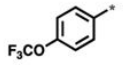
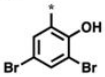
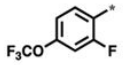
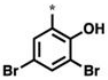
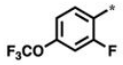
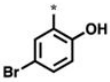
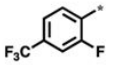
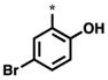
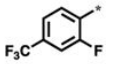
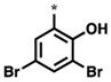
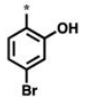
Compound	Ring A	Ring B	MIC_{80}[a]	K100[b]	A549[c] (LD_{50})	HepG2[c] (LD_{50})	SI A549[d]	SI HepG2[d]
5.8			0.06	0.12 (24 h)	64	64	1066.6	1066.6
5.8a			0.03	1 (6 h)	32	32	1066.6	1066.6
5.8b			0.06	1 (48 h)	16	32	266.6	533.3
5.8c			0.12	Fungistatic	4	16	33.3	133.3
5.8d			0.25	1 (72 h)	8	8	32	32
5.8e			0.12	0.5 (72 h)	16	16	133.3	133.3
5.8f			0.12	0.5 (72 h)	4	8	33.3	66.6
5.9			0.06	Fungistatic	16	16	266.6	266.6
5.9a			0.25	0.5 (24 h)	2	4	8	16
5.9b			0.5	0.5 (24 h)	64	64	128	128
5.9c			1	1 (48 h)	4	16	4	16

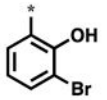
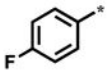
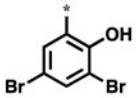
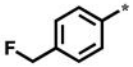
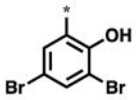
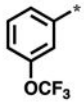
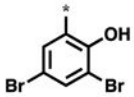
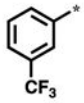
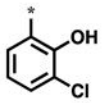
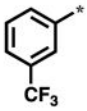
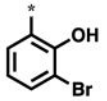
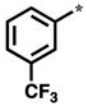
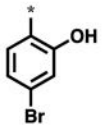
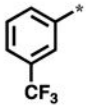
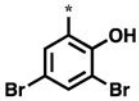
5.9d			0.12	1 (12 h)	16	16	133.3	133.3
5.9e			>16	-	-	-	-	-
5.9f			0.5	1 (24 h)	32	16	64	32
5.10			>16	-	-	-	-	-
5.10a			0.5	2 (48 h)	32	16	64	32
5.10b			>16	-	-	-	-	-
5.10c			>16	-	-	-	-	-
5.10d			1	1 (48 h)	64	16	64	16

[a-d] See the captions in Table 1.

Table 3.

Acylhydrazones bearing bromine-bioisosteres or fluorine-contaning groups in ring A and their biological activities (μg/mL)

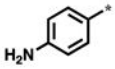
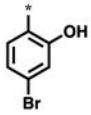
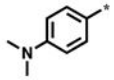
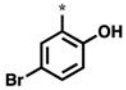
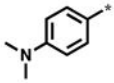
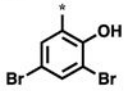
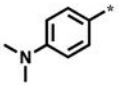
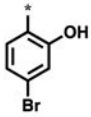
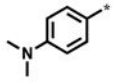
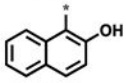
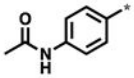
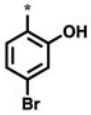
Compound	Ring A	Ring B	MIC_{80}[a]	K100[b]	A549[c] (LD_{50})	HepG2[c] (LD_{50})	SI[d] A549	SI[d] HepG2
5.11			0.06	1 (72 h)	64	16	1066.6	266.6
5.12			1	1 (72 h)	16	16	16	16
5.13			0.12	1 (12 h)	4	8	33.3	33.3
5.14			0.12	1 (24 h)	8	64	66.6	533.3
5.14a			0.12	0.25 (96 h)	16	16	133.3	133.3
5.15			0.12	1 (24 h)	32	32	266.6	266.6
5.15a			0.25	2 (72 h)	32	16	128	64
5.15b			0.12	1 (6 h)	16	16	133.3	133.3
5.16			0.06	1 (12 h)	4	16	66.6	266.6
5.16a			0.5	4 (72 h)	64	16	128	32
5.17			0.06	0.5 (48 h)	16	16	266.6	266.6
5.17a			0.5	0.5 (72 h)	64	8	128	16
5.18			0.5	1 (48 h)	16	16	32	32
5.18a			0.12	0.5 (48 h)	64	16	533.3	133.3
5.19			0.5	Fungistatic	32	32	64	64

5.19a			1	2 (12 h)	16	16	16	16
5.20			0.12	0.25 (24 h)	32	32	266.6	266.6
5.21			0.5	0.5 (24 h)	16	16	32	32
5.22			4	-	-	-		
5.23			1	2 (96 h)	32	32	32	32
5.23a			1	2 (48 h)	16	16	16	16
5.23b			0.5	1 (48 h)	64	16	128	32
5.23c			2	-	-	-	-	-

[a-d] See the captions in Table 1.

Table 4.

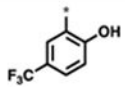
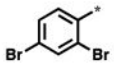
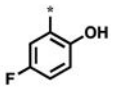
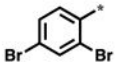
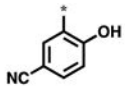
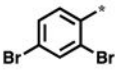
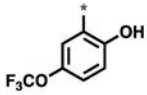
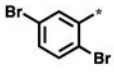
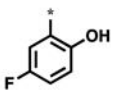
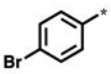
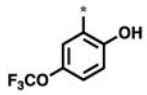
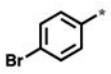
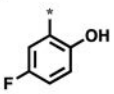
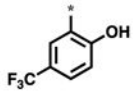
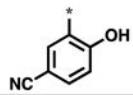
4-Aminobenzoylhydrazones 5.24~5.26 and their biological activities (μg/mL)

Compound	Ring A	Ring B	MIC_{80}[a]	K100[b]	A549[c] (LD_{50})	HepG2[c] (LD_{50})	SI A549[d]	SI HepG2[d]
5.24			4	-	-	-	-	-
5.25			>16	-	-	-	-	-
5.25a			0.5	Fungistatic	16	16	32	32
5.25b			0.25	Fungistatic	16	16	64	64
5.25c			0.5	2 (24 h)	32	16	64	64
5.26			>16	-	-	-	-	-

[a-d] See the captions in Table 1.

Table 5.

Acylhydrazones with CN, CF_3, F or OCF_3 substitution in ring B and their biological activities (μg/mL)

Compound	Ring A	Ring B	MIC_{80}[a]	K100[b]	A549[c] (LD_{50})	HepG2[c] (LD_{50})	SI[d] A549	SI[d] HepG2[d]
5a			0.5	Fungistatic	16	16	32	32
5.2f			1	2 (96 h)	16	16	16	16
5.2g			8	-	-	-	-	-
5.2h			0.25	2 (24 h)	32	64	128	256
5.3f			>16	-	-	-	-	-
5.4a			0.25	1 (72 h)	16	64	64	256
5.4b			1	4 (96 h)	8	16	8	16
5.27			8	-	-	-	-	-
5.27a			4	-	-	-	-	-

[a-d] See the captions in Table 1.

Table 6.

Triclyclic, *N*-methylated and *C*-methylated acylhydrazones, bis-hydrazones and their MIC_{80} values (μg/mL)

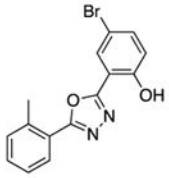
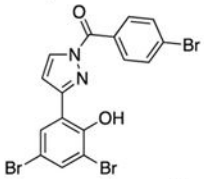
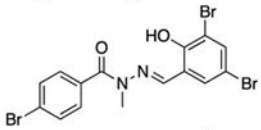
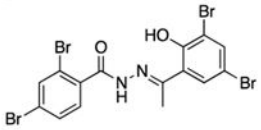
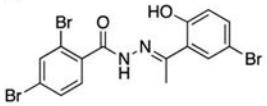
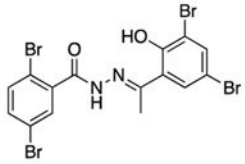
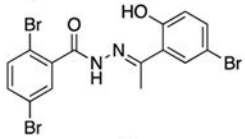
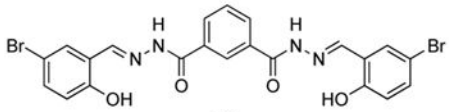
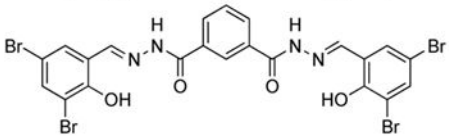
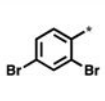
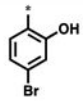
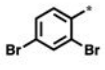
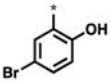
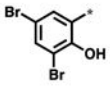
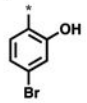
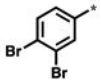
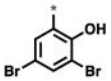
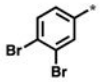
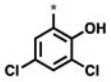
Compound	Structure	MIC_{80}
6a		>16
6b		>16
5.28		>16
5.2i		>16
5.2j		>16
5.3g		>16
5.3h		>16
5.29		>16
5.30		>16

Table 7.

Five most potent and selective acylhydrazones and their biological activities (μg/mL)

Compound	Ring A	Ring B	MIC_{80}[a]	K100[b]	A549[c] (LD_{50})	HepG2[c] (LD_{50})	SI[d] A549	SI[d] HepG2
5.2d			0.06	0.06 (72 h)	64	128	1066.6	2133.3
5.2e			0.06	0.06 (72 h)	64	64	1066.6	1066.6
5.6a			0.06	0.25 (12 h)	32	32	533.3	533.3
5.8			0.06	0.12 (24 h)	64	64	1066.6	1066.6
5.8a			0.03	1 (6 h)	32	32	1066.6	1066.6

[a-d] See the captions in Table 1.

Table 8.

MIC_{80} (μg/mL) values of lead compounds against different pathogenic strains of fungi

Compound	*C. neoformans*	*C. albicans*	*C. auris*	*C. krusei*	*C. krusei* R	*C. parapsilosis*	*A. fumigatus*
5.2d	0.06	>16	>16	>16	>16	8	>16
5.2e	0.06	>16	>16	8	>16	8	>16
5.6a	0.06	2	0.5	0.25	0.25	2	4
5.8	0.06	>16	16	>16	4	16	>16
5.8a	0.03	2	0.5	0.25	0.25	1	8

Table 9.

FICI values of 5.2d combined with clinical drugs against 7 fungal strains

Fungal strain	5.2d/Flu*	5.2d/Vori*	5.2d/Itra*	5.2d/Caspo*	5.2d/AB*
C. neoformans	0.065	0.072	0.072	ND	0.086
C. albicans	0.625	0.256	0.128	0.502	ND
Ck Flu R	2	0.281	0.252	2	ND
Ck6258	2	1.250	1.063	2	ND
C. auris	2	1.016	0.508	2	ND
C. parapsilosis	1.002	0.076	0.124	2	ND
A. fumigatus	2	0.515	0.484	ND	ND

*Flu: fluconazole; Vori: voriconazole; Itra/itraconazole; Caspo: caspofungin; AB: amphotericin B.

Table 10.

FICI values of 5.2e combined with clinical drugs against 7 fungal strains

Fungal strain	5.2e/Flu	5.2e/Vori	5.2e/Itra	5.2e/Caspo	5.2e/AB
C. neoformans	0.065	0.072	0.072	ND	0.086
C. albicans	2	0.256	0.128	0.502	ND
Ck Flu R	2	1.063	0.375	2	ND
Ck6258	1.002	1.060	2.015	1.002	ND
C. auris	2	1.125	1.016	2	ND
C. parapsilosis	1.015	0.135	2.098	1.5	ND
A. fumigatus	2	0.515	0.484	ND	ND

For abbreviation of drug names, see the captions in Table 9.

Table 11.

FICI values of 5.6a combined with clinical drugs against 7 fungal strains

Fungal strain	5.6a/Flu	5.6a/Vori	5.6a/Itra	5.6a/Caspo	5.6a/AB
C. neoformans	0.240	0.240	0.247	ND	0.261
C. albicans	2	0.375	0.188	0.508	ND
Ck Flu R	0.00016	0.018	0.023	0.0005	ND
Ck6258	0.501	0.129	0.183	2.002	ND
C. auris	0.531	0.252	0.180	2	ND
C. parapsilosis	1.015	0.135	0.508	2	ND
A. fumigatus	2	0.188	1.016	ND	ND

For abbreviation of drug names, see the captions in Table 9.

Table 12.

FICI values of 5.8 combined with clinical drugs against 7 fungal strains

Fungal strain	5.8/Flu	5.8/Vori	5.8/Itra	5.8/Caspo	5.8/AB
C. neoformans	0.237	0.247	0.247	ND	0.247
C. albicans	0.563	0.271	0.370	0.500	ND
Ck Flu R	0.501	0.310	0.528	0.121	ND
Ck6258	1	0.254	0.153	0.125	ND
C. auris	1.001	1.001	1.12	0.501	ND
C. parapsilosis	2.015	1.03	2.007	1.001	ND
A. fumigatus	2	1.031	0.281	ND	ND

For abbreviation of drug names, see the captions in Table 9.

Table 13:

FICI values of 5.8a combined with clinical drugs against 7 fungal strains

Fungal strain	5.8a/Flu	5.8a/Vori	5.8a/Itra	5.8a/Caspo	5.8a/AB
C. neoformans	0.237	0.247	0.247	ND	0.247
C. albicans	0.252	0.077	0.091	1.00	ND
Ck Flu R	0.501	1.030	1.240	1.004	ND
Ck6258	0.250	0.560	0.530	1.001	ND
C. auris	0.500	0.500	1.12	0.500	ND
C. parapsilosis	0.258	1.500	1.250	0.501	ND
A. fumigatus	2	1.031	1.008	ND	ND

For abbreviation of drug names, see the captions in Table 9.