

Acylhydrazide Schiff Bases: Synthesis and Antiglycation Activity

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Summary: Acylhydrazide Schiff bases **1-27** were synthesized and their *in vitro* antiglycation potential was evaluated. Compounds **16** (IC₅₀ = 199.82 ± 10.6 μM), **27** (IC₅₀ = 234.83 ± 10.28 μM), **2** (IC₅₀ = 240.99 ± 4.2 μM), and **14** (IC₅₀ = 276.2 ± 2.3 μM) showed antiglycation potential comparable to the standard rutin (IC₅₀ = 294.50 ± 1.5 μM). From this study we identified a new series of potent antiglycating agents. A structure-activity relationship has been described, while all compounds were characterized by using different spectroscopic techniques.

Keywords: Acylhydrazide Schiff bases, antiglycation, AGEs.

Introduction

Schiff bases have a range of applications in many fields, such as medicinal and analytical chemistry [1-3]. Schiff bases of various heterocyclic compounds were reported to possess antiproliferative [4], anticonvulsant [5], cytotoxic [6], anticancer, antifungal, and anti-HIV activities [7, 8]. A number of acylhydrazide Schiff bases have shown interesting bioactivities such as antibacterial, antifungal, anticonvulsant, antiinflammatory, antimalarial, analgesic, antiplatelets, antituberculosis, anticancer [9-17], insecticidal, antilishmanial, antimycobacterial, adriamycin immunokonjugates, and proteinase inhibition against *Trypanosoma brucei* [18-22]. *N*-Cyanoethyl hydrazide derivatives have shown β-glucuronidase inhibition [23]. Furthermore, substituted acylhydrazide Schiff bases heterocyclic derivative also have been reported as anticancer [24], antiinflammatory, and antitubercular activity [25]. Substituted hydrazines have found to be considerable commercial applications [26]. These compounds are used as reagents in organic chemistry, e.g. phenyl hydrazine the oldest hydrazine reagent, played an important role in the synthesis of crystalline derivatives of carbohydrates. A wide spectrum of heterocycles becomes easily accessible by hydrazide derivatives [27] e.g. diacylhydrazides can be cyclized to 1,3-oxa- and thiazoles and 1,2,4-triazoles [28].

In recent past we have reported antilishmanial activities of acylhydrazides [29].

In continuation of our search for new *in vitro* antiglycation agent, a series of twenty seven (27) acylhydrazides Schiff bases have been evaluated. Hyperglycemia during diabetes is responsible of long-term complications such as cataract, retinopathy, neuropathy, nephropathy, atherosclerosis, embryopathy, and delayed healing of wounds [30-32]. The exact mechanism underlying the pathogenesis is the initial step in the Maillard reaction and proceeds when a sugar carbonyl group condenses. Food scientists have been interested in the Maillard reaction for many years because it is related with food spoilage and changed taste. Protein glycation, also referred to as non-enzymatic glycosylation, is the initial step in the Maillard reaction and proceed when a sugar carbonyl group condenses with a protein amino group to form a labile Schiff base that afterward rearranges to Amadori product. Formation of labile Schiff bases take hours, whereas formation of Amadori products take number of days. Glycation products form spontaneously whenever proteins are mixed with reducing sugars and are reliant on the degree and period of hyperglycemia *in vivo*. Glycated proteins can suffer further changes involving dicarbonyl

intermediates which may lead to the formation of advanced glycation end products (AGEPs). Little is known about the chemistry of AGEPs, and only a few have been studied and characterized. In general, AGEPs can form fluorescent crosslinked structures, for example pentosidine [33], non-fluorescent crosslinked compounds such as arginine-lysine imidazole cross-link [34], or non cross-linked structures, for instance pyrrolidine [35]. Development of safe and attractive antiglycation agents is a key approach for the genuine treatment of late diabetic complications. So far only a few antiglycating agents have been discovered [36], and the need of new antiglycating agents is remain unmet [37].

Results and Discussion

Chemistry

In the first step of the synthesis of acylhydrazone Schiff bases, esters were prepared from different (aromatic heterocyclic or aliphatic) acids. Then the esters were treated with hydrazine hydrate and reflux for 2 h to obtain the corresponding acylhydrazone, which were recrystallized by methanol in good yields. Acylhydrazone Schiff bases were synthesized by condensing different acylhydrazides with different aromatic aldehydes and acetophenones by refluxing in ethanol for 2 to 3 hours and reactions were monitored by TLC (Scheme-1). The crude products were re-crystallized by methanol and needle-like crystal was obtained in most of the cases. The structures of acylhydrazone Schiff bases **1-27** were determined by using spectroscopic techniques including, ¹H NMR, and mass spectrometry. All compounds gave satisfactory elemental analysis.

Antiglycation Studies

We have recently published unsymmetrical disubstituted urea derivatives as new class of potent antiglycating agents [38]. In the present study, we have synthesized acylhydrazone Schiff bases **1-27** and evaluated for their *in vitro* antiglycation potential. Compounds **1-27** showed a varying degree of antiglycation activities having IC₅₀ values ranging between 199.48-652.62 μM, comparable with the standard rutin (IC₅₀ = 294.46 ± 1.50 μM) (Table-1). Compounds **16** (IC₅₀ = 199.82 ± 10.6 μM), **27** (IC₅₀ = 234.83 ± 10.28 μM), **2** (IC₅₀ = 240.99 ± 4.2 μM), and **14** (IC₅₀ = 276.23 ± 2.3 μM) showed outstanding antiglycation activity, better than the standard rutin. Compounds **7** (IC₅₀ = 346.21 ± 1.4 μM), **11** (IC₅₀ = 356.73 ± 7.3 μM), **9** (IC₅₀ = 365.67 ± 1.4 μM), **10** (IC₅₀ = 406.62 ± 5.2 μM), and **4** (IC₅₀ = 422.95 ± 10.8 μM), also possess good antiglycation potential.

Similarly compounds **6** (IC₅₀ = 545.90 ± 9.8 μM), **12** (IC₅₀ = 652.62 ± 7.0 μM) and **17** (IC₅₀ = 664.81 ± 12.57 μM) were the least active among the series. Compounds **1, 2, 3, 5, 8, 13, 15, 17, and 18-26** were found to be completely inactive.

An initial exploration of structure-activity relationship (SAR) studies was carried out with the synthesis of a range of analogues bearing substitutions on each ring acyl hydrazides Schiff bases. Substitution on both benzylidene and acylium part of the Schiff base affect the activity (Figure-1). The comparison of the activity of most active compound **16** (IC₅₀ = 199.82 ± 10.6 μM) of the series and its analogs **2** (IC₅₀ = 240.99 ± 4.2 μM), **14** (IC₅₀ = 276.23 ± 2.3 μM), **7** (IC₅₀ = 346.21 ± 1.4 μM), **11** (IC₅₀ = 356.73 ± 7.3 μM), **9** (IC₅₀ = 365.67 ± 1.4 μM), **10** (IC₅₀ = 406.62 ± 5.2 μM), and **4** (IC₅₀ = 422.95 ± 10.8 μM), indicated that the substitution on benzylidene portion of molecules effect the activity which contain dihydroxy functionalities. Activity related to all these compounds might be due to acetal formation between dihydroxy groups of the molecules and the carbonyl groups of methylglyoxal. The difference in activity of the different analogs is largely depending upon its efficiency for acetal formation ability. The compound **16** was found to be the most active, whereas, **2** and **11** having same structural features, except the substitution on double bond in the benzylidene part of the molecule showed less antiglycation activity. Compounds **2** having (CH₃) on benzylidene part and **11** having only (H), the difference in activity between these analogs may due to difference in substitution on double bond of benzylidene part of molecule. If one of the hydroxyl groups is *para* to double bond, as in case of compound **16**, it shown utmost activity. The hydroxyl groups at *para* to double bond, enhances the ability of acetal formation with methylglyoxal, resulted in excellent antiglycation activity.

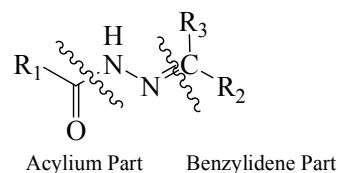
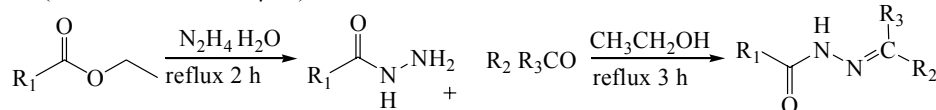


Fig. 1: Acylium and Bezylidene Parts of Molecule

The compound **14** which has a nitro group at *para* position of benzene ring in acylium part instead of *ortho* hydroxyl group as in compound **16**, has an IC₅₀ value 276.23 ± 2.3 μM, whereas compound **10** have a bromo substituent at the same position showed an IC₅₀ = 406.62 ± 5.2 μM. Compounds **4** (IC₅₀

422.95 ± 10.8 μM), **10** (IC₅₀ 406.62 ± 5.2 μM), **14** (IC₅₀ 276.23 ± 2.3 μM) have methoxy, bromo and nitro group at *para* position of benzene ring in acylium part instead of *ortho* hydroxyl group as in compound **16** (IC₅₀ 199.825 ± 10.6 μM). Similarly compound **9** (IC₅₀ 365.67 ± 1.4 μM) has different

acylium part, but all these compounds have the same benzene ring substitution that is *ortho* and *para* hydroxyl group. The difference in activity of these compounds may be due to change in substitution in acylium part of the molecule.



Scheme-1: Synthesis of Schiff base of acylhydrazone **1-27**

Table-1: Synthesis and *in vitro* antiglycation activity of compounds **1-27**.

Comp	R ₁	R ₂	R ₃	IC ₅₀ ± SEM ^a (μM)
1			CH ₃	NA ^b
2			CH ₃	240.99 ± 4.2
3			H	NA ^b
4			CH ₃	422.95 ± 10.8
5			H	NA ^b
6	H ₃ C-(CH ₂) ₇		H	545.90 ± 9.8
7			CH ₃	346.21 ± 1.4
8			H	NA ^b
9			CH ₃	365.67 ± 1.4
10			H	406.62 ± 5.2
11			H	356.73 ± 7.3
12			H	652.62 ± 7.0
13			H	NA ^b
14			H	276.23 ± 2.3
15			H	NA ^b
16			H	199.82 ± 10.6
17			H	664.81 ± 12.57
18			H	NA ^b
19			H	NA ^b
20			H	NA ^b
21			H	NA ^b
22			CH ₃	NA ^b
23			CH ₃	NA ^b
24			H	NA ^b

25			H	NA ^b
26			H	NA ^b
27			H	234.83 ± 10.28
			Rutin ^c	294.46 ± 1.50

SEM^a is the standard error of the mean, NA^b Not active, Rutin,^c standard inhibitor for anti-glycation activity.

Compounds **2** and **7** are structurally similar except their acylium part and the obvious activity difference may be due to an additional acetal formation by hydroxyl group present on benzene ring in acylium part of molecule **2**, whereas it lacks in compound **7**.

A major activity difference between compounds **7** ($IC_{50} = 364.21 \pm 1.4 \mu M$), **17** ($IC_{50} = 664.81 \pm 12.57 \mu M$) and **27** ($IC_{50} = 234.83 \pm 10.28 \mu M$) may be due to change in the benzilidium part of these molecules. Compound **27** contains a *para* hydroxyl group on the benzene ring present in benzylidene part, which enhances ability for acetal formation than an *ortho* hydroxyl group, as in case of compounds **7** and **17**, which have *ortho* and *para* hydroxyl group, respectively. Contrary the compounds **3**, **20**, **23** and **25** have same acylium group but having *p*-chloro, *o*-hydroxy, *o*-bromo in **3**, **23** and **25**, respectively, and compound **20** have a naphthyl group, caused the complete loss of activity. These results indicate that hydroxyl group at *para* position is responsible for the good activity. Although compound **6** ($IC_{50} = 545.90 \pm 9.8 \mu M$) has a *para* hydroxyl group but showed very low activity which may be due to aliphatic acylium part of the molecule. Compounds **1**, **5**, **13**, **15**, **22**, and **23** containing *ortho* hydroxyl groups in benzilidium part of the molecules but having different acylium part were found to be completely inactive due to steric hindrance. This may result in their failure to form acetal linkage with methyl glyoxal. Furthermore there was a possibility of unstable hemiacetal formation which does not contribute in the overall antiglycation effect of these molecules.

Compounds **8** and **12** having the same benzilidium part but acylium part is *para*-chloro substituted benzene in compound **8** while in compound **12** it is methyl substituted instead of chloro group. Compound **12** was found to be weakly active ($IC_{50} = 652.62 \pm 7.0 \mu M$), whereas compound **8** was

found to be completely inactive due to difference in substituents on acylium part.

Compounds **3**, **18-21**, and **24-26** were found completely inactive due to inappropriate substituted benzilidium as well as acylium parts of the compounds which is prerequisite in this type of molecules and already been established in the foregoing discussion.

From this study it is established that hydroxyl groups at an appropriate position of benzilidium or acylium or both parts of acylhydrazone Schiff bases play important role in antiglycation activity of this class of molecules which is also confirmed by considering the structure of standard rutin that has a number of hydroxyl groups in its skeleton which is responsible for its antiglycation potential.

In conclusion, compounds **16** ($IC_{50} = 199.82 \pm 10.6 \mu M$), **27** ($IC_{50} = 234.83 \pm 10.28 \mu M$), **2** ($IC_{50} = 240.99 \pm 4.2 \mu M$), and **14** ($IC_{50} = 276.2 \pm 2.3 \mu M$) have shown excellent antiglycation potential more potent than the standard rutin, ($IC_{50} = 294.50 \pm 1.5 \mu M$). These compounds can therefore serve as lead compounds for further studies in this field.

General Experimental

NMR experiments were performed on Avance Bruker AM 300 and 500 MHz, CHN analysis was performed on a Carlo Erba Strumentazione-Mod-1106, Italy. Ultraviolet Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

Assay for Antiglycation

Chemicals

Bovine serum albumin (BSA) was purchased from the Research Organics (Cleveland, USA), while other chemicals {glucose anhydrous, trichloroacetic acid (TCA) sodium azide (NaN_3), dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate (NaH_2PO_4), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), and sodium hydroxide (NaOH) were purchased from Sigma Aldrich, USA. Sodium phosphate buffer (pH 7.4), was prepared by mixing

Na_2HPO_4 and NaH_2PO_4 (67 mM) containing sodium azide (3 mM). Phosphate buffer saline (PBS) was prepared by mixing NaCl (137 mM), Na_2HPO_4 (8.1 mM), KCl (2.68 mM), and KH_2PO_4 (1.47 mM) and pH 10 was adjusted with NaOH (0.25 mM). BSA (10 mg/mL) and glucose anhydrous (50 mg/mL) solutions were prepared in sodium phosphate buffer. Test samples were prepared in DMSO (1 mM/mL).

This test was used to evaluate the ability of the candidate compounds to inhibit the methyl glyoxal mediated development of fluorescence of BSA. Activity was performed by using the reported method [39, 40] with the following modifications:

Triplicate samples of BSA 100 mg/mL, 14 mM MGO, 0.1M phosphate buffer (pH 7.4) containing NaN_3 (30 mM) was incubated under aseptic conditions at 37 °C for 9 days in the presence or absence of various concentrations of the test compounds. After 9 days of incubation, each sample was examined for the development of specific fluorescence (excitation, 330 nm; emission, 440 nm) on a microtitre plate spectrophotometer (Spectra Max, Molecular Devices, USA). Rutin was used as a positive control ($\text{IC}_{50} = 294 \mu\text{M} \pm 1.50 \text{ SEM}$).

The percent inhibition of AGE formation in the test sample versus control was calculated for each compound by using the following formula:

$$\% \text{ inhibition} = (1 - \text{fluorescence of test sample} / \text{fluorescence of the control group}) \times 100$$

General Procedure for the Synthesis of Compounds 1-27

The acylhydrazide Schiff bases were synthesized by refluxing in ethanol a mixture 2 mmol of acylhydrazine with 2 mmol of different aldehyde or acetophenone and catalytical amount of acetic acid for 3 h. The progress of reaction was monitored by TLC. After completion of reaction, the solvent was evaporated by vacuum to afford crude products **1-27**, which were further recrystallized in methanol and got needle like pure product in good to excellent yields.

N'-(1-(2-Hydroxyphenyl)ethylidene)nicotino-hydrazide (**1**)

Yield: 0.90 g (91%); $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$): δ 13.26 (s, 1H, OH), 11.51 (br s, 1H, NH), 9.07 (d, 1H, $J_{2,4} = 1.0$ Hz, H-2), 8.77 (dd, 1H, $J_{4,5} = 5.0$ Hz, $J_{4,2} = 1.0$ Hz, H-4), 8.27 (dd, 1H, $J_{3',4'} = 7.5$ Hz, $J_{3',5'} = 1.0$ Hz, H-3'), 7.64 (d, 1H, $J_{6',5'} = 7.5$ Hz, H-6'), 7.57 (dd, 1H, $J_{5,4} = 5.0$ Hz, $J_{5,6} = 8.0$ Hz, H-5), 7.31 (dt, 1H, $J_{5',3'} = 1.0$ Hz, $J_{5'(4',6')} = 7.5$ Hz, H-

5'), 6.91 (d, 1H, $J_{6,5} = 8.0$ Hz, H-6), 6.90 (t, 1H, $J_{4(3,5')} = 7.5$ Hz, H-4'), 3.32 (s, 3H, CH_3), Anal. Calcd for $\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}$ C = 69.69, H = 6.27, N = 17.41, Found C = 69.67, H = 6.23, N = 17.40; EI MS m/z (% rel. abund.): 255 (M^+ , 58.7), 240 (42.2), 135 (11.6), 106 (100), 78 (91.1), 51 (24.1).

N'-(1-(2,5-Dihydroxyphenyl)ethylidene)-2-hydroxybenzohydrazide (**2**)

Yield: 0.75 g (85%); $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 12.38 (s, 1H, NH), 11.52 (br s, 2H, OH), 8.92 (br s, 1H, OH), 7.98 (dd, 1H, $J_{4',6'} = 1.5$ Hz, $J_{4',3'} = 8.7$ Hz, H-4'), 7.46 (dt, 1H, $J_{5,3} = 1.5$ Hz, $J_{5,(4,6)} = 8.4$ Hz, H-5), 7.05-6.98 (m, 3H, H-3, H-4, H-6), 6.78 (d, 1H, $J_{3',4'} = 8.7$ Hz, H-3'), 6.74 (d, 1H, $J_{4',6'} = 1.5$ Hz, H-6') 2.49 (s, 3H, CH_3); Anal. Calcd for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_3$ C = 66.16, H = 5.92, N = 10.29, Found C = 66.12, H = 5.88, N = 10.25; EI MS m/z (% rel. abund.): 286 (M^+ , 67.9), 166 (100), 149 (66.2), 121 (97.7), 65 (14.2).

N'-(4-Chlorobenzylidene)thiophene-2-carbohydrazide (**3**)

Yield: 0.9 g (90%); $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 11.92 (s, 1H, NH), 8.42 (s, 1H, N=CH-Ar), 7.78 (d, 2H $J_{2',3'} = J_{6',5'} = 8.7$ Hz, H-2'/H-6'), 7.64 (d, 1H, $J_{4,3} = 5.1$ Hz, H-4), 7.13 (d, 1H, $J_{2,3} = 3.3$ Hz, H-2), 7.15 (dd, 1H, $J_{3,2} = 3.3$, $J_{3,4} = 1.2$ Hz, H-3), 7.78 (d, 2H, 2H $J_{3',2'} = J_{5',6'} = 8.7$ Hz, H-3'/H-5'); Anal. Calcd for $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_2\text{S}$, C = 58.52, H = 4.09, N = 11.37, Found C = 58.47, H = 4.05, N = 11.35; EI MS m/z (% rel. abund.): 286.12 (M^+ , 14.9), 127 (87.6), 111 (100), 83 (9.1), 40 (14.5).

N'-(1-(2,4-Dihydroxyphenyl)ethylidene)-4-methoxybenzohydrazide (**4**)

Yield: 0.85 g (81%); $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 11.78 (s, 1H, NH), 11.55 (s, 1H, OH), 9.93 (br s, 1H, OH), 7.91 (d, 2H, 2H $J_{2,3} = J_{6,5} = 8.7$ Hz, H-2/H-6), 7.28 (d, 1H, $J_{6',5'} = 8.4$ Hz, H-6'), 7.08 (d, 2H, $J_{3,2} = J_{5,6} = 8.7$ Hz, H-3/H-5), 6.36 (dd, 1H, $J_{5',3'} = 2.1$ Hz, $J_{5',6'} = 8.4$ Hz, H-5'), 6.33 (d, 1H, $J_{3',5'} = 2.1$ H-3'), 3.89 (s, 3H, OCH_3), 3.24 (s, 3H, CH_3); Anal. Calcd for $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_4$, C = 63.99, H = 5.37, N = 9.33, Found C = 63.95, H = 5.34, N = 9.30; EI MS m/z (% rel. abund.): 300 (M^+ , 14), 286 (2.54), 151 (24.36), 135 (100), 13.77 (13.77), 77 (21.36).

N'-(1-(2-Hydroxy-3-methoxyphenyl)methylidene)-4-methoxybenzohydrazide (**5**)

Yield: 0.54 g (86%); $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 11.96 (s, 1H, NH), 11.07 (s, 1H, OH),

8.61 (s, 1H, N=CH-Ar), 7.93 (d, 2H, $J_{2,3} = J_{6,5} = 8.4$ Hz, H-2/H-6), 7.12-7.00 (m, 4H, H-3, H-5/H-6'/H-4'), 6.87 (t, 1H, $J_{5(4',6')} = 7.8$ Hz, H-5'), 3.83 (s, 3H, OCH₃), 3.80 (s, 3H, OCH₃); Anal. Calcd for C₁₆H₁₆N₂O₄, C = 63.99, H = 5.37, N = 9.33, Found C = 63.97, H = 5.35, N = 9.31; EI MS m/z (% rel. abund.): 300 (M⁺, 3.33), 151 (18.01), 135 (100), 107 (9.55), 92 (9.73), 64 (6.15).

N'-(4-Hydroxybenzylidene)nonanehydrazide (**6**)

Yield: 0.83 g (88%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.07 (s, 1H, NH), 9.84 (s, 1H, OH), 8.02 (s, 1H, N=CH-Ar), 7.48 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.7$ Hz, H-2'/H-6'), 6.80 (d, 2H, $J_{3',2'} = J_{5',6'} = 8.7$ Hz, H-3'/H-5'), 2.26 (t, 2H, $J = 6.3$ Hz, COCH₂), 1.55 (m, 2H, CH₂), 1.28-1.21 (m, 10H, 5xCH₂), 0.85 (t, 3H, $J = 5.4$ Hz, CH₃); Anal. Calcd for C₁₆H₂₄N₂O₂, C = 69.53, H = 8.75, N = 10.14, Found C = 69.51, H = 8.70, N = 10.09; EI MS m/z (% rel. abund.): 276 (M⁺, 23.8), 158 (26.4), 136 (100), 120 (24.1), 72 (24.2), 59 (80.8).

N'-(1-(2,4-Dihydroxyphenyl)ethylidene)thiophene-2-carbohydrazide (**7**)

Yield: 0.76 g (92%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 12.41 (br s, 1H, NH), 11.21 (br s, 1H, OH), 8.90 (s, 1H, OH), 8.02 (d, 1H, $J_{4,3} = 4.5$ Hz, H-4), 7.91 (dd, 1H, $J_{2,3} = 4.5$ Hz, $J_{2,4} = 1.0$ Hz, H-2), 7.21 (t, 1H, $J_{3(2,4)} = 4.5$ Hz, H-3), 6.96 (d, 1H, $J_{3',5'} = 1.5$ Hz, H-3'), 6.76 (t, 2H, $J_{5(6',3')} = 8.7$ Hz, H-5'/H-6'), 2.40 (s, 3H, CH₃); Anal. Calcd for C₁₃H₁₂N₂O₃S, C = 56.51, H = 4.38, N = 10.14, Found C = 56.48, H = 4.35, N = 10.11; EI MS m/z (% rel. abund.): 276 (M⁺, 16.8), 148 (8.8), 111 (100), 83 (11.6), 52 (6.0).

4-Chlorobenzylidene)-*N'*-(3-hydroxybenzylidene)-benzohydrazide (**8**)

Yield: 0.92 g (91%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.70 (s, 1H, NH), 9.93 (br s, 1H, OH), 8.33 (s, 1H, N=CH-Ar), 7.92 (d, 2H, $J_{2,3} = J_{6,5} = 8.4$ Hz, H-2/H-6), 7.60 (d, 2H, $J_{2',CH} = J_{4',5'} = 9.9$ Hz, H-2'/H-4'), 7.57 (t, 2H, $J_{5(4',6')} = J_{6(5',CH)} = 9.9$ Hz, H-5'/H-6') 6.82 (d, 2H, $J_{3,2} = J_{5,6} = 8.4$ Hz, H-3/H-5); Anal. Calcd for C₁₄H₁₁ClN₂O₂, C = 61.21, H = 4.04, N = 10.20, Found C = 61.22; H = 4.01, N = 10.16; EI MS m/z (% rel. abund.): 274 (M⁺, 7.57), 156 (43), 139 (100), 120 (20), 75 (16.0).

N'-(1-(2,4-Dihydroxyphenyl)ethylidene)nicotino-hydrazide (**9**)

Yield: 0.75 g (87%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 12.49 (s, 1H, NH), 11.45 (br s, 1H,

OH), 9.06 (s, 1H, OH), 8.92 (br s, 1H, H-2), 8.77 (d, 1H, $J_{6',4'} = 2.5$ Hz, H-6'), 8.26 (d, 1H, $J_{6,5} = 8.0$ Hz, H-6), 7.56 (dt, 1H, $J_{5,2} = 2.5$, $J_{5(4,6)} = 8.0$ Hz, H-5), 6.97 (d, 1H, $J_{4',2'} = 2.0$ Hz, H-4'), 6.75 (d, 1H, $J_{2',4'} = 2.0$ Hz, H-2'), 6.73 (d, 1H, $J_{4,5} = 8.0$ Hz, H-4), 2.41 (s, 3H, CH₃); Anal. Calcd for C₁₄H₁₃N₃O₃, C = 61.99, H = 4.83, N = 15.49, Found C = 61.96, H = 4.78, N = 15.45; EI MS m/z (% rel. abund.): 271 (M⁺, 24.5), 254 (30.9), 148 (10.1), 106 (78.3), 79 (81.4), 51(41.8).

4-Bromo-*N'*-(2,4-dihydroxybenzylidene)benzohydrazide (**10**)

Yield: 0.69 g (89%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.96 (s, 1H, N-H), 11.38 (s, 1H, OH), 9.96 (br s, 1H, OH), 8.49 (s, 1H, N=CH-Ar), 7.87 (d, 2H, $J_{2,3} = J_{6,5} = 8.4$ Hz, H-2/H-6), 7.72 (d, 2H, $J_{3,2} = J_{5,6} = 8.4$ Hz, H-3/H-5), 7.32 (d, 1H, $J_{6',5'} = 8.4$ Hz, H-6'), 6.35 (dd, 1H, $J_{5',3'} = 1.8$ Hz, $J_{5',6'} = 8.4$ Hz, H-5'), 6.31(d, 1H, $J_{3',5'} = 1.8$ Hz, H-3'); Anal. Calcd for C₁₄H₁₁BrN₂O₃, C = 50.17, H = 3.31, N = 8.36, Found C = 50.12, H = 3.29, N = 8.31; EI MS m/z (% rel. abund.): 336 (M⁺, 8.27), 334 (9.15) 202 (34.41), 183 (100), 135 (40), 76 (30.14).

N'-(2,5-Dihydroxybenzylidene)-2-hydroxybenzohydrazide (**11**)

Yield: 0.82 g (91%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.92 (br s, 2H, NH, OH), 10.30 (s, 1H, OH), 8.98 (s, 1H, OH), 8.60 (s, 1H, N=CH-Ar), 7.90 (dd, 1H, $J_{4',3'} = 7.8$ Hz, $J_{4',6'} = 1.2$ Hz, H-4'), 7.43 (dt, 1H, $J_{4,6} = 1.5$ Hz, $J_{4(3,5)} = 8.4$ Hz, H-4), 6.98-6.95 (m, 2H, H-3/H-6), 6.94 (dt, 1H, $J_{5,3} = 1.8$ Hz, $J_{5(4,6)} = 8.4$ Hz, H-5), 6.78 (d, 1H, $J_{3',4'} = 8.7$ Hz, H-3'), 6.73 (d, 1H, $J_{6',4'} = 2.4$ Hz, H-6'); EI MS m/z (% rel. abund.): 286 (M⁺, 10), Anal. Calcd for C₁₄H₁₂N₂O₄, C = 61.76, H = 4.44, N = 10.29, Found C = 61.72, H = 4.42, N = 10.27; EI MS m/z (% rel. abund.): 286 (M⁺, 10), 272 (18), 152 (34.41), 135(38), 121 (100), 93 (21), 65 (34).

N'-[(3-Hydroxyphenyl)methylidene]-4-methylbenzohydrazide (**12**)

Yield: 0.85 g (81%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.71 (s, 1H, N-H), 9.62 (s, 1H, OH), 8.35 (s, 1H, N=CH-Ar), 7.82 (d, 2H, $J_{2,3} = J_{6,5} = 8.7$ Hz, H-2/H-6), 7.40 (d, 2H, $J_{3,2} = J_{5,6} = 8.7$ Hz, H-3/H-5), 7.26-7.06 (m, 2H, H-2', H-5'), 6.82 (d, 1H, $J_{4',5'} = 7.2$ Hz, H-4'), 6.82 (d, 1H, $J_{6',5'} = 6.6$ Hz, H-6'); Anal. Calcd for C₁₅H₁₄N₂O₂, C = 70.85; H = 5.55, N = 11.02, Found C = 70.83; H = 5.52, N = 11.01; EI MS m/z (% rel. abund.): 254 (M⁺, 3), 135 (47), 119 (100), 92 (16), 77 (12), 65 (50).

N'-(2-Hydroxy-5-methylbenzylidene)-4-methoxybenzohydrazide (13)

Yield: 0.69 g (90%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.96 (s, 1H, NH), 11.09 (s, 1H, OH), 8.56 (s, 1H, N=CH-Ar), 7.92 (d, 2H, $J_{2,3} = J_{6,5} = 8.7$ Hz, H-2/H-6), 7.31 (br s, 1H, H-6'), 7.08 (d, 1H, $J_{4',3'} = 8.1$ Hz, H-4'), 7.07 (d, 2H, $J_{3,2} = J_{5,6} = 8.7$ Hz, H-3/H-5), 6.81 (d, 1H, $J_{3',4'} = 8.1$ Hz, H-3'), 3.83 (s, 3H, OCH₃), 2.24 (s, 3H, CH₃); Anal. Calcd for C₁₆H₁₆N₂O₃, C = 67.59, H = 5.67, N = 9.85, Found C = 67.55, H = 5.70, N = 9.81; EI MS *m/z* 284 (M⁺, 9.5), 151 (12.5), 135 (100), 107 (6.4), 92 (9), 77 (11.3).

N'-(2,4-Dihydroxybenzylidene)-4-nitrobenzohydrazide (14)

Yield: 0.85 g (81%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 12.16 (s, 1H, N-H), 11.28 (s, 1H, OH), 9.99 (s, 1H, OH), 8.50 (s, 1H, N=CH-Ar), 8.30 (d, 2H, $J_{2,3} = J_{6,5} = 9.0$ Hz, H-2/H-6), 8.15 (d, 2H, $J_{3,2} = J_{5,6} = 9.0$ Hz, H-3/H-5), 7.36 (d, 1H, $J_{6',5'} = 8.4$ Hz, H-6'), 6.36 (dd, 1H, $J_{5',3'} = 2.1$ Hz, $J_{5',6'} = 8.4$ Hz, H-5'), 6.33 (d, 1H, $J_{3',5'} = 2.1$ Hz, H-3'); Anal. Calcd for C₁₄H₁₁N₃O₅, C = 55.82, H = 3.68, N = 13.95, Found C = 55.84, H = 3.67, N = 13.92; *m/z* 301 (M⁺, 36.6), 167 (42), 151 (14.4), 135 (100), 120 (25), 104 (33), 76 (17.5).

4-Fluoro-N'-(2-hydroxy-3-methoxybenzylidene)benzohydrazide (15)

Yield: 0.65 g (88%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 12.08 (s, 1H, NH), 10.90 (br s, 1H, OH), 8.63 (s, 1H, N=CH-Ar), 8.01 (dd, 2H, $J_{2,3} = J_{6,5} = 5.7$ Hz, $J_{2,F} = J_{6,F} = 2.5$ Hz, H-2/H-6), 7.39 (t, 2H, $J_{3(2,F)} = J_{5(6,F)} = 5.4$ Hz, H-3/H-5), 7.15 (d, 1H, $J_{6',5'} = 7.7$ Hz, H-6'), 7.03 (d, 1H, $J_{4',5'} = 7.7$ Hz, H-4'), 6.88 (t, 1H, $J_{5'(4',6')} = 7.7$ Hz, H-5'), 3.80 (s, 3H, OCH₃); Anal. Calcd for C₁₅H₁₃FN₂O₃, C = 62.50, H = 4.55, N = 9.72, Found C = 62.45, H = 4.51, N = 9.75; EI MS *m/z* (% rel. abund.): 288 (M⁺, 6.5), 149 (23), 123 (100), 95 (30), 75 (10).

N'-(2,4-Dihydroxybenzylidene)-2-hydroxybenzohydrazide (16)

Yield: 0.82 g (90%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.87 (br s, 2H, OH, NH), 11.33 (s, 1H, OH), 9.99 (s, 1H, OH), 8.53 (s, 1H, N=CH-Ar), 7.86 (d, 1H, $J_{6,5} = 7.2$ Hz, H-6), 7.45 (t, 1H, $J_{4(3,5)} = 7.2$ Hz, H-4), 7.34 (d, 1H, $J_{6',5'} = 8.4$ Hz, H-6'), 6.97 (t, 1H, $J_{5(4,6)} = 7.2$ Hz, H-5), 6.94 (d, 1H, $J_{3,4} = 7.2$ Hz, H-3), 6.37 (dd, 1H, $J_{5',3'} = 1.8$ Hz, $J_{5',6'} = 8.4$ Hz, H-5'), 6.33 (d, 1H, $J_{3',5'} = 1.8$ Hz, H-3'); Anal. Calcd for

C₁₄H₁₂N₂O₄, C = 61.76, H = 4.44, N = 10.29, Found C = 61.72, H = 4.40, N = 10.27; EI MS *m/z* (% rel. abund.): 272 (M⁺, 42), 152 (53), 137 (53), 121 (100), 65 (45).

N'-[-(2-Hydroxyphenyl)methylidene]-2-thiophene-carbohydrazide (17)

Yield: 0.70 g (85%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 13.21 (s, 1H, NH), 11.27 (s, 1H, OH), 8.40 (s, 1H, N=CH-Ar), 8.03 (d, 1H, $J_{2,3} = 3.0$ Hz, H-2), 7.90 (d, 1H, $J_{4,3} = 4.8$ Hz, H-4), 7.62 (d, 1H, $J_{6',5'} = 8.1$ Hz, H-6'), 7.30 (d, 1H, $J_{3',4'} = 8.1$ Hz, H-3'), 7.23 (dd, 1H, $J_{3,2} = 3.0$ Hz, $J_{3,4} = 4.8$ Hz, H-3), 6.89 (dd, 2H, $J_{4',6'} = 2.1$ Hz, $J_{4',5'} = J_{4',3'} = 8.1$ Hz, $J_{5',4'} = J_{5',6'} = 8.4$ Hz, H-4'/H-5'); Anal. Calcd for C₁₂H₁₀N₂O₂S, C = 58.52, H = 4.09, N = 11.37, Found C = 58.47, H = 4.07, N = 11.35; 246 (M⁺, 38), 128 (39), 111 (100), 83 (11.9), 40(12.4).

4-Chloro-N'-(naphthalen-2-ylmethylene)benzohydrazide (18)

Yield: 0.85 g (81%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.69 (s, 1H, NH), 9.93 (br s, 1H, N=CH-Ar), 8.33 (s, 1H, H-1'), 7.92 (d, 2H, $J_{2,3} = J_{6,5} = 8.4$ Hz, H-2/H-6), 7.57 (t, 6H $J = 8.4$ Hz, H-3', H-4', H-5', H-6', H-7', H-8'), 6.83 (d, 2H, $J_{3,2} = J_{5,6} = 8.4$ Hz, H-3/H-5); Anal. Calcd for C₁₈H₁₃ClN₂O, C = 70.02, H = 4.24, N = 9.07, Found C = 70.01, H = 4.20, N = 9.02; EI MS *m/z* (% rel. abund.): 308 (M⁺, 2.2), 153 (77.62), 139. (100), 111 (42), 75 (22), 51 (10).

4-Chloro-N'-(4-chlorobenzylidene)benzohydrazide (19)

Yield: 0.95 g (81%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.9 (s, 1H, NH), 8.4 ((s, 1H, N=CH-Ar), 7.90 (d, 2H, $J_{2,3} = J_{6,5} = 8.7$ Hz, H-2/H-6), 7.70 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.4$ Hz, H-2'/H-6'), 7.6 (d, 2H, $J_{3',2'} = J_{5',6'} = 8.4$ Hz, H-3'/H-5'), 7.5 (d 2H, $J_{3,2} = J_{5,6} = 8.7$ Hz, H-3/H-5); Anal. Calcd for C₁₄H₁₀Cl₂N₂O, C = 57.36, H = 3.44, N = 9.56, Found C = 57.32, H = 3.40, N = 9.57; EI MS *m/z* (% rel. abund.): 292. (M⁺, 16.2), 155 (91), 139. (100), 113 (28) 75 (26), 50 (7).

N'-[2-Naphthylmethylidene]-2-thiophenecarbohydrazide (20)

Yield: 0.78 g (88%); ¹H-NMR (300 MHz, DMSO-*d*₆): δ 11.93 (s, 1H, NH), 8.14 ((s, 1H, N=CH-Ar), 8.01-7.93 (m, 7H, H-1', H-3', H-4', H-5', H-6', H-7', H-8'), 7.59-54 (m, 2H, H-2, H-4), 7.25 (dd, 1H, $J_{3,4} = 3.8$ Hz, $J_{3,2} = 5.0$ Hz, H-3); Anal. Calcd for C₁₆H₁₂N₂OS, C = 68.55, H = 4.31, N =

9.99, Found C = 68.51, H = 4.30, N = 9.98; EI MS m/z (% rel. abund.): 280 (M^+ , 87), 152 (100), 127 (100), 111 (100), 83 (35), 44(20).

N'-(3-Bromo-4-chlorobenzylidene)-4-chlorobenzohydrazide (**21**)

Yield: 0.76 g (91%); $^1\text{H-NMR}$ (300 MHz, DMSO- d_6): δ 10.87 (s, 1H, NH), 10.59 (s, 1H, N=CH-Ar), 8.44 (d, 1H, $J_{2,6'} = 2.1$ Hz, H-2'), 8.09 (dd, 1H, $J_{6',5'} = 8.7$ Hz, $J_{6',2'} = 2.1$ Hz, H-6'), 7.98 (d, 1H, $J_{5',6'} = 8.7$ Hz, H-5'), 7.71 (d, 2H, $J_{2,3} = J_{6,5} = 8.4$ Hz, H-2/H-6), 7.54 (d, 2H, $J_{3,2} = J_{5,6} = 8.4$ Hz, H-3/H-5); Anal. Calcd for $\text{C}_{14}\text{H}_9\text{BrCl}_2\text{N}_2\text{O}$, C = 45.20, H = 2.44, N = 7.53, Found C = 45.17, H = 2.40, N = 7.47; EI MS m/z (% rel. abund.): 372 (M^+ , 20), 221 (7.3) 156 (20.1), 139 (100), 113 (21.9), 75 (32.4).

4-Bromo-N'-[1-(2-hydroxyphenyl)ethylidene]-benzohydrazide (**22**)

Yield: 0.72 g (92%); $^1\text{H-NMR}$ (300 MHz, DMSO- d_6): δ 13.28 (s, 1H, NH), 11.37 (s, 1H, OH), 7.88 (d, 2H, $J_{2,3} = J_{6,5} = 8.4$ Hz, H-2/H-6), 7.75 (d, 2H, $J_{3,2} = J_{5,6} = 8.4$ Hz, H-3/H-5), 7.63 (d, 1H, $J_{3',4'} = 7.5$ Hz, H-3'), 7.31(t, 1H, $J_{4'(3',5')} = 7.5$ Hz, H-4') 6.90 (t, 1H, $J_{5'(4',6')} = 7.5$ Hz, H-5'), 6.89 (d, 1H, $J_{6',5'} = 7.5$ Hz, H-6'); Anal. Calcd for $\text{C}_{15}\text{H}_{13}\text{BrClN}_2\text{O}_2$, C = 54.07, H = 3.93, N = 8.41, Found C = 54.05, H = 3.87, N = 8.38; EI MS m/z (% rel. abund.): 333 (M^+ , 2.43), 318 (23), 183 (100), 155(27), 91 (29).

N'-[1-(2-Hydroxyphenyl)ethylidene]-2-thiophene carbohydrazide (**23**)

Yield: 0.70 g (85%); $^1\text{H-NMR}$ (300 MHz, DMSO- d_6): δ 13.23 (s, 1H, NH), 11.27 (s, 1H, OH), 8.03 (d, 1H, $J_{2,3} = 3.3$ Hz, H-2), 7.91 (d, 1H, $J_{4,3} = 4.8$ Hz, H-4), 7.63 (d, 1H, $J_{6',5'} = 8.1$ Hz, H-6'), 7.32 (d, 1H, $J_{3',4'} = 7.2$ Hz, H-3'), 7.31 (dd, 1H, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 4.8$ Hz, H-3), 6.90 (t, 2H, $J_{4'(5',6')} = 7.2 = J_{5'(4',6')} = 7.2$ Hz, H-4'/H-5'), 3.21 (s, 3H, CH_3); Anal. Calcd for $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_2\text{S}$, C = 59.98, H = 4.65, N = 10.76, Found C = 59.95, H = 4.61, N = 10.72; EI MS m/z (% rel. abund.): 260 (M^+ , 8), 245 (7.3), 111 (100), 91 (21.9), 65 (12.4).

N'-[-(2-Bromophenyl)methylidene]-4-chlorobenzohydrazide (**24**)

C Yield: 0.85 g (81%); $^1\text{H-NMR}$ (300 MHz, DMSO- d_6): δ 12.01 (s, 1H, NH), 8.41 (s, 1H, N=CH-Ar), 7.94 (d, 2H, $J_{2,3} = J_{6,5} = 8.3$ Hz, H-2/H-6), 7.67 (br s, 4H, H-3',H-4', H-5', H-6'), 7.60 (d, 2H, $J_{3,2} = J_{5,6} = 8.3$ Hz, H-3/H-5), Anal. Calcd for $\text{C}_{14}\text{H}_{10}\text{BrClN}_2\text{O}$, C = 49.81, H = 2.99, N = 8.30, Found C = 49.79, H = 2.95, N = 8.26; EI MS m/z (%

rel. abund.): 339 (M^+ , 5.5), 157 (58.6), 139 (100), 113 (29.1), 89(24.5).

N'-(2-Bromobenzylidene)thiophene-2-carbohydrazide (**25**)

Yield: 0.85 g (81%); $^1\text{H-NMR}$ (300 MHz, DMSO- d_6): δ 11.90 (s, 1H, N-H), 8.39 (s, 1H, N=CH-Ar), 8.12 (d, 1H, $J_{2,3} = 3.1$ Hz, H-2), 7.94 (d, 1H, $J_{4,3} = 4.5$ Hz, H-4), 7.67 (d, 1H, $J_{6',5'} = 8.2$ Hz, H-6'), 7.42 (d, 1H, $J_{3',4'} = 7.2$ Hz, H-3'), 7.33 (dd, 1H, $J_{3,2} = 3.1$ Hz, $J_{3,4} = 4.5$ Hz, H-3), 6.90 (t, 2H, $J_{4'(3',5')} = J_{5'(4',6')} = 7.2$ Hz, H-4'/H-5'); Anal. Calcd for $\text{C}_{12}\text{H}_9\text{BrN}_2\text{OS}$, C = 46.62, H = 2.93, N = 9.06, Found C = 46.59, H = 2.88, N = 9.03; EI MS m/z (% rel. abund.): 308 (M^+ , 5.5), 127 (78.6), 111 (100), 89 (9.1), 41(8.2).

N'-(2-Bromobenzylidene)-4-methylbenzohydrazide (**26**)

Yield: 0.85 g (81%); $^1\text{H-NMR}$ (300 MHz, DMSO- d_6): δ 11.83 (s, 1H, NH), 8.41(s, 1H, N=CH-Ar), 7.83 (d, 2H, $J_{2,3} = J_{6,5} = 7.5$ Hz, H-2/H-6), 7.66 (s, 4H, H-3',H-4', H-5', H-6'), 7.33 (d, 2H, $J_{3,2} = J_{5,6} = 7.5$ Hz, H-3, H-5); Anal. Calcd for $\text{C}_{14}\text{H}_{10}\text{BrClN}_2\text{O}$, C = 49.81, H = 2.99, N = 8.30, Found C = 49.79, H = 2.95, N = 8.26; EI MS m/z (% rel. abund.): 316.18 (M^+ , 5), 135 (67.6), 119 (100), 91 (29.1), 65 (14.5).

N'-(4-Hydroxybenzylidene)thiophene-2-carbohydrazide (**27**)

Yield: 0.85 g (81%); $^1\text{H-NMR}$ (500 MHz, DMSO- d_6): δ 11.56 (s, 1H, NH), 10.09 (br s, 1H, OH), 8.62 (s, N=CH-Ar), 7.78 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.7$ Hz, H-2'/H-6'), 7.64 (d, 1H, $J_{4,3} = 4.8$ Hz, H-4), 7.13 (br d, 1H, $J_{2,3} = 3.9$ Hz, H-2), 7.20 (dd, 1H, $J_{3,2} = 3.9$ Hz, $J_{3,4} = 4.8$ Hz, H-3), 7.78 (d, 2H, $J_{3',2'} = J_{5',6'} = 8.7$ Hz, H-3'/H-5'); Anal. Calcd for $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_2\text{S}$, C = 58.52, H = 4.09, N = 11.37, Found C = 58.47, H = 4.05, N = 11.35; EI MS m/z (% rel. abund.): 286.12 (M^+ , 14.9), 127 (87.6), 111 (100), 83 (9.1), 40 (14.5).

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