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SYNTHESIS AND ANTIGLYCATION ACTIVITY OF BENZENE-1,3,5-TRICARBOXYLIC ACID MEDIATED NEW SERIES OF SCHIFF BASE DERIVATIVES

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ABSTRACT

A series of benzene-1,3,5-tricarboxylic acid mediated Schiff base derivatives **4-15** were synthesized and characterized as novel antiglycating agents. The *in vitro* antiglycation activity of these compounds were evaluated and compared with standard Rutin (41.9 µM). Preliminary structure-activity relationship revealed that the compounds **6, 8, 12 and 14** with electron donating moiety (OH and OCH_3) were found to be excellent antiglycating agents and compounds **5, 9, 11 and 13** with electron withdrawing (Cl and NO_2) group showed least antiglycating activity. This study offers an opportunity for further structural modifications and extensions that could give rise to compounds with improved inhibitory profile against protein glycation.

KEYWORDS: Schiff's base, Antiglycation, Electronic effect.

INTRODUCTION

The Schiff's base family is composed of natural products with critical pharmacophores.[1] It can be used as ideal lead structures to develop agrochemicals and medicines, including antiglycation,[2] bactericide,[3] antivirals,[4] antioxidants,[5] antiproliferative[6] and antimicrobial drug.[7] Various natural alkaloids with critical pharmacophores contain quinazolinone groups. For example, febrifugine, isofebrifugine, thiabutazide, (-)-benzomalvin A, 2-(4-hydroxybutyl) quinazolin-4-one, and luotonin F were found in the plants, animals, and microorganisms.[8-9] Moreover, The quinazolinone nucleus and its derivatives have been extensively studied because of their wide range of pharmacological activities. As medicines, many of them display antitubercular,[10] anti-inflammatory,[11] anticonvulsant,[12] antidepressant,[13] antiulcer[14] and analgesic[15] activities.

In the present study, in vitro antiglycation activity of a series of Schiff base derivatives **1–15** has been evaluated. Discovery of antiglycation agents is an important approach for the treatment of late diabetic complications. Since currently number of effective antiglycating agents is very small, the need of new antiglycating agents is still unmet.[16] As the unpleasant incident of type-2 diabetes is increasing, its injurious effects are mostly attributed to the formation of sugar-derived substances called advanced glycation end products (AGE-Ps)[17] which are important pathogenic mediators of almost all diabetic complications.[18]

Schiff bases are formed initially by reaction between protein and glucose without any enzyme, whereas the rearrangement of Schiff base intermediate to Amadori product takes number of days. Extensive effort has been focused on the discovery of new inhibitors of glycation, because of their therapeutic potential.[19] Certain molecules have been developed that can cleave AGEPs cross-links and perhaps open the possibility of reversing the steady process of diabetic complications.[20] It has been found that aged garlic extract (AGE) inhibit the formation of AGEPs in vitro and prevents the formation of glycation-derived free radicals. S-Allylcysteine is a very important component of aged garlic extract that acts as a potent antioxidant and thus inhibit the AGEPs formation.[21, 22]

Aminoguanidine, an inhibitor of AGEPs formation was found to prevent retinopathy in diabetic animals and protect them from developments of diabetic vascular complications. However, amino-guanidine has encountered some toxicity problems in phase III clinical trials.[23] Efforts have now been made to develop new and safe synthetic antiglycation agents.[24] It has been demonstrated that polyamines, spermine and spermidine have potent antiglycation effects, comparable to those of aminoguanidine and carnosine. This prompted us to

develop some new chemotherapeutic agents by joining in one single structure these important biologically active scaffolds seeking an improvement in the activity.

RESULT AND DISCUSSION

CHEMISTRY

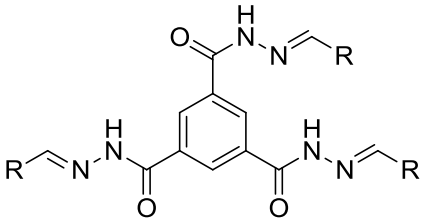
Syntheses of the desired compounds were achieved according to the steps illustrated in **Scheme**. benzene-1,3,5-tricarboxylic acid was methylated using trimethylsilylchloride (TMS-Cl) and methanol at room temperature, which upon reaction with excess of hydrazine hydrate afforded the corresponding tribenzylidenebenzene-1,3,5-tricarbohydrazides
hydrazides (**3**). The Schiff's bases (**4-15**) were obtained by reacting **3** with different aromatic aldehydes in presence of catalytic amount of glacial acetic acid. To synthesize compounds (**4-7**) reaction was carried out with 3 equivalents of different aromatic aldehydes in presence of catalytic amount of glacial acetic acid where as for compounds ***(*8-15*)*** carried out with 2 equivalents of different aromatic aldehydes. All the derivatives were obtained in high yield and the methods employed are very simple. The structures of all the newly synthesized compounds including intermediates were confirmed by IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis. The formation of methyl esters (**2**) were confirmed by the appearance of a siglet at 3.73δ for OCH_3 and absence of COOH proton peak at 12.10δ in ^{1}HNMR spectrum. In IR spectra, bands at 3315 and 3220cm^{-1} for NH_2-NH groups indicates the conversion of methyl esters into hydrazides. The formation of Schiff's bases were confirmed by the presence of absorption at 1610-1630 for imines i.e., -N=CH- in IR spectra. The presence of all requisite peaks and absence of extraneous peaks in ^{1}HNMR and ^{13}CNMR confirms the structures.

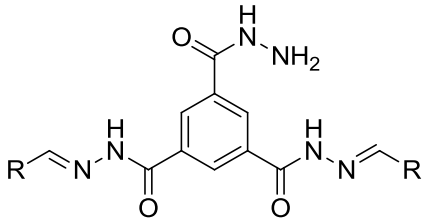
BIOLOGY

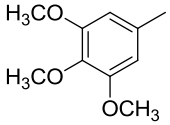
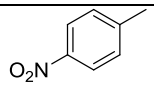
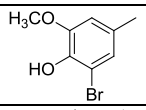
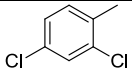
In vitro antiglycation activity of all the synthesized compounds including intermediates were evaluated by method of Nakagawa et al.,[25] which is a rapid and convenient technique for screening. The values of IC_{50}, the effective concentration at which 50% inhibition observed, were calculated to evaluate the protein glycation inhibition activity. A lower IC_{50} value indicated greater antiglycation activity. The results were shown in **Table.** Most of the synthesized compounds showed potent antiglycation activity. Compounds **6 (**IC_{50} = 5.2 ± 0.3 µM**), 7 (**IC_{50} = 21.2 ± 0.6 µM**), 8 (**IC_{50} = 3.4 ± 0.6 µM) and **12 (**IC_{50} = 9.5 ± 0.1 µM) showed excellent antiglycation activity much better than the standard Rutin (IC_{50} = 41.9 ± 0.7µM). The compounds **10 (**IC_{50} = 16.3 ± 0.4 µM) and **14 (**IC_{50} = 14.5 ± 0.3 µM) also exhibited striking antioxidant activity which is better than the standard.

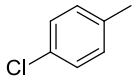
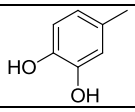
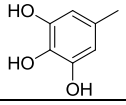
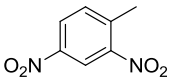
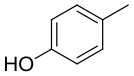
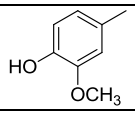
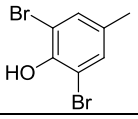
The compounds **4 (**IC_{50} = 28.4 ± 0.2 µM**), 7 (**IC_{50} = 21.2 ± 0.6 µM) and **15 (**IC_{50} = 39.4 ± 0.5 µM) showed excellent antioxidant activities with IC_{50} values much lower than the standards. The IC_{50} values of these compounds **5 (**IC_{50} = 53.0 ± 0.2 µM**), 9 (**IC_{50} = 61.0 ± 0.2 µM**), 11 (**IC_{50} = 53.0 ± 0.2 µM**), 13 (**IC_{50} = 72.0 ± 0.2 µM), 1 (**IC_{50} = 260.0 ± 0.6 µM), 2 (**IC_{50} = 164.0 ± 0.4 µM) and 3(**IC_{50} = 77.0 ± 0.2 µM) were found to be less active among the series. On the basis of the above observation, compounds having –OH (phenolic) and –OCH_3 (anisole) groups in the phenyl ring (**6, 7, 8 and 12)** were found to be the most potent antioxidants. The compounds with electron withdrawing Cl and NO_2 substituents (**5, 9, 11 and 13**) showed least antiglycation activity.

Table: Antiglycation activity of the synthesized compounds

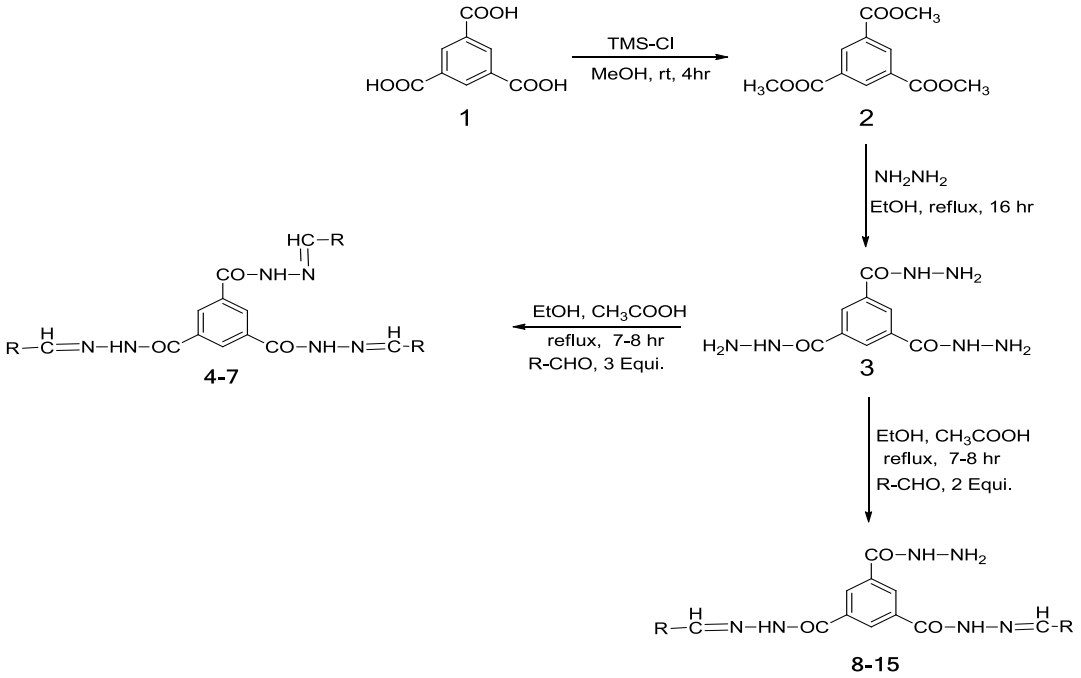




	R	IC_{50}		R	IC_{50}
(1)	Acid	260.0 ± 0.6	***(8)***		3.4 ± 0.6
(2)	Ester	164.0 ± 0.4	***(9)***		61.0 ± 0.2
(3)	Hydrazide	77.0 ± 0.2	***(10)***		16.3 ± 0.4
(4)		28.4 ± 0.2	***(11)***		53.0 ± 0.3

(5)		53.0 ± 0.1	***(12)***		9.5 ± 0.1
(6)		5.2 ± 0.3	***(13)***		72.0 ± 0.7
(7)		21.2 ± 0.6	***(14)***		14.5 ± 0.3
	Rutin (std)	41.9 ± 0.7	***(15)***		39.4 ± 0.5

*****The IC_{50}s are reported in µM concentration. Values are mean of three determinations (SEM), the ranges of which are<5% of the mean in all cases.



Scheme: **Synthesis of Schiff's base derivatives**

CONCLUSION

In conclusion, we have designed and synthesized a series of benzene-1,3,5-tricarboxylic acid mediated Schiff's bases with different groups in benzene ring. Of all the compounds synthesized, compounds (**6, 7, 8 and 12)** with OH and OCH_3 groups in benzene ring (electron donating) exhibited stronger antiglycation activity. The compounds with electron withdrawing Cl and NO_2 substituents (**5, 9, 11 and 13**) showed least antiglycation activity.

EXPERIMENTAL

GENERAL

Aldehydes, were purchased from Sigma Aldrich (India). All other chemicals and reagents obtained from Merck (India) and Avra Synthesis (India) were used without further purification. Melting points were determined on a Superfit melting point apparatus (India) and are uncorrected. FT-IR was performed using a Jasco spectrometer (Japan) using nujol media. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Agilent Technologies (USA) using DMSO (d_6) as solvent. High resolution mass spectroscopic analysis was performed on a Bruker MicroTOF QII mass spectrometer in positive mode. Progress of the reaction was monitored by TLC using silica gel coated on glass plates with the solvent system comprising chloroform/ methanol/acetic acid in the ratio 98:02:03 (R_f^a) and 95:05:03 (R_f^b) and the compounds on the TLC plates were detected by iodine vapors. Based on the above facts and in continuation of our drug development program, the present work involves the synthesis of a new series of benzene-1,3,5-tricarboxylic acid mediated Schiff's base derivatives as potential antiglycation agents.

Experimental procedure for the synthesis of 2: To a solution of benzene-1,3,5-tricarboxylic acid **1** (0.02 mol, 4.36 g) separately in methanol (40 mL),

trimethylsilylchloride (0.02 mol, 3.80 mL) was added slowly. The reaction mixture was stirred for 4 hrs to complete the reaction (monitored by TLC). The solvent was removed under reduced pressure and the resultant precipitate was washed with ice cold water and filtered to yield the desired products **2** (Yield = 4.90 g, 91.41%, m.p. 184-185 °C)**.**

***Experimental procedure for the synthesis of 3*:** To a solution of **2** (0.015 mol, 4.02 g) separately in ethanol (40 mL), hydrazine hydrate (0.020 mol, 0.97 mL) was added. The reaction mixture was refluxed for 16 hrs for completion of the reaction (monitored by TLC). The solvent was removed under reduced pressure and cooled by adding ice cold water. The resulting precipitate was filtered, washed with cold water and recrystallized from ethanol to get the desired compound. (Yield = 2.90 g, 83.33%, m.p. 220-221 °C)**.**

General procedure for the synthesis of Schiff's bases (4-15): An equimolar amount of **3** (1 mmol) was dissolved separately in ethanol (10 mL/g of compound) and treated with appropriate aldehydes (3 mmol/ 2 mmol) in the presence of catalytic amount of glacial acetic acid. The reaction mixtures were refluxed for 7–8 hr and the completion of reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and cooled by adding ice cold water. The resulting precipitate was filtered, washed with water and recrystallized from ethanol to obtain the desired Schiff's bases **(4-15)**. The yields, % of yield and m.p are recorded.

ANTIGLYCATION ASSAY

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) + KH_2PO_4 (1.47 mM) and pH 10 was adjusted with NaOH (0.25 mM), while BSA (10 mg/ml) and anhydrous glucose (50 mg/ml) solutions were prepared in sodium phosphate buffer.

Bovine serum albumin (10 mg/ml) was incubated with glucose anhydrous (50 mg/ml) in sodium phosphate buffer (pH 7.4). DMSO used for dissolving the compounds was found to have no effect on the reaction at <2% (v/v).Glycated control contains 20 µl BSA + 20 µl glucose + 20 µl sodium phosphate buffer, while blank control contains 20 µl BSA and 40 µl sodium phosphate buffer. The mixture was incubated at 37 °C for 7 days. After incubation, 6 µl (100%) of TCA (Trichloroacetic acid) was added into each well and centrifuged (15,000 rpm) for 4 min at 4 °C. After centrifugation, the pellets were rewashed with 60 µl (10%) of TCA. The supernatant containing glucose, inhibitor and interfering substance was removed and pellet containing advanced glycated end product-BSA were dissolved in 60 µl phosphate buffer solution (PBS). Evaluation of fluorescence spectrum (excitation 370 nm), and change in fluorescence intensity (excitation 370 nm to emission 440 nm), based on AGEs were monitored by using spectrofluorimeter (RF-1500, Shimadzu, Japan). % Inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 1 - \frac{\text{Fluorescence of sample}}{\text{Fluorescence of glycated sample}} \times 100$$

Characterization of synthesized compounds

Trimethyl benzene-1,3,5-tricarboxylate (2)

Yield 91.41%, R_f^a = 0.66, R_f^b = 0.70, m.p. 184-185 °C, IR KBr (cm^{-1}): 1630, 1660, 1668; ^{1}H NMR (DMSO-d_6) δ ppm: 8.58 (s, 3H, Ar-H), 3.90 (s, 9H, 3OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 172.1, 161.7, 134.2, 125.9, 51.5; HRMS m/z, (M+1): 253.1021

Benzene-1,3,5-tricarbohydrazide (3)

Yield 82.36%, R_f^a = 0.39, R_f^b = 0.40, m.p. 201-202 °C, IR KBr (cm^{-1}): 1628, 1640, 1680; ^{1}H NMR (DMSO-d_6) δ ppm: 9.99 (s, 2H, NH), 9.80 (s, 1H, NH), 8.46 (s, 3H, Ar-H), 3.91-3.88 (m, 6H, NH_2); ^{13}C NMR (DMSO-d_6) δ ppm: 171.5, 161.7, 134.1, 125.8; HRMS m/z, (M+1): 253.5644;

(N'^3E)-N'^1,N'^3,N'^5-tribenzylidenebenzene-1,3,5-tricarbohydrazide (4)

Yield 89.18%, R_f^a = 0.58, R_f^b = 0.64, m.p. 242-244 °C, IR KBr (cm^{-1}): 1610, 1630, 1647, 1680, 3210 ; ^{1}H NMR (DMSO-d_6) δ ppm: 12.20 (s, 3H, NH), 8.73 (s, 3H, -N=CH), 8.49 (s, 3H, Ar-H), 7.75-7.45 (m, 15H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 172.0, 161.5, 148.7, 137.1, 134.1, 128.9, 128.1, 126.7, 125.8; HRMS m/z, (M+1): 517.2364

(N'3E)-N'1,N'3,N'5-tris(4-chlorobenzylidene)benzene-1,3,5-tricarbohydrazide (5)

Yield 87.18%, R_f^a = 0.52, R_f^b = 0.57, m.p. 220-221 °C, IR KBr (cm^{-1}): 1620, 1637, 1649, 1673, 3210, 3318 ; ^{1}H NMR (DMSO-d_6) δ ppm: 12.20 (s, 3H, NH), 8.70 (s, 3H, -N=CH), 8.46 (s, 3H, Ar-H), 7.60-7.44 (m, 12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 171.0, 162.5, 143.7, 136.1, 135.6, 131.9, 130.1, 128.7, 127.8; HRMS m/z, (M+1): 620.4521

(N'3E)-N'1,N'3,N'5-tris(3,4,5-trihydroxybenzylidene)benzene-1,3,5-tricarbohydrazide (6)

Yield 84.18%, R_f^a = 0.37, R_f^b = 0.40, m.p. 190-191 °C, IR KBr (cm^{-1}): 1618, 1640, 1652, 1680, 3280, 3516; ^{1}H NMR (DMSO-d_6) δ ppm: 11.40 (s, 3H, NH), 9.20 (s, 3H, 9OH), 8.53 (s, 3H, -N=CH), 8.40 (s, 3H, Ar-H), 7.30-6.68 (m, 6H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 170.2, 161.6, 148.7, 143.6, 137.1, 134.6, 129.3, 128.1, 107.8; HRMS m/z, (M+1): 661.3121

(N'3E)-N'1,N'3,N'5-tris(4-hydroxybenzylidene)benzene-1,3,5-tricarbohydrazide (7)

Yield 82.15%, R_f^a = 0.42 R_f^b = 0.46, m.p. 186-187 °C, IR KBr (cm^{-1}): 1613, 1640, 1658, 1677, 3278, 3564 ; ^{1}H

NMR (DMSO-d_6) δ ppm: 12.01 (s, 3H, NH), 9.54 (s, 3H, 3OH), 8.51 (s, 3H, -N=CH), 8.37 (s, 3H, Ar-H), 7.42-7.28 (m, 12H, Ar-H); ^{13}C NMR (DMSO-d_6) δ ppm: 173.0, 161.5, 160.4, 144.2, 133.1, 130.6, 128.6, 117.7; HRMS m/z, (M+1): 565.3218

N'1,N'3-bis(3,4,5-trimethoxybenzylidene)benzene-1,3,5-tricarbohydrazide (8)

Yield 92.13%, R_f^a = 0.57, R_f^b = 0.63, m.p. 206-207 °C, IR KBr (cm^{-1}): 1620, 1635, 1648, 1670, 3210, 3320, 3330; ^{1}H NMR (DMSO-d_6) δ ppm: 12.18 (s, 2H, NH), 8.68 (s, 3H, Ar-H), 8.40 (s, 2H, -N=CH), 7.02-6.88 (m, 4H, Ar-H), 3.83 (s, 18H, 6OCH$_3$), 3.69 (s, 3H, NH-NH$_2$); ^{13}C NMR (DMSO-d_6) δ ppm: 162.7, 162.3, 153.5, 146.4, 142.9, 134.6, 130.2, 127.1, 126.3, 126.0, 104.2, 60.5, 57.2; HRMS m/z (M+1): 609.0452,

N'1,N'3-bis(4-nitrobenzylidene)benzene-1,3,5-tricarbohydrazide (9)

Yield 85.23%, R_f^a = 0.52, R_f^b = 0.55, m.p. 190-191 °C, IR KBr (cm^{-1}): 1608, 1640, 1656, 1677, 3220, 3308; ^{1}H NMR (DMSO-d_6) δ ppm: 11.28 (s, 2H, NH), 8.46 (s, 3H, Ar-H), 8.23 (s, 2H, -N=CH), 7.12-6.80 (m, 8H, Ar-H), 3.80 (s, 3H, NH-NH$_2$); ^{13}C NMR (DMSO-d_6) δ ppm: 168.7, 161.3, 150.5, 143.4, 138.9, 134.3, 129.6, 128.6, 125.3, 124.0; HRMS m/z (M+1): 519.4231,

N'1,N'3-bis(3-bromo-4-hydroxy-5-methoxybenzylidene)benzene-1,3,5-tricarbohydrazide (10)

Yield 81.18%, R_f^a = 0.53, R_f^b = 0.57, m.p. 214-215 °C, IR KBr (cm^{-1}): 1622, 1624, 1636, 1678, 3247, 3317, 3546; ^{1}H NMR (DMSO-d_6) δ ppm: 12.16 (s, 2H, NH), 9.45 (s, 3H, 3OH), 8.60 (s, 3H, Ar-H), 8.12 (s, 2H, -N=CH), 7.22-6.70 (m, 4H, Ar-H), 3.79 (s, 6H, 2OCH$_3$), 3.56 (s, 3H, NH-NH$_2$); ^{13}C NMR (DMSO-d_6) δ ppm: 170.2, 161.8, 155.5, 144.1, 142.6, 133.9, 130.5, 129.2, 128.1, 123.3, 114.0, 111.2, 55.5; HRMS m/z (M+1): 679.2464,

N'1,N'3-bis(2,4-dichlorobenzylidene)benzene-1,3,5-tricarbohydrazide (11)

Yield 83.56%, R_f^a = 0.56, R_f^b = 0.60, m.p. 244-245 °C, IR KBr (cm^{-1}): 1616, 1632, 1641, 1678, 3210, 3314; ^{1}H NMR (DMSO-d_6) δ ppm: 12.01 (s, 2H, NH), 8.32 (s, 3H, Ar-H), 8.33 (s, 2H, -N=CH), 7.15-6.89 (m, 6H, Ar-H), 3.90 (s, 3H, NH-NH$_2$); ^{13}C NMR (DMSO-d_6) δ ppm: 171.2, 162.5, 142.9, 134.2, 132.7, 131.2, 130.5, 129.1, 128.3, 127.2, 127.6; HRMS m/z (M+1): 724.6214,

N'1,N'3-bis(3,4-dihydroxybenzylidene)benzene-1,3,5-tricarbohydrazide (12)

Yield 84.40%, R_f^a = 0.64, R_f^b = 0.71, m.p. 221-223 °C, IR KBr (cm^{-1}): 1621, 1631, 1645, 1678, 3210, 3100, 3546; ^{1}H NMR (DMSO-d_6) δ ppm: 11.94 (s, 2H, NH), 9.40 (s, 4H, OH), 8.63 (s, 3H, Ar-H), 8.29 (s, 2H, -N=CH), 7.24-6.76 (m, 6H, Ar-H), 3.91 (s, 3H, NH-NH$_2$); ^{13}C NMR (DMSO-d_6) δ ppm: 173.1, 167.6, 149.7, 147.0, 146.5, 134.7, 127.2, 126.4, 126.1, 125.0, 121.2; HRMS m/z: 492.9648.

N'1,N'3-bis(2,4-dinitrobenzylidene)benzene-1,3,5-tricarbohydrazide (13)

Yield 87.56%, R_f^a = 0.54, R_f^b = 0.59, m.p. 196-198 °C, IR KBr (cm^{-1}): 1623, 1620, 1635, 1680, 3230, 3319; ^{1}H NMR (DMSO-d_6) δ ppm: 11.56 (s, 2H, NH), 8.12 (s, 3H, Ar-H), 7.96 (s, 2H, -N=CH), 7.22-6.70 (m, 6H, Ar-H), 3.44 (s, 3H, NH-NH$_2$); ^{13}C NMR (DMSO-d_6) δ ppm: 170.2, 161.9, 152.9, 149.2, 136.7, 134.2, 132.5, 130.1, 129.3, 129.3, 128.2, 120.6; HRMS m/z (M+1): 609.2345,

N'1,N'3-bis(4-hydroxy-3-methoxybenzylidene)benzene-1,3,5-tricarbohydrazide (14)

Yield 85.56%, R_f^a = 0.50, R_f^b = 0.53, m.p. 212-213 °C, IR KBr (cm^{-1}): 1607, 1624, 1641, 1678, 3214, 3512; ^{1}H NMR (DMSO-d_6) δ ppm: 11.80 (s, 2H, NH), 9.12 (s, 2H, 2OH), 8.10 (s, 3H, Ar-H), 7.99 (s, 2H, -N=CH), 7.25-6.82 (m, 6H, Ar-H), 3.78 (s, 6H, 2OCH$_3$), 3.62 (s, 3H, NH-NH$_2$); ^{13}C NMR (DMSO-d_6) δ ppm: 171.2, 163.9, 154.3, 150.9, 143.2, 134.7, 129.6, 128.5, 122.1, 117.3, 112.8, 128.2. 56.7; HRMS m/z (M+1): 521.3264,

N'1,N'3-bis(3,5-dibromo-4-hydroxybenzylidene)benzene-1,3,5-tricarbohydrazide (15)

Yield 81.56%, R_f^a = 0.46, R_f^b = 0.51, m.p. 197-199 °C, IR KBr (cm^{-1}): 1615, 1626, 1645, 1680, 3233, 3529; ^{1}H NMR (DMSO-d_6) δ ppm: 11.56 (s, 2H, NH), 9.80 (s, 2H, 2OH), 8.15 (s, 3H, Ar-H), 7.86 (s, 2H, -N=CH), 7.21-6.78 (m, 4H, Ar-H), 5.82 (s, 1H, NH), 3.80 (s, 2H, NH$_2$); ^{13}C NMR (DMSO-d_6) δ ppm: 172.2, 161.9, 159.3, 143.2, 135.1, 130.6, 129.5, 129.1, 128.6, 110.3; HRMS m/z (M+1): 777.1458,

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