Synthesis, Structural Characterization, and Antioxidant Activities of 2,4-Dinitrophenyl-Hydrazone Derivatives

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Summary: Thirty-two derivatives of 2,4-dinitro phenylhydrazone **1-32** were synthesized by one step reaction and characterized by spectroscopic techniques such as EI-MS and ^{1}H-NMR. Compounds **1-32** were screened for their *in vitro* antioxidant activities. DPPH radical scavenging, ferrous ion-chelating, ferric ion reducing, total antioxidants, and hydroxyl radical scavenging assays were used to check the antioxidant potential of the synthetic derivatives. Compounds showed good to moderate antioxidant activities as compared to the standard vitamin C (for DPPH radical scavenging (IC_{50} = 77.83 ± 16.56 μM), ferric ion reducing (IC_{50} = 92.16 ± 17.74 μM), total antioxidant (IC_{50} = 97.85 ± 17.21 μM), and hydroxyl radical scavenging (IC_{50} = 96.128 ± 17.50 μM) activities) and EDTA (for ferrous ion-chelating assay (IC_{50} = 101.86 ± 17.84 μM)). This study has identified potential leads for future research on antioxidant agents of this class of compounds.

Keywords: Schiff bases; 2,4-dinitro-phenyl hydrazone; Reactive oxygen species; Antioxidant; *In vitro*.

Introduction

Schiff bases are condensation products of primary amines and carbonyl compounds, possesses oxygen and nitrogen donor atoms, and represented by general formula $R_2R_3C{=}NR_1$ [1,2]. Schiff bases were first time discovered by German Scientist, Hugo Schiff in 1864 [2]. Schiff bases are also known as imine or azomethine which are the nitrogen analogue of an aldehydes or ketones in which the carbonyl group is replaced by an imine or azomethine group [3,4]. Imine or azomethine groups are existing in different natural, naturally derived and non-natural compounds. Imine containing compounds have been displayed a wide range of biological activities [5, 6]. In organic synthesis the formation of carbon-nitrogen double bond is of great interest and can be achieved in acidic medium by the reaction of aldehydes/ketones and amines [7, 8]. Similarly, in pharmaceutical and medicinal field, Schiff bases are playing an important role [9]. Such class of compounds displayed various biological activities such as antibacterial, antifungal, antitumor and antiherbicidal activities etc. [10].

Antioxidants are the agents which prevent blood vessel membranes from oxidative damage thus ensure proper blood circulation and avoid cardiovascular diseases. They provide defense against cancer-causing DNA damages. Modes of action of antioxidants are attributed to their radical scavenging activity by contributing one electron to the free radicals and to convert them into harmless molecules. Antioxidants thus protect cells from the oxidative damage that leads to aging and other diseases [11-13].

Proteins, nucleic acids, lipids, and extra-cellular matrix glycosaminoglycans (*e.g.* hyaluronic acid) are found to have strong affinity for free radicals and cause different diseases. The polyunsaturated fatty acids are more vulnerable to free radical damage, causing cerebral ischemia and other CNS diseases [14]. Free radicals have also been found to contribute in the pathology of arteriosclerosis, malaria and rheumatoid arthritis, in neurodegenerative disease, and aging [15,16]. Thus, the discovery and development of effective antioxidants, capable of supplementing body's antioxidant system is an important area of research.

Our group has discovered a variety of Schiff bases for their antioxidant activities (Fig. 1) [17-19] which encouraged us to investigate 2,4-dinitro phenylhydrazone **1-32** in order to identify more diversified and strong antioxidant agents.

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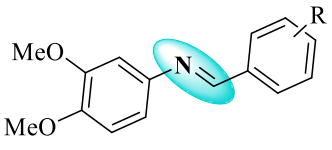
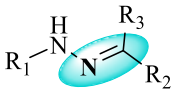
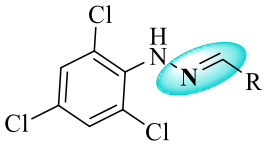


3,4-Dimethoxybenzenamine-Schiff Bases



Acylhydrazide Schiff Bases



2,4,6-Trichlorophenylhydrazine-Schiff Bases

Fig. 1: Identified leads for the antioxidant activity.

Experimental

Materials and Methods

Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT-311A, Germany. ^{1}H-NMR experiments were performed in DMSO-d_6 using AVANCE Bruker AM 400 MHz spectrometer. Splitting patterns were as follows; s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet). Chemical shift are reported in δ (ppm) and coupling constant are given in Hz. CHN Elemental analysis was done with Carlo Erba Strumentazion-Mod-1106, Italy. Progress of all reactions was monitored by thin layer chromatography (TLC) which was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). The chromatograms were visualized by ultraviolet light at 254 and 365 nm.

General Procedure for the Synthesis of Compounds **1-32**

2,4-Dinitro phenyl hydrazine (2 mmol) and different aromatic aldehydes (2 mmol) in absolute methanol (10 mL) were refluxed for 3-5 h. Acetic acid was used as a catalyst. In all cases, solid product was formed which was filtered, washed with absolute methanol and dried under vacuum. The pure compounds **1-32** were obtained as fluffy solids having satisfactory spectroscopic data.

N-(2,4-Dinitrophenyl)-N'-(2'-methylbenzylidene)hydrazone **(1)**

Yield: 0.22 g, 83%; ^{1}H-NMR (DMSO-d_6): δ 3.28 (s, 1H, NH), 8.83 (d, J = 2.7 Hz, 1H, H-3), 8.34 (dd, J = 2.7 Hz, J = 2.7 Hz, 1H, H-5), 7.88 (d, J = 7.8 Hz, 1H, H-6), 2.46 (s, 3H, CH_3), 7.24 (t, J = 8.1 Hz, 2H, H-3', H-5'), 7.30 (m, 1H, H-4'), 8.04 (d, J = 9.6 Hz, 1H, H-6'), 8.98 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 300 (M^+, 100), 285 (63), 209 (71), 196 (81), 167 (41), 121 (26); Anal. Calcd for $C_{14}H_{12}N_4O_4$ (300.27); C, 56.00; H, 4.03; N, 18.66; Found; C, 55.97; H, 4.00; N, 18.62.

N-(2,4-Dinitrophenyl)-N'-(4'-thiomethylbenzylidene)hydrazone **(2)**

Yield: 0.23 g, 87%; ^{1}H-NMR (DMSO-d_6): δ 3.29 (s, 1H, NH), 8.83 (dd, J = 2.7 Hz, 1H, H-3), 8.34 (dd, J = 2.7 Hz, J = 2.7 Hz, 1H, H-5), 7.32 (d, J = 8.7 Hz, 1H, H-6), 8.05 (d, J = 9.6 Hz, 2H, H-2', H-6'), 7.69 (d, J = 8.7 Hz, 2H, H-3', H-5'), 2.49 (bds, 3H, CH_3), 8.62 (s, C-H); EI-MS *m/z* (% rel. abund.): 317 (M^+, 100), 284 (61), 209 (73), 196 (79), 167 (34); Anal. Calcd for $C_{14}H_{12}N_4O_4S$ (332.33); C, 50.60; H, 3.64; N, 16.86; S, 9.65; Found; C, 50.57; H, 3.62; N, 16.82; S, 9.61.

N-(2,4-Dinitrophenyl)-N'-(2',4'-dimethoxybenzylidene)hydrazone **(3)**

Yield: 0.33 g, 98%; ^{1}H-NMR (DMSO-d_6): δ 3.82 (s, 1H, NH), 8.83 (d, J = 2.4 Hz, 1H, H-3), 8.32 (dd, J = 2.7 Hz, J = 2.7 Hz, H-5), 7.39 (d, J = 7.8 Hz, 1H, H-6), 3.28 (s, 6H, OCH_3), 7.02 (d, J = 8.4 Hz, 1H, H-3'), 7.23 (dd, J = 1.8 Hz, J = 1.8 Hz, 1H, H-5'), 8.07 (d, J = 9.6 Hz, 1H, H-6'), 8.57 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 346 (M^+, 97), 315 (36), 209 (100), 150 (24), 137 (34); Anal. Calcd for $C_{15}H_{14}N_4O_6$ (346.30); C, 52.03; H, 4.08; N, 16.18; Found; C, 52.01; H, 4.05; N, 16.14.

N-(2,4-Dinitrophenyl)-N'-(4'-methoxybenzylidene)hydrazone **(4)**

Yield: 0.25 g, 87%; ^{1}H-NMR (DMSO-d_6): δ 3.82 (s, I-H, NH), 8.83 (d, J = 2.7 Hz, 1H, H-3), 8.31 (dd, J = 2.7 Hz, J = 2.7 Hz, 1H, H-5), 7.02 (d, J = 8.7 Hz, 1H, H-6), 8.04 (d, J = 9.6 Hz, 2H, H-2', H-6'), 7.72 (d, J = 8.7 Hz, 2H, H-3', H-5'), 3.28 (s, 3H, OCH_3), 8.61 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 316 (M^+, 100), 285 (57), 209 (68), 196 (83), 167 (34); Anal. Calcd for $C_{14}H_{12}N_4O_5$ (316.27); C, 53.17; H, 3.82; N, 17.72; Found; C, 53.14; H, 3.80; N, 17.69.

N-(2,4-Dinitrophenyl)-N'-(4'-methylbenzylidene)hydrazone **(5)**

Yield: 0.26 g, 86%; ^{1}H-NMR (DMSO-d_6): δ 3.27 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.33 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.27 (d, *J* = 7.8 Hz, 1H, H-6), 8.06 (d, *J* = 9.6 Hz, 2H, H-2', H-6'), 7.66 (d, *J* = 8.1 Hz, 2H, H-3', H-5'), 2.33 (s, 3H, CH_3), 8.63 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 300 (M^+ , 100), 284 (61), 196 (84), 167 (24), 107 (34); Anal. Calcd for $C_{14}H_{12}N_4O_4$ (300.27); C, 56.00; H, 4.03; N, 18.66; Found; C, 55.97; H, 4.01; N, 18.62.

N-(2,4-Dinitrophenyl)-N'-(3',4',5'-trimethoxybenzylidene)hydrazone **(6)**

Yield: 0.24 g, 86%; ^{1}H-NMR (DMSO-d_6): δ 3.28 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.33 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz 1H, H-5), 8.09 (d, *J* = 9.6 Hz, 1H, H-6), 7.06 (s, 2H, H-2', H-6'), 3.28 (s, 9H, 3OCH_3), 8.55 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 376 (M^+ , 100), 330 (36), 209 (68), 196 (88), 180 (22); Anal. Calcd for $C_{13}H_{10}N_4O_7$ (376.33); C, 46.72; H, 3.02; N, 16.76; Found; C, 46.69; H, 2.99; N, 16.74.

N-(2,4-Dinitrophenyl)-N'-(2',3'-dihydroxybenzylidene)hydrazone **(7)**

Yield: 0.23 g, 82%; ^{1}H-NMR (DMSO-d_6): δ 3.27 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.33 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.97 (d, *J* = 4.2 Hz, 1H, H-6), 5.02 (bds, 2H, 2OH), 6.83(dd, *J* = 1.2 Hz, *J* = 1.2 Hz, 1H, H-4'), 6.69 (t, *J* = 12.00 Hz, 1H, H-5'), 7.97 (d, *J* = 4.2 Hz, 1H, H-6'), 8.93 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 318 (M^+ , 100), 272 (37), 196 (84), 151 (26), 109 (67); Anal. Calcd for $C_{13}H_{10}N_4O_6$ (318.25); C, 49.06; H, 3.17; N, 17.61; Found; C, 49.03; H, 3.13; N, 17.59.

N-(2,4-Dinitrophenyl)-N'-(3'-hydroxybenzylidene)hydrazone **(8)**

Yield: 0.24, 86%; ^{1}H-NMR (DMSO-d_6): δ 3.27 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.36 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.24 (d, *J* = 7.8 Hz, 1H, H-6), 7.16 (t, *J* = 3.6 Hz, 2H, H-2', H-5'), 5.03 (bds, 1H, OH), 6.83 (m, 1H, H-4'), 8.01 (d, *J* = 9.6 Hz, 1H, H-6'), 8.59 (s,1H, C-H); EI-MS *m/z* (% rel. abund.): 302 (M^+ , 23), 209 (100), 196 (91), 135 (26), 106 (67); Anal. Calcd for $C_{13}H_{10}N_4O_5$ (302.25); C, 51.66; H, 3.34; N, 18.54; Found; C, 51.64; H, 3.31; N, 18.51.

N-(2,4-Dinitrophenyl)-N'-(2'-hydroxybenzylidene)hydrazone **(9)**

Yield: 0.28, 89%; ^{1}HNMR (400 MHz, DMSO-d_6): δ 3.29 (s, 1H, NH), 8.84 (d, *J* = 2.4 Hz, 1H, H-3), 8.36 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.17 (d, *J* = 7.8 Hz, 1H, H-6), 5.00 (bds, 1H, OH), 6.84 (m, 2H, H-3', H-5'), 7.25 (t, *J* = 15.9 Hz, 1H, H-4'), 8.01 (d, *J* = 9.6 Hz, 1H, H-6'), 8.59 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 302 (M^+ , 100), 285 (61), 196 (87), 135 (49), 93 (37); Anal. Calcd for $C_{13}H_{10}N_4O_5$ (302.25); C, 51.66; H, 3.34; N, 18.54; Found; C, 51.63; H, 3.31; N, 18.50.

N-(2,4-Dinitrophenyl)-N'-(2',4'-dihydroxybenzylidene)hydrazone **(10)**

Yield: 0.25, 84%; ^{1}H-NMR (DMSO-d_6): δ 3.29 (s, 1H, NH), 8.82 (d, *J* = 2.7 Hz, 1H, H-3), 8.29 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.62 (d, *J* = 9.3 Hz, 1H, H-6), 5.02 (bds, 2H, 2OH), 6.33 (m, 2H, H-3', H-5'), 7.92 (d, *J* = 9.3 Hz, 1H, H-6'), 8,78 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 318 (M^+ , 100), 284 (53), 209 (31), 196 (78), 167 (26), Anal. Calcd for $C_{13}H_{10}N_4O_6$ (318.25); C, 49.06; H, 3.17; N, 17.61.16; Found; C, 49.03; H, 3.15; N, 17.58.

N-(2,4-Dinitrophenyl)-N'-(2'-hydroxy-5'-nitrobenzylidene)hydrazone **(11)**

Yield: 0.24 g, 88%; ^{1}H-NMR (DMSO-d_6): δ 3.29 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.42 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 6.03 (d, *J* = 9.00 Hz, 1H, H-6), 5.01 (bds, 1H, OH), 7.02 (d, *J* = 9.64 Hz, 1H, H-3'), 7.11 (dd, *J* = 2.7 Hz, *J* = 3.00 Hz, 1H, H-4'), 8.60 (d, *J* = 3.01 Hz, 1H, H-6'), 8.96 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 347 (M^+ , 100), 330 (26), 313 (41), 284 (51), 196 (84), 180 (67), Anal. Calcd for $C_{13}H_9N_5O_7$ (347.24); C, 44.97; H, 2.61; N, 20.17; Found; C, 44.95; H, 2.58; N, 20.14; O, 32.23.

N-(2,4-Dinitrophenyl)-N'-(4'-chlorobenzylidene)hydrazone **(12)**

Yield: 0.21 g, 78%; ^{1}H-NMR (DMSO-d_6): δ 3.27 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.34 (dd, J = 2.1 Hz, *J* = 2.1 Hz, 1H, H-5), 7.52 (d, *J* = 6.6 Hz, 1H, H-6), 8.08 (d, *J* = 7.2 Hz, 2H, H-2', H-6'), 7.79 (d, *J* = 6.6 Hz, 2H, H-3', H-5'), 8.67 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 320 (M^+ , 100), 285 (61), 274 (78), 228 (26), 196 (71); Anal. Calcd for $C_{13}H_9ClN_4O_4$ (320.69); C, 48.69; H, 2.83; N, 17.47; Found; C, 48.63; H, 2.80; N, 17.43.

N-(2,4-Dinitrophenyl)-N'-(3'-nitrobenzylidene)hydrazone **(13)**

Yield: 0.28 g 84%; ^{1}H-NMR (DMSO-d_6): δ 3.30 (s, 1H, NH), 8.84 (d, *J* = 2.7 Hz, 1H, H-3), 8.42 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 8.12 (d, *J* = 9.6 Hz, 1H, H-6), 8.76 (d, *J* = 6.3 Hz, 1H, H-2'), 8.25 (dd, *J* = 2.4 Hz, J = 2.4 Hz, 1H, H-4'), 7.75 (d, *J* = 16.2Hz, 1H, H-5'), 8.21 (d, *J* = 7.8 Hz, 1H, H-6'), 8.78 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 331 (M^+ , 100), 285 (67), 209 (78), 196 (81), 122 (51); Anal. Calcd for $C_{13}H_9N_6O_6$ (331.24); C, 47.14; H, 2.74; N, 21.14; Found; C, 47.10; H, 2.70; N, 21.11.

N-(2,4-Dinitrophenyl)-N'-(2'-chlorobenzylidene)hydrazone **(14)**

Yield: 0.25 g, 86%; ^{1}H-NMR (DMSO-d_6): δ 3.29 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.35 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.42 (d, *J* = 1.8 Hz, 1H, H-6), 7.51 (d, *J* = 1.5 Hz, 1H, H-3'), 7.45 (t, *J* = 6.00 Hz, 2H, H-4', H-5'), 7.53 (d, *J* = 2.1 Hz, 1H, H-6'), 9.08 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 320 (M^+ , 100), 284 (65), 209 (81), 198 (81), 196 (68); Anal. Calcd for $C_{13}H_9ClN_4O_4$ (320.69); C, 48.69; H, 2.83; N, 17.47; Found; C, 48.66; H, 2.79; N, 17.44.

N-(2,4-Dinitrophenyl)-N'-(2'-bromobenzylidene)hydrazone **(15)**

Yield: 0.21 g, 71%; ^{1}H-NMR (DMSO-d_6): δ 3.27 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.35 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.38 (d, *J* = 1.5 Hz, 1H, H-6), 7.68 (d, *J* = 6.9 Hz, 2H, H-3', H-6'), 7.46 (t, *J* = 9.3 Hz, 2H, H-4', H-5'), 9.01 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 363 (M^+ , 100), 284 (67), 209 (81), 196 (81); Anal. Calcd for $C_{13}H_9BrN_4O_4$ (365.14); C, 42.76; H, 2.48; N, 15.34; Found; C, 42.73; H, 2.46; N, 15.30.

N-(2,4-Dinitrophenyl)-N'-(4'-dimethylaminebenzylidene)hydrazone **(16)**

Yield: 0.28 g, 77%; ^{1}H-NMR (DMSO-d_6): δ 3.27 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.33 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.58 (d, *J* = 6.9 Hz, 1H, H-6), 7.97 (d, *J* = 4.2 Hz, 2H, H-2', H-6'), 6.74 (d, *J* = 6.6 Hz, 2H, H-3', H-5'), 2.96 (s, 6H, $2CH_3$-N), 8.93 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 329 (M^+ , 100), 285 (65), 209 (81), 162 (49), 120 (37); Anal. Calcd for $C_{15}H_{15}N_5O_4$ (329.32); C, 54.71; H, 4.59; N, 21.27; Found; C, 54.67; H, 4.57; N, 21.24.

N-(2,4-Dinitrophenyl)-N'-(anthracen-9-ylmethylene)hydrazone **(17)**

Yield: 0.28 g, 77%; ^{1}H-NMR (DMSO-d_6): δ 3.27 (s, 1H, NH), 8.90 (d, *J* = 1.8 Hz, 1H, H-3), 8.41 (dd, *J* = 1.8 Hz, *J* = 1.8 Hz, 1H, H-5), 8.01 (d, *J* = 7.2 Hz, 1H, H-6), 8.15 (d, *J* = 6.5 Hz, 4H, H-3', H-6', H-10', H-13'), 7.65 (m, 4H, H-4', H-5', H-11', H-12'), 8.78 (d, *J* = 6.6 Hz, 1H, H-8'), 8.74 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 386 (M^+ , 100), 340 (53), 294 (37), 219 (67), 177 (26); Anal. Calcd for $C_{21}H_{14}N_4O_4$ (386.37); C, 65.28; H, 3.65; N, 14.50; Found; C, 65.26; H, 3.61; N, 14.48.

N-(2,4-Dinitrophenyl)-N'-(2',4'-dichlorobenzylidene)hydrazone **(18)**

Yield: 0.25 g, 85%; ^{1}H-NMR (DMSO-d_6): δ 3.83 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.31 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.02 (d, *J* = 8.7 Hz, 1H, H-6), 7.06 (s,1H, H-3'), 7.72 (d, *J* = 8.7 Hz, 1H, H-5'), 8.04 (d, *J* = 9.6 Hz, 1H, H-6'), 8.61 (s, 1H C-H); EI-MS *m/z* (% rel. abund.): 353 (M^+ , 37), 283 (100), 209 (78), 191 (63), 75 (26); Anal. Calcd for $C_{13}H_8Cl_2N_4O_4$ (355.13); C, 43.97; H, 2.27; N, 15.78; Found; C, 43.94; H, 2.25; N, 15.76.

N-(2,4-Dinitrophenyl)-N'-(4'-hydroxybenzylidene)hydrazone **(19)**

Yield: 0.25 g, 86%; ^{1}H-NMR (DMSO-d_6): δ 3.23 (s, 1H, NH), 8.84 (d, *J* = 1.8 Hz, 1H, H-3), 8.33 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.88 (d, *J* = 7.5 Hz, 1H, H-6), 7.12 (d, *J* = 6.3 Hz, 2H, H-2', H-6'), 6.42 (d, *J* = 6.3 Hz, 2H, H-3', H-5'), 5.02 (bds, 1H, OH), 8.81 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 302 (M^+ , 100), 285 (78), 256 (61), 209 (71); Anal. Calcd for $C_{13}H_{10}N_4O_5$ (302.25); C, 51.66; H, 3.34; N, 18.54; Found; C, 51.64; H, 3.30; N, 18.51.

N-(2,4-Dinitrophenyl)-N'-(3',4'-dimethylbenzylidene)hydrazone **(20)**

Yield: 0.26 g, 87%; ^{1}H-NMR (DMSO-d_6): δ 3.33 (s, 1H, NH), 8.83 (d, *J* = 2.7 Hz, 1H, H-3), 8.33 (dd, *J* = 2.7 Hz, *J* = 2.7 Hz, 1H, H-5), 7.06 (d, *J* = 13.5 Hz, 1H, H-6), 7.42 (d, *J* = 4.5 Hz, 2H, H-2', H-6'), 2.50 (s, 6H, $2CH_3$), 7.26 (dd, *J* = 3.3 Hz, J = 5.7 Hz, 1H, H-5'), 8.63 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 314 (M^+ , 100), 299 (53), 284 (61), 209 (79), 105 (67); Anal. Calcd for $C_{15}H_{14}N_4O_4$ (314.30); C, 57.32; H, 4.49; N, 17.83; Found; C, 57.29; H, 4.45; N, 17.80.

N-(2,4-Dinitrophenyl)-N'-(2',3',4'-trihydroxybenzylidene)hydrazone **(21)**

Yield: 0.27 g, 90%; ^{1}H-NMR (DMSO-d_6): δ 3.34 (s, 1H, NH), 8.84 (d, J = 1.8 Hz, 1H, H-3), 8.33 (dd, J = 2.7 Hz, J = 2.7 Hz, 1H, H-5), 7.88 (d, J = 7.5 Hz, 1H, H-6), 5.03 (bds, 3H, 3OH), 6.42 (d, J = 6.00 Hz, 1H, H-5'), 7.12 (d, J = 7.5 Hz, 1H, H-6'), 8.81 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 334 (M^+, 100), 302 (49), 286 (67), 209 (81), 167 (37); Anal. Calcd for $C_{13}H_{10}N_4O_7$ (334.24); C, 46.72; H, 3.02; N, 16.76; Found; C, 46.69; H, 2.99; N, 16.73.

N-(2,4-Dinitrophenyl)-N'-(3',4'-dihydroxybenzylidene)hydrazone **(22)**

Yield: 0.24 g, 87%; ^{1}H-NMR (DMSO-d_6): δ 3.33 (s, 1H, NH), 8.85 (d, J = 1.8 Hz, 1H, H-3), 8.36 (dd, J = 2.1 Hz, J = 1.8 Hz, 1H, H-5), 7.66 (d, J = 7.5 Hz, 1H, H-6), 7.24 (d, J = 3.6 Hz, 1H, H-2'), 5.01 (bd, s, 2H, 2OH), 7.03 (dd, J = 1.5 Hz, J = 1.5 Hz, 1H, H-5'), 7.98 (d, J = 7.2 Hz, 1H, H-6'), 8.51 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 318 (M^+, 67), 302 (100), 286 (67), 209 (61), 151 (34); Anal. Calcd for $C_{13}H_{10}N_4O_6$ (318.25); C, 49.06; H, 3.17; N, 17.61; Found; C, 49.03; H, 3.14; N, 17.58.

N-(2,4-Dinitrophenyl)-N'-(2'-nitrobenzylidene)hydrazone **(23)**

Yield: 0.17 g, 55%; ^{1}H-NMR (DMSO-d_6): δ 3.33 (s, 1H, NH), 8.83 (d, J = 1.2 Hz, 1H, H-3), 8.36 (dd, J = 1.8 Hz, J = 1.8 Hz, 1H, H-5), 6.42 (d, J = 6.3 Hz, 1H, H-6), 8.83 (d, J = 1.8 Hz, 1H, H-3'), 7.66 (m, 1H, H-4'). 7.80 (m, 1H, H-5'), 8.16 (d, J = 1.8 Hz, 1H, H-6'), 9.03 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 331 (M^+, 61), 285 (100), 209 (71), 164 (26); Anal. Calcd for $C_{13}H_9N_6O_6$ (331.24); C, 47.14; H, 2.74; N, 21.14; Found; C, 47.09; H, 2.71; N, 21.10.

N-(2,4-Dinitrophenyl)-N'-(3'-ethoxy-2'-hydroxybenzylidene)hydrazone **(24)**

Yield: 0.25 g, 83%; ^{1}H-NMR (DMSO-d_6): δ 3.30 (s, 1H, NH), 8.83 (d, J = 2.1 Hz, 1H, H-3), 8.33 (dd, J = 4.2 Hz, J = 2.1 Hz, 1H, H-5), 7.40 (d, J = 6.3 Hz, 1H, H-6), 5.02 (bd, s, 1H, OH), 1.33 (t, J = 10.2 Hz, 3H, CH_3), 4.05 (q, J = 15.6 Hz, 2H, CH_2), 6.99 (dd, J = 0.9 Hz, J = 0.9 Hz, 1H, H-4'), 6.81 (t, J = 12.0 Hz, 1H, H-5'), 7.99 (d, J = 7.2 Hz, 1H, H-6'), 8.96 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 346 (M^+, 100), 330 (37), 301 (26), 209 (53), 179 (22); Anal. Calcd for $C_{15}H_{14}N_4O_6$ (346.30); C, 52.03; H, 4.08; N, 16.18; Found; C, 52.00; H, 4.06; N, 16.14.

N-(2,4-Dinitrophenyl)-N'-(2'-hydroxy-3'-methoxybenzylidene)hydrazone **(25)**

Yield: 0.22 g, 69%; ^{1}H-NMR (DMSO-d_6): δ 3.80 (s, 1H, NH), 8.82 (d, J = 2.1 Hz, 1H, H-3), 8.32 (dd, J = 2.7 Hz, J = 2.7 Hz, 1H, H-5), 7.41 (d, J = 6.0 Hz, 1H, H-6), 3.89 (bds, 1H, OH), 3.21 (s, 3H, OCH_3), 7.00 (dd, J = 0.9 Hz, J = 1.2 Hz, 1H, H-4'), 6.82 (t, J = 12.0 Hz, 1H, H-5'), 8.00 (d, J = 7.2 Hz, 1H, H-6'), 8.95 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 332 (M^+, 100), 316 (53), 301 (37), 285 (75), 209 (69), 165 (36); Anal. Calcd for $C_{14}H_{12}N_4O_6$ (332.27); C, 50.61; H, 3.64; N, 16.86; Found; C, 50.57; H, 3.61; N, 16.83.

N-(2,4-Dinitrophenyl)-N'-(2'-bromo-6'-hydroxybenzylidene)hydrazone **(26)**

Yield: 0.25 g, 86%; ^{1}H-NMR (DMSO-d_6): δ 3.28 (s, 1H, NH), 8.82 (d, J = 2.1 Hz, 1H, H-3), 8.34 (dd, J = 2.1 Hz, J = 2.1 Hz, 1H, H-5), 7.93 (d, J = 8.1 Hz, 1H, H-6), 3.43 (bd, s, 1H, OH), 6.85 (d, J = 6.6 Hz, 1H, H-3'), 7.39 (dd, J = 2.1 Hz, J = 2.1 Hz, 1H, H-4'), 8.07 (d, J = 7.2 Hz, 1H, H-6'), 8.87 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 379 (M^+, 67), 300 (100), 284 (49), 209 (81); Anal. Calcd for $C_{13}H_9BrN_4O_5$ (381.14); C, 40.97; H, 2.38; N, 14.70; Found; C, 40.95; H, 2.35; N, 14.66.

N-(2,4-Dinitrophenyl)-N'-(4'-nitrobenzylidene)hydrazone **(27)**

Yield: 0.25, 84%; ^{1}H-NMR (DMSO-d_6): δ 3.31 (s, 1H, NH), 8.82 (d, J = 2.1 Hz, 1H, H-3), 8.34 (dd, J = 2.1 Hz, J = 2.1 Hz, 1H, H-5), 8.04 (d, J = 6.9 Hz, 1H, H-6), 8.15 (d, J = 7.2 Hz, 2H, H-2', H-6'), 8.31 (d, J = 6.6 Hz, 2H, H-3, H-5'), 8.87 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 331 (M^+, 63), 285 (100), 196 (37), 164 (53); Anal. Calcd for $C_{13}H_9N_6O_6$ (331.24); C, 47.14; H, 2.74; N, 21.14; Found; C, 47.09; H, 2.71; N, 21.12.

N-(2,4-Dinitrophenyl)-N'-(naphthalen-1-ylmethylene)hydrazone **(28)**

Yield: 0.23 g, 82%; ^{1}H-NMR (DMSO-d_6): δ 3.32 (s, 1H, NH), 8.82 (d, J = 2.1 Hz, 1H, H-3), 8.39 (dd, J = 1.8 Hz, J = 2.1 Hz, 1H, H-5), 8.01 (d, J = 7.8 Hz, 1H, H-6), 8.07 (d, J = 8.1 Hz, 1H, H-2'), 7.96 (t, J = 7.2 Hz, 1H, H-3'), 8.06 (d, J = 6.3 Hz, 1H, H-4'), 8.18 (d, J = 7.2 Hz, 2H, H-6', H-9'), 7.58 (m, 2H, H-7', H-8'), 8.87 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 336 (M^+, 100), 260 (49), 196 (67), 164 (83); Anal. Calcd for $C_{17}H_{12}N_4O_4$ (336.31); C, 60.71; H, 3.60; N, 16.66; Found; C, 60.67; H, 3.03; N, 16.62.

N-(2,4-Dinitrophenyl)-N'-(2',3',4'-trimethoxybenzylidene)hydrazone **(29)**

Yield: 0.22 g, 83%; ^{1}H-NMR (DMSO-d_6): δ 3.82 (s, 1H, NH), 8.83 (d, J = 2.7 Hz, 1H, H-3), 8.31 (dd, J = 2.1 Hz, J = 2.1 Hz, 1H, H-5), 7.72 (d, J = 8.7 Hz, 1H, H-6), 3.32 (s, 9H, 3OCH_3), 7.02 (d, J = 8.7 Hz, 1H, H-5'), 8.04 (d, J = 9.6 Hz, 1H, H-6'), 8.61 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 376 (M^+, 100), 345 (70), 314 (21), 283 (34), 209 (41); Anal. Calcd for $C_{16}H_{16}N_4O_7$ (376.33); C, 51.07; H, 4.29; N, 14.89; Found; C, 51.04; H, 4.25; N, 14.83.

N-(2,4-Dinitrophenyl)-N'-(naphthalen-2-ylmethylene)hydrazone **(30)**

Yield: 0.26 g, 86%; ^{1}H-NMR (DMSO-d_6): δ 3.33 (s, 1H, NH), 8.88 (d, J = 2.1 Hz, 1H, H-3), 8.39 (dd, J = 1.8 Hz, J = 2.1 Hz, 1H, H-5), 7.39 (d, J = 7.8 Hz, 1H, H-6), 8.08 (d, J = 7.8 Hz, 1H, H-2'), 8.07 (d, J = 6.6 Hz, 1H, H-3'), 8.19 (d, J = 7.2 Hz, 2H, H-5', H-8'), 7.58 (m, 2H, H-6', H-7'), 8.20 (s, 1H, H-10'), 8.86 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 336 (M^+, 100), 260 (61), 196 (51), 169 (49), 140 (17); Anal. Calcd for $C_{17}H_{12}N_4O_4$ (336.31); C, 60.71; H, 3.60; N, 16.66; Found; C, 60.68; H, 3.56; N, 16.64.

N-(2,4-Dinitrophenyl)-N'-(3',4'-dimethoxybenzylidene)hydrazone **(31)**

Yield: 0.28, 89%; ^{1}H-NMR (DMSO-d_6): δ 3.84 (s, 1H, NH), 8.85 (d, J = 2.1 Hz, 1H, H-3), 8.34 (dd, J = 1.8 Hz, J = 1.8 Hz, 1H, H-5), 7.41 (d, J = 1.5 Hz, 1H, H-6), 7.05 (d, J = 6.3 Hz, 1H, H-2'), 3.32 (s, 2H, 2OCH_3), 7.26 (dd, J = 1.5 Hz, J = 1.5 Hz, 1H, H-5'), 8.10 (d, J = 7.2 Hz, 1H, H-6'), 8.60 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 346 (M^+, 100), 315 (49), 284 (67), 209 (51), 196 (31); Anal. Calcd for $C_{15}H_{14}N_4O_6$ (346.30); C, 52.03; H, 4.08; N, 16.18; Found; C, 52.00; H, 4.05; N, 16.14.

N-(2,4-Dinitrophenyl)-N'-(2',3'-dimethoxybenzylidene)hydrazone **(32)**

Yield: 0.26 g, 87%; ^{1}H-NMR (DMSO-d_6): δ 3.84 (s, 1H, NH), 8.80 (d, J = 1.8 Hz, 1H, H-3), 8.35 (dd, J = 1.5 Hz, J = 2.1 Hz, 1H, H-5), 7.42 (d, J = 1.5 Hz, 1H, H-6), 3.35 (s, 6H, 2OCH_3), 7.05 (d, J = 6.3 Hz, 1H, H-4'), 7.26 (m, 1H, H-5'), 8.10 (d, J = 6.9 Hz, 1H, H-6'), 8.60 (s, 1H, C-H); EI-MS *m/z* (% rel. abund.): 346 (M^+, 100), 317 (34), 286 (71), 181 (66); Anal. Calcd for $C_{15}H_{14}N_4O_6$ (346.30); C, 52.03; H, 4.08; N, 16.18; Found; C, 52.01; H, 4.05; N, 16.14.

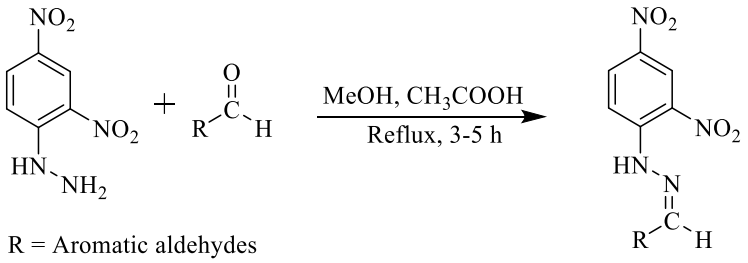
Results and discussion

Chemistry

A variety of 2,4-dinitro phenylhydrazone derivatives **1-32** were synthesized from commercially available 2,4-dinitro phenyl hydrazine by refluxing with different aromatic aldehydes in methanol (Scheme-1) in the presence of catalytic amount of glacial acetic acid. The progress of reaction was monitored by TLC (ethyl acetate:*n*-hexane, 1:1). Products were afforded in the form of crystals which were re-crystallized from methanol to get the pure crystals. The structure of synthesized compounds **1-32** were determined by using ^{1}H-NMR and EI-MS spectroscopy.

In vitro antioxidant activities

All 2,4-dinitro phenyl hydrazone derivatives **1-32** were subjected to *in vitro* antioxidant activities, including DPPH (2,2-diphenyl-1-picrylhydrazyl), hydroxyl radicals scavenging, ferrous ion-chelating, ferric reducing, total antioxidant activities. Results depicted in table-2 showed that all compounds showed good to moderate antioxidant activities.



Scheme-1: Synthesis of 2,4-dinitro phenyl hydrazone derivatives **1-32.**

Table-1: 2,4-Dinitro phenyl hydrazone derivatives **1-32.**

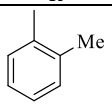
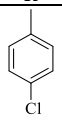
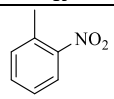
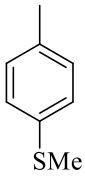
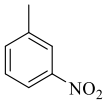
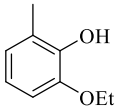
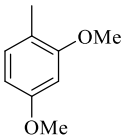
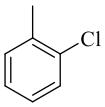
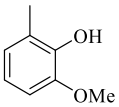
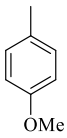
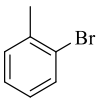
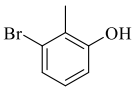
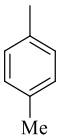
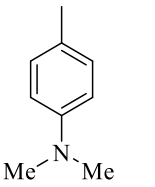
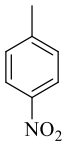
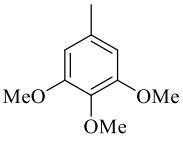
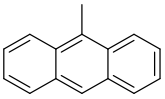
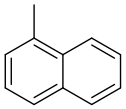
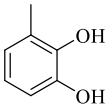
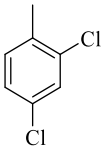
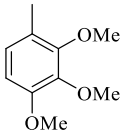
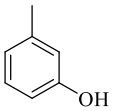
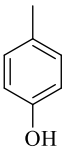
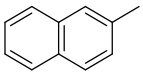
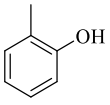
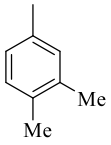
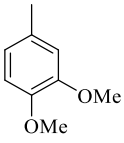
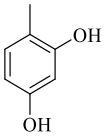
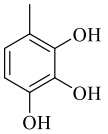
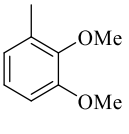
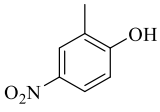
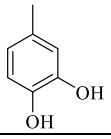
Compound	R	Compound	R	Compound	R
1		**12**		**23**	
2		**13**		**24**	
3		**14**		**25**	
4		**15**		**26**	
5		**16**		**27**	
6		**17**		**28**	
7		**18**		**29**	
8		**19**		**30**	
9		**20**		**31**	
10		**21**		**32**	
11		**22**		-	-

Table-2: *In vitro* antioxidant activities of 2,4-dinitro phenyl hydrazone derivatives **1-32.**

Compounds	DPPH Assay	Iron Chelation Assay	Iron Reducing Assay	Total Antioxidant Assay	Hydroxyl Assay
	IC_{50} ± SEM[a]	IC_{50} ± SEM[a]	IC_{50} ± SEM[a]	IC_{50} ± SEM[a]	IC_{50} ± SEM[a]
1	122.18 ± 11.99	111.00 ± 14.67	131.83 ± 131.83	144.15 ± 13.38	202.50 ± 9.589
2	164.53 ± 9.51	246.42 ± 8.111	198.05 ± 13.58	219.04 ± 9.094	227.59 ± 8.727
3	135.43 ± 9.64	149.92 ± 13.43	422.52 ± 9.938	485.17 ± 4.018	177.88 ± 9.705
4	129.68 ± 13.46	226.37 ± 8.298	179.01 ± 4.618	194.70 ± 10.07	168.34 ± 11.05
5	129.47 ± 12.64	317.47 ± 6.332	143.98 ± 10.50	158.54 ± 12.12	146.46 ± 13.62
6	124.98 ± 13.73	151.48 ± 11.65	117.06 ± 12.56	126.16 ± 14.9	133.32 ± 13.16
7	67.94 ± 15.54	118.85 ± 13.50	283.48 ± 14.70	262.11 ± 7.237	140.32 ± 12.64
8	133.26 ± 14.58	105.19 ± 14.92	212.76 ± 6.985	232.71 ± 8.100	186.24 ± 10.28
9	84.61 ± 14.95	250.38 ± 8.003	350.89 ± 8.394	462.14 ± 4.340	247.02 ± 7.989
10	88.06 ± 12.39	160.67 ± 12.46	116.03 ± 5.886	124.29 ± 15.40	120.64 ± 15.24
11	172.19 ± 11.97	126.83 ± 14.43	162.04 ± 15.38	160.28 ± 12.39	176.01 ± 11.08
12	220.96 ± 9.17	99.53 ± 17.63	161.07 ± 11.25	209.56 ± 9.502	156.16 ± 12.68
13	159.05 ± 12.86	101.57 ± 1.888	152.78 ± 1.734	172.28 ± 11.34	142.12 ± 12.99
14	177.14 ± 12.22	130.87 ± 14.41	230.73 ± 12.36	210.89 ± 9.111	191.08 ± 10.56
15	187.12 ± 11.28	226.90 ± 8.627	232.97 ± 8.655	235.09 ± 7.973	128.56 ± 14.07
16	451.96 ± 3.30	182.29 ± 9.728	289.33 ± 8.618	302.41 ± 6.178	159.02 ± 12.45
17	74.91 ± 13.27	155.54 ± 13.42	209.13 ± 6.824	230.70 ± 8.440	136.77 ± 14.06
18	126.39 ± 15.59	251.19 ± 7.657	123.84 ± 8.857	138.66 ± 13.88	230.22 ± 8.629
19	190.70 ± 1.86	207.93 ± 9.032	133.46 ± 14.34	128.60 ± 14.22	181.08 ± 10.98
20	484.82 ± 3.76	152.31 ± 1.424	197.91 ± 13.60	184.27 ± 9.994	158.61 ± 11.72
21	112.79 ± 14.66	111.60 ± 17.26	325.69 ± 6.071	290.58 ± 6.387	134.39 ± 13.83
22	283.72 ± 5.64	172.43 ± 10.19	322.67 ± .7230	202.19 ± 1.063	146.41 ± 12.72
23	106.57 ± 10.19	143.99 ± 12.65	187.32 ± 10.30	170.33 ± 10.50	161.52 ± 12.13
24	166.69 ± 11.97	118.22 ± 16.60	132.75 ± 14.03	154.46 ± 12.65	163.74 ± 14.97
25	249.67 ± 8.32	279.52 ± 7.133	306.99 ± 6.136	237.01 ± 7.927	186.21 ± 10.60
26	213.47 ± 9.55	176.96 ± 10.85	146.65 ± 13.13	184.51 ± 11.55	198.88 ± 10.16
27	140.35 ± 12.73	223.93 ± 8.536	111.05 ± 15.65	120.94 ± 15.85	138.69 ± 14.09
28	337.82 ± 0.83	155.70 ± 11.65	139.93 ± 13.32	126.89 ± 14.79	124.03 ± 14.16
29	260.99 ± 5.94	128.34 ± 1.479	132.92 ± 14.52	126.80 ± 15.23	166.64 ± 13.31
30	234.63 ± 8.74	195.09 ± 9.759	801.06 ± 2.424	781.19 ± 2.539	193.41 ± 10.13
31	278.34 ± 6.89	75.81 ± 15.98	277.83 ± 6.892	311.89 ± 6.188	138.78 ± 14.08
32	65.04 ± 13.35	416.80 ± 4.669	225.67 ± 8.813	228.49 ± 8.398	206.21 ± 9.450
Vitamin C[b]	77.83 ± 16.56	-	92.16 ± 17.74	97.85 ± 17.21	96.128 ± 17.50
EDTA[c]	-	101.86 ± 17.84	-	-	-

SEM[a] (standard error of mean); Vitamin C[b] (Standard for DPPH assay, iron reducing assay, total antioxidant assay, hydroxyl assay); EDTA[c] (Standard for iron chelation assay)

DPPH Radical scavenging activity

All 2,4-dinitro phenyl hydrazone derivatives **1-32** showed excellent to moderate DPPH free radical scavenging activities in the range of 65.04 ± 13.35-484.82 ± 3.76 μM as compared to standard vitamin C (IC_{50} = 77.83 ± 16.56 μM). Amongst the most active analogs, compound **32** (IC_{50} = 65.04 ± 13.35 μM) with 2,3-dimethoxy substitutions, was found to be the most potent compound of this series. Its excellent activity might be due to the presence of two vicinal methoxy groups which may involve in the free radical stabilization. Similarly, extended conjugation may also help in the stabilization. Another compound **7** (IC_{50} = 67.94 ± 15.54 μM) with 2,3-dihydroxy groups, was found to be the second most potent analog of this series. Its potent activity might be due to the presence of two hydroxy groups which can better stabilize the free radical. Other hydroxy substituted compounds **9** (IC_{50} = 84.61 ± 14.95 μM) and **10** (IC_{50} = 88.06 ± 12.39 μM) were found to be slightly less active than standard. Anthracenyl substituted derivative **17** (IC_{50} = 74.91 ± 13.27 μM) was found to be the third most active compound. Its good activity might be due to the presence of anthracenyl ring which can form the stable radical due to an extended conjugation (Fig. 2).

Ferrous ion-chelation activity

Compounds **1-32** were demonstrated good to moderate ferrous ion-chelation activity in the range of 75.81 ± 15.98-416.80 ± 4.669 μM as compared to standard EDTA (IC_{50} = 101.86 ± 17.84 μM). Again dimethoxy substituted compound **31** (IC_{50} = 75.81 ± 15.98 μM) was found to be the most potent analog. Compound **12** (IC_{50} = 99.53 ± 17.63 μM) with 4-chloro substitution was found to be slightly more active than the standard EDTA. Good activity of these compounds might be due to the presence of electronegative chloro group which can better chelate with the ferrous ion. 3-Nitro substituted analog **13** (IC_{50} = 101.57 ± 1.888 μM) displayed same ferrous ion-chelation activity as standard. In this case nitro group might involve in chelation with the ferrous ion. Compounds **1** (IC_{50} = 111.00 ± 14.67 μM), **8** (IC_{50} = 105.19 ± 14.92 μM), and **21** (IC_{50} = 111.60 ± 17.26 μM) showed slightly lesser ferrous ion-chelation activity (Fig-3).

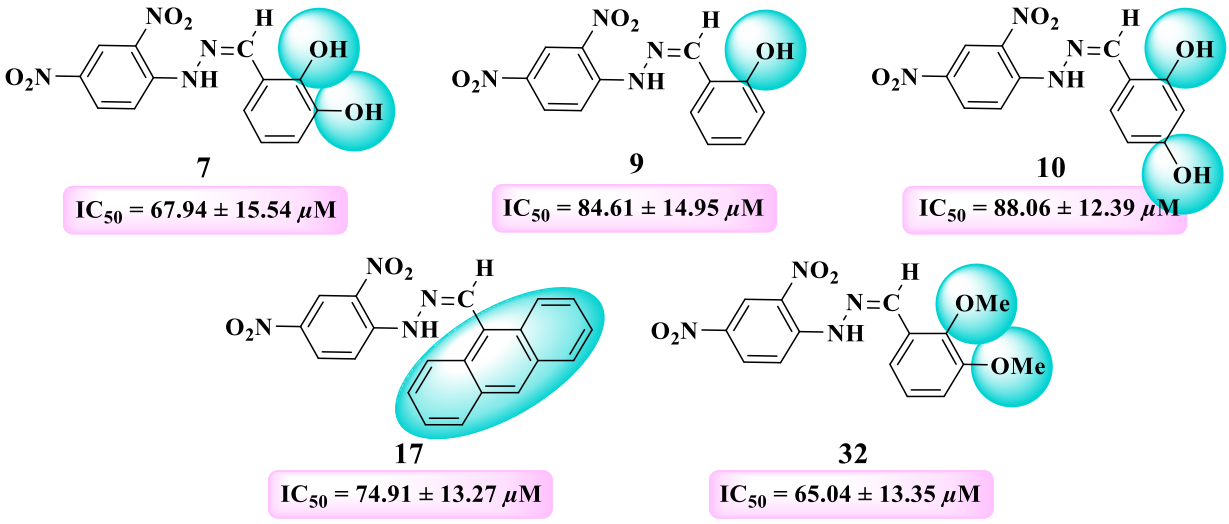


Fig-2: Structure-activity relationship of compound **7, 9, 10, 17,** and **32.**

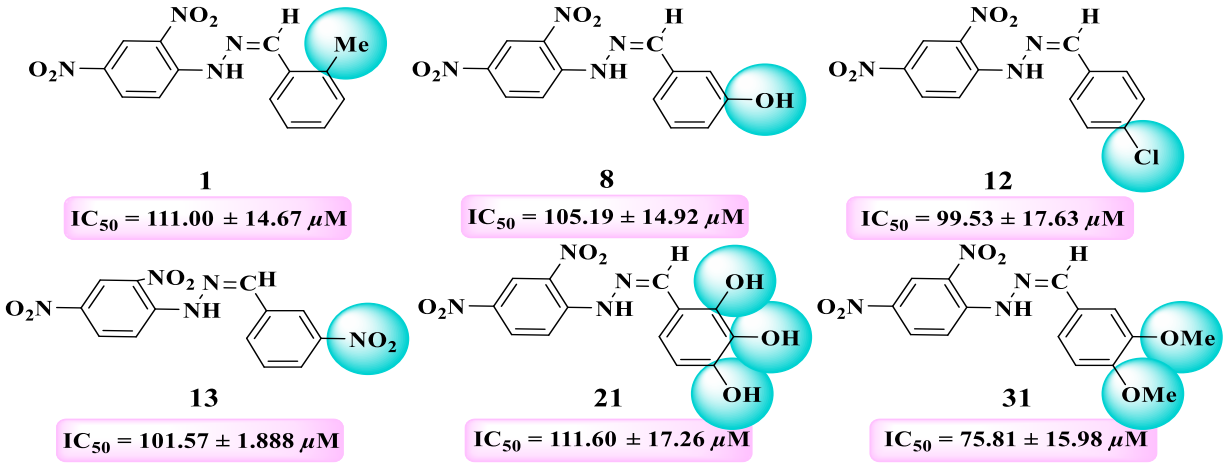


Fig. 3: Structure-activity relationship of compound **1, 8, 12, 13, 21, and 31.**

Ferric ion reducing activity

Compounds **1-32** were also subjected to *in vitro* ferric reducing assay. None of the compound was found to be more active than the standard vitamin C (IC_{50} = 92.16 ± 17.74 μM). Three compounds **6** (IC_{50} = 117.06 ± 12.56 μM), **10** (IC_{50} = 116.03 ± 5.886 μM), and **27** (IC_{50} = 111.05 ± 15.65 μM), were found to be moderately active (Fig-4) while rest of the compounds showed activity in the range of 123.84 ± 8.857-801.06 ± 2.424 μM as compared to standard.

Total antioxidant activity

All molecules **1-32** were also screened for *in vitro* total antioxidant activity and showed their moderate to weak potential in the range of 120.02 ± 15.90-781.19 ± 2.539 μM as compared to standard vitamin C (IC_{50} = 97.85 ± 17.21 μM). None of the compound was found to be more active than the standard. However, compounds **19** (IC_{50} = 128.60 ± 14.22 μM), **27** (IC_{50} = 120.94 ± 15.85 μM), **28** (IC_{50} = 126.89 ± 14.79 μM), and **29** (IC_{50} = 126.80 ± 15.23 μM), were found to be activities better than other analogs (Fig-5).

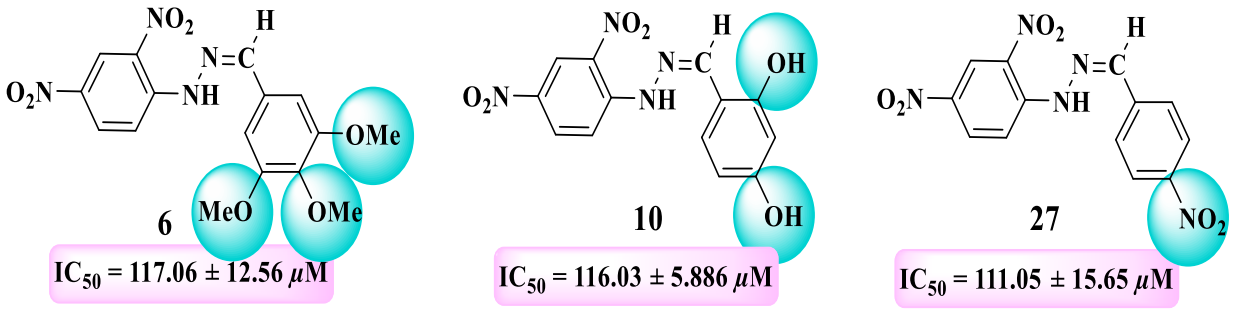


Fig. 4: Structure-activity relationship of compound **6, 10,** and **27.**

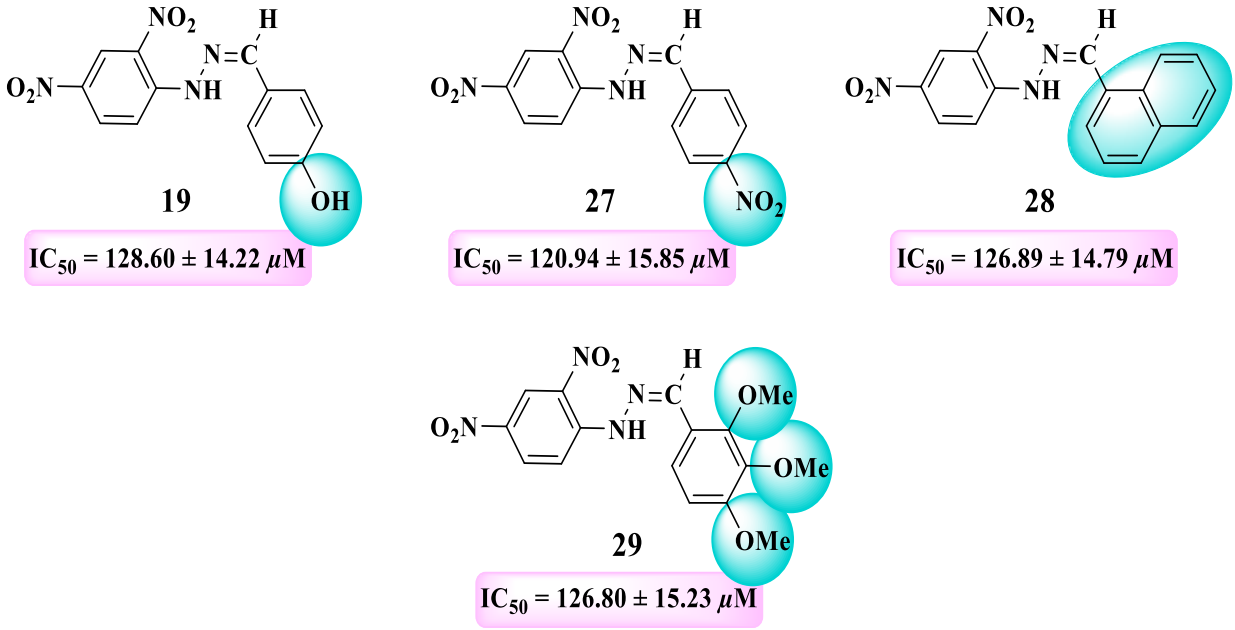


Fig. 5: Structure-activity relationship of compound **19, 27, 28, and 29.**

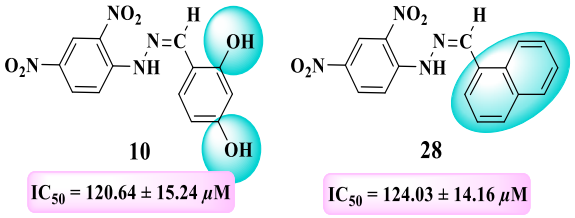


Fig. 6: Structure-activity relationship of compound **10, 28, and 32.**

Hydroxyl radical scavenging activity

Compounds **1-32** were further subjected to hydroxyl radical scavenging assay and showed the radical scavenging activity in the range of 120.64 ± 15.24-247.02 ± 7.989 μM as compared to standard vitamin C (IC_{50} = 96.128 ± 17.50 μM). None of the compounds was found to be good radical scavenger than standard. Two analogs **10** (IC_{50} = 120.64 ± 15.24 μM), and **28** (IC_{50} = 124.03 ± 14.16 μM) were showed somehow better activity than the other derivatives (Fig-6).

Conclusion

Synthetic 2,4-dinitro phenyl hydrazone derivatives **1-32** were evaluated for antioxidant activities. Compounds showed excellent to weak DPPH radical scavenging (IC_{50} = 65.04 - 484.82 μM), ferric ion reducing (IC_{50} = 111.0-801.06 μM), ferrous ion-chelating (IC_{50} = 75.81 - 416.80 μM), total antioxidant (IC_{50} = 120.02 - 781.19 μM), and

hydroxyl radical scavenging (IC_{50} = 120.64 -247.02 μM) antioxidant activities as compared to standards. Compounds with electron donating groups as well as groups having good chelating ability with ferrous and ferric ions were found to be significantly active. This study has identified several lead molecules which may serve in the future advanced research in order to identify more powerful antioxidant agents.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

The antioxidant activity of synthetic compounds was carried out using the stable DPPH free radical according to (Shanab *et al*., 2012) [20]. Various concentrations (12.5, 25, 50, 100 and 200 μM) of synthetic compounds were mixed with an ethyl acetate solution containing 85 μM DPPH radical. The mixture solutions were incubated for 30 min at room temperature and the decrease in absorbance was measured at 518 nm using an UV spectrophotometer. Ascorbic acid at the same concentrations of drugs was used as a positive control. The experiment was carried out in triplicate. Percentage inhibition of the drugs as well as ascorbic acid was calculated by using the following formula:

DPPH Inhibition effect (%) = $(A_c - A_{s\,/}\, A_c) \times 100$

$\mathbf{A_c}$ = Absorbance reading of the control
$\mathbf{A_s}$ = Absorbance reading of the sample

Ferrous ion-chelating assay

The ferrous ion chelating activities of synthetic compounds were evaluated by a standard method (Puntel *et al*., 2005) [21]. Various concentrations (12.5, 25, 50, 100, and 200 μM) of synthetic compounds were mixed with 0.2 mL of 3.6 mM ferrous sulphate, 0.3 mL of 100 mM Tris-HCl (pH = 7.4), 0.1 mL of 9 mM *O*-phenanthroline and diluted up to 3.0 mL with ultra-pure distilled water. The reaction mixture was shaken vigorously, incubated for 10 minutes and the decrease in absorbance was determined at 510 nm. EDTA (ethylenediaminetetraacetic acid) at the same concentrations utilized as a reference standard and without Schiff bases complexes sample mixture as control. The Fe^{2+} chelating capacity was calculated by using the following formula:

Chelating effect (%) = $(A_c - A_{s\,/}\, A_c) \times 100$

$\mathbf{A_c}$ = Absorbance reading of the control
$\mathbf{A_s}$ = Absorbance reading of the sample

Ferric Reducing / Antioxidant Power Assay

The ferric reducing power of synthetic compounds was determined as described by (Kumar *et al*., 2012) [22]. Different concentrations (12.5, 25, 50, 100 and 200 μM) of synthetic compounds, 0.2 mL of 3.6 mM ferric chloride, 0.3 mL of 100 mM *tris*-buffer (pH = 7.4), 0.1 mL of 9 mM *O*-phenanthroline and diluted up to 3.0 mL with ultra-pure distilled water was shaken vigorously and left to stand at room temperature for 10 min. The increase in absorbance of the sample solution was measured at 510 nm using an UV spectrophotometer. Ascorbic acid at the same concentrations was utilized as a reference standard and without compounds sample mixture as control. The reducing power comparable with ascorbic acid was calculated by using the following formula:

Reducing Power (%) = $(A_s - A_{c\,/}\, A_s) \times 100$

$\mathbf{A_c}$ = Absorbance reading of the control
$\mathbf{A_s}$ = Absorbance reading of the sample

Total antioxidant activity (Phosphomolybdenum assay)

The total antioxidant capacities of synthetic compounds were evaluated by phosphomolybdenum assay assessed by (Sahaa *et al*., 2008) [23]. Reagent solution containing various concentrations (12.5, 25, 50, 100 and 200 μM) of synthetic compounds aliquot in ethyl acetate, 0.7 mL of 0.6 M sulphuric acid, 1.0 mM ammonium molybdate, 1.0 mL of 28 mM potassium phosphate and ultra pure distilled water was incubated at 95 $^{\circ}$C for 90 min. After cooling to room temperature, the increase in absorbance of the mixture is measured at 695 nm using an UV spectrophotometer. Ascorbic acid was utilized as reference standard and without compounds sample mixture as control. The reducing power of drugs as well as ascorbic acid was calculated by using the following farmula:

Reducing Power (%) = $(A_s - A_{c\,/}\, A_s) \times 100$

A_s = Absorbance reading of the control
A_c = Absorbance reading of the sample

Hydroxyl radical scavenging activity

The scavenging activity of synthetic compounds for hydroxyl radicals was measured with Fenton reaction described by (Huo *et al*., 2011) [24]. Reaction mixture of various concentrations (12.5, 25, 50, 100 and 200 μM) of synthetic compounds, 0.1 mL of 7.5 mM *o*-phenanthroline, 0.5 mL of 0.2 M

phosphate buffer (pH 6.6), 0.1 mL of 7.5 mM ferrous sulfate and 0.1 mL of H_2O_2 (0.1%) and diluted up to 3 mL with distilled water. The reaction mixture incubated at room temperature for 30 min and the absorbance was measured at 510 nm using an UV spectrophotometer. The reaction mixture without Schiff bases complexes has been used as control and without Schiff bases complexes and H_2O_2 as a blank. The DPPH radical scavenging activity of Schiff base complexes and ascorbic acid were calculated by using the following furmula:

Scavenging power (%) $=(A_s - A_c / A_b - A_s) \times 100$

$\mathbf{A_s}$ = Absorbance reading of the sample
$\mathbf{A_c}$ = Absorbance reading of the control
$\mathbf{A_b}$ = Absorbance reading of the blank

Statistical analysis

Linear regression analysis was used to calculate $IC_{50} \pm$ SEM values from data and graphs by using Graph pad prism 6. Significant differences among the means of data were tested by the one-way ANOVA followed by the student's t-test with significance level ($P<0.05$). All the tests were conducted in triplicate.

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