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Antioxidant and DNA damage inhibition activities of 4-Aryl-N-(4-aryl-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamides

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Abstract. A series of 4-aryl-*N*-(4-pheny-thiazol-2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamides were synthesized by condensing 4-aryl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylic acid with 2-amino-4-aryl-thiazole derivatives. The newly synthesized molecules were characterized by spectral analysis and subjected to antioxidant and DNA damage inhibition studies.

Keywords. HOBt; EDC; HCl; 1,3,4-oxadiazine carboxamides; antioxidant.

1. Introduction

Oxadiazines are interesting and promising six-membered heterocyclic compounds with oxygen and nitrogen atoms. 1,3,4-oxadiazine derivatives exhibit biological activities such as cardiovascular, antibacterial, plant growth regulating, miticidal and nematocidal, acricidal, insecticidal and anticonvulsive activities.[1] In addition, oxadiazine derivatives are also used as drugs for the treatment of anaemia[2] and viral diseases.[3] Thiazoles are important class of natural and synthetic compounds and display a wide range of biological activities such as cardiotonic,[4] sedative,[5] anaesthetic,[6] bactericidal[7] and anti-inflammatory.[8]

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals or reactive oxygen species (ROS) are present in biological systems from a wide variety of sources.[9] These free radicals can cause a number of chain reactions and may oxidize nucleic acids, proteins, lipids or DNA and can initiate several diseases.[10] Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions, thus inhibiting the oxidative mechanism that leads to degenerative diseases. They do this by oxidizing themselves, so antioxidants are often reducing agents. Antioxidants are widely used as dietary supplements and are being investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness.[11]

DNA damage can lead to mutation if replication proceeds without proper repair. Oxidative stress-induced DNA damage by the reactive oxygen species and chemical carcinogens can lead to mutations and is suspected to be a major cause of cancer.[12] In order to protect the genome from deleterious effects of ROS, cells have antioxidants, ROS-eliminating enzymes and efficient DNA repair pathways. Among DNA damage causing cancer development, approximately 80% of the damage is caused by ROS such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radical ($^{\bullet}OH$). Therefore, protection of oxidative DNA damage induced by ROS is very important for cancer prevention.[13]

Benzoxadiazines derived from embelin (herbs extract) is reported to exhibit antioxidant and free radical scavenging activities.[14] Various oxadiazines linked with other heterocyclic moieties show enhanced biological activities.[15–17] In addition, oxadiazines bearing thiazole moiety exhibit antifungal activities.[18]

In order to achieve promising antioxidants, we have synthesized a new series of 4-aryl-*N*-(4-aryl-thiazol-2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamides (**7a–l**) by condensing 4-aryl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylic acid (**6a–d**) with 2-amino-4-aryl-thiazole derivatives (**2a–d**) as illustrated in schemes 1 and 2.

2. Experimental

The melting points were measured with micro melting point apparatus and are uncorrected. All chemicals/reagents used were purchased from Merck Chemicals (India), Fluka Chemicals (India) and IR spectra were recorded in KBr pellets on Shimadzu 8300

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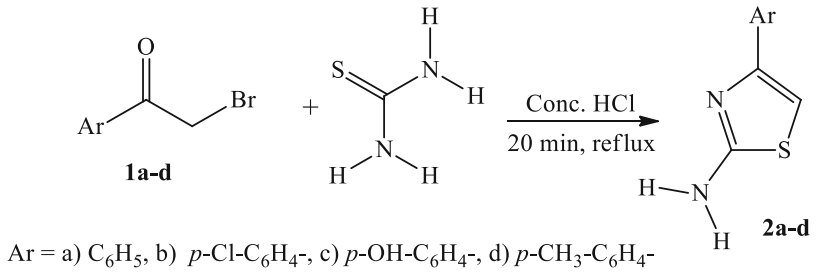
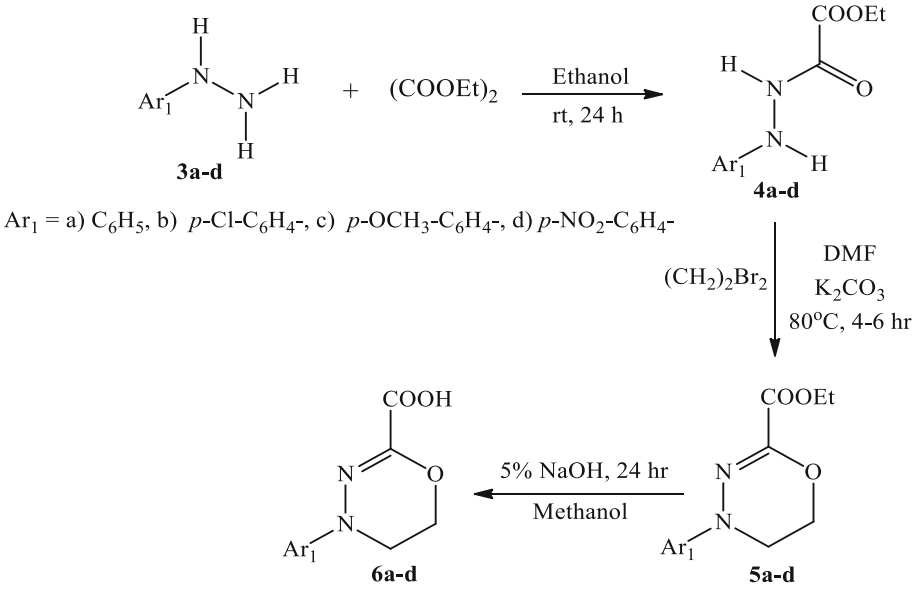
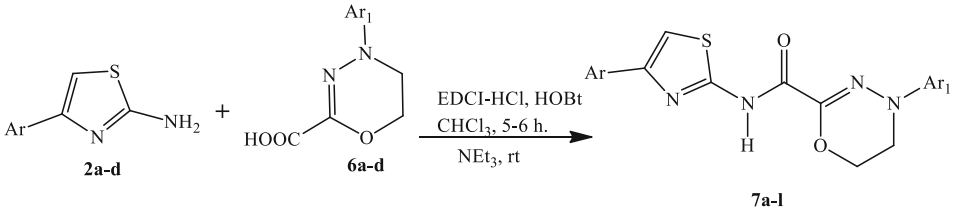


Scheme 1. Synthesis of 2-amino-4-aryl-thiazole.



Scheme 2. Synthesis of 4-aryl-5,6-dihydro-4H-1,3,4- 51 oxadiazine-2-carboxylic acid.



7a: Ar = C_6H_5-, Ar_1 = C_6H_5;
7b: Ar = *p*-Cl-C_6H_4, Ar_1 = C_6H_5;
7c: Ar = *p*-OH-C_6H_4, Ar_1 = C_6H_5;
7d: Ar = *p*-CH_3-C_6H_4, Ar_1 = C_6H_5;
7e: Ar = *p*-Cl-C_6H_4, Ar_1 = *p*-Cl-C_6H_4;
7f: Ar = *p*-OH-C_6H_4, Ar_1 = *p*-Cl-C_6H_4;
7g: Ar = *p*-CH_3-C_6H_4, Ar_1 = *p*-Cl-C_6H_4;
7h: Ar =*p*-Cl-C_6H_4 Ar_1 =*p*-OCH_3-C_6H_4;
7i: Ar = *p*-OH-C_6H_4, Ar_1 = *p*-OCH_3-C_6H_4;
7j: Ar = *p*-CH_3-C_6H_4, Ar_1 = *p*-OCH_3-C_6H_4;
7k: Ar = *p*-Cl-C_6H_4, Ar_1 = *p*-NO_2-C_6H_4;
7l: Ar = *p*-CH_3-C_6H_4-, Ar_1 = *p*-NO_2-C_6H_4

Scheme 3. Synthesis of 4-aryl-N-(4-aryl-thiazol-2-yl)-5,6-dihydro-4h-1,3,4-oxadiazine-2-carboxamides.

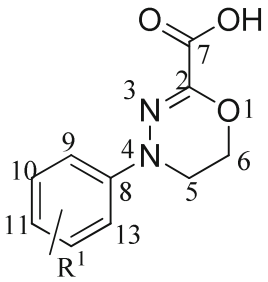
spectrometer. The ^{1}H NMR spectra were recorded on a Bruker supercon 400 MHz spectrophotometer using DMSO-d_6 as solvent and Tetra methyl silane (TMS) as an internal standard. The chemical shifts were expressed in ppm. Mass spectra were obtained on LC-MSD-Trap-XCT-Plus spectrophotometer. Thin layer chromatography (TLC) was performed on pre-coated Silica Gel sheet (HF 254, Sd-fine) and visualization of the spots was done in iodine vapour and UV light. Chromatographic separations were carried out on silica gel (60-120) mesh using petroleum ether: acetone (9:1) as eluent (scheme 3).

2.1 *General procedure for the synthesis of 2-amino-4-aryl-thiazole 2a-d*

2-amino-4-aryl thiazole derivatives were prepared[19–21] using different substituted phenacyl bromides by

refluxing with excess thiourea in presence of conc. HCl for about 20 min. After the completion of reaction, the reaction mixture was cooled to room temperature; the pale yellow solid thus separated was filtered, washed with chloroform, dried and recrystallized from ethanol to obtain 2-amino-4-aryl thiazole derivatives.

2.2 *Typical procedure for the synthesis of 4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxylic acid: (**6a**)*



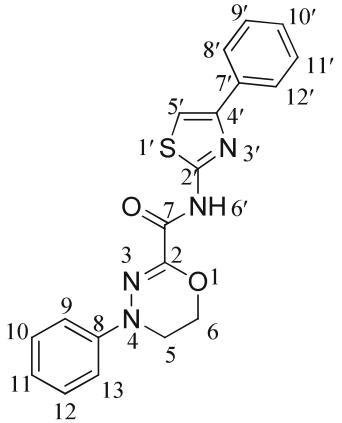
An oven-dried two-neck round bottom flask was charged with a solution of 4-phenyl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylate[22–24] (**5a**, 2.35 g, 10.00 mmol) in 25 mL of methanol. A volume of 10 mL of 5% NaOH solution was added dropwise to the reaction mixture and allowed to stir at room temperature for 24 h. After completion of reaction (monitored by TLC), methanol was evaporated under reduced pressure and the residue was acidified with dil. HCl. The white solid thus separated was filtered, dried and recrystallized from 20 mL ethanol to obtain 4-phenyl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylic acid (**6a**, 1.64 g) 80% yield; **IR** (KBr cm^{-1}): ν 3061 (Ar-CH), 1133 (C-O-C), 1680 (>C=O), 2737 (-OH), 1256 (C-O), 1579 (Ar-C=C); ^{1}**H NMR** (DMSO-d_6): δ 3.81 (t, 2H, J = 5.2 Hz), 4.43 (t, 2H, J = 5.6 Hz), 6.95–7.42 (m, 5H), 11.82 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 54.2 (C-6), 63.1 (C-5), 113.8 (C-9 and C-13), 121.3 (C-11), 129.8 (C-10 and C-12), 144.1 (C-8), 150.9 (C-2), 168.1 (C-7). **LC-MS:** 207.0 $(M+H)^+$. **Anal. % Calcd for** $C_{10}H_{10}N_2O_3$: C: 58.51, H: 4.97, N: 13.36; Found; C: 58.25, H: 4.89, N: 13.45. Same procedure was followed for all the derivatives.

2.2a *4-(4-Chlorophenyl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxylic acid (**6b**)*: Obtained from **5b** (2.70 g, 10.00 mmol), yield 82% (1.98 g); **IR** (KBr cm^{-1}): ν 2350 (Ar-Cl), 3063 (Ar-CH), 1130 (C-O-C), 1685 (>C=O), 2734 (-OH), 1242 (C-O), 1578 (Ar-C=C); ^{1}**H NMR** (DMSO-d_6): δ 3.81 (t, 2H, J = 5.2 Hz), 4.43 (t, 2H, J = 5.6 Hz), 7.16–7.44 (m, 4H), 11.78 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 54.2 (C-6), 63.0 (C-5), 116.2 (C-9 and C-13), 126.4 (C-11), 129.9 (C-10 and C-12), 141.9 (C-8), 150.9 (C-2), 168.1 (C-7); **LC-MS:** 242.6 $(M+H)^+$. **Anal. % Calcd for** $C_{10}H_9ClN_2O_3$: C: 50.02, H: 4.89, N: 11.73. Found: C: 49.91, H: 4.72, N: 11.64.

2.2b *4-(4-Methoxyphenyl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxylic acid (**6c**)*: Obtained from **5c** (2.64 g, 10.00 mmol), yield 81% (1.91 g); **IR** (KBr cm^{-1}): ν 2845 (-OCH_3), 1683 (>C=O), 2735 (-OH), 1242 (C-O), 3061 (Ar-CH), 1132 (C-O-C), 1580 (Ar-C=C); ^{1}**H NMR** (DMSO-d_6): δ 3.82 (t, 2H, J = 5.2 Hz), 3.90 (s, 3H, -OCH_3) 4.43 (t, 2H, J = 5.6 Hz), 6.64–7.11 (m, 4H), 11.8 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 54.2 (C-6), 55.9 (-OCH_3), 63.1 (C-5), 114.8 (C-9 and C-13), 115.3 (C-10 and C-12), 136.4 (C-8), 152.4 (C-11), 150.8 (C-2), 168.1 (C-7); **LC-MS:** 237.3 $(M+H)^+$; **Anal. % Calcd for** $C_{11}H_{12}N_2O_4$: C: 55.95, H: 5.12, N: 11.86. Found: C: 55.91, H: 5.05, N: 11.74.

2.2c *4-(4-Nitrophenyl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxylic acid (**6d**)*: Obtained from 5d (2.80 g, 10.00 mmol), yield 85% (2.14 g); IR (KBr cm^{-1}): ν 1545 and 1350 (-NO_2), 1695 (>C=O), 2742 (-OH), 1248 (C-O), 3071 (Ar-CH), 1142 (C-O-C), 1582 (Ar-C=C); ^{1}**H NMR** (DMSO-d_6): δ 3.82 (t, 2H, J = 5.2 Hz), 4.42 (t, 2H, J = 5.6 Hz), 7.32–8.24 (m, 4H), 11.8 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 54.3 (C-6), 63.1 (C-5), 114.7 (C-9 and C-13), 124.9 (C-10 and C-12), 141.3 (C-11), 150.1 (C-8), 150.9 (C-2), 168.1 (C-7); **LC-MS**: 252.3 $(M+H)^+$; **Anal. % Calcd for** $C_{10}H_9N_3O_5$: C: 47.77, H: 3.57, N: 16.78. Found: C: 47.88, H: 3.61, N: 16. 63.

2.3 *Typical procedure for the synthesis of 4-phenyl-N-(4-phenyl-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamides (**7a**)*



4-phenyl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylic acid (**6a**, 2.30 g, 11.00 mmol) was taken in round

bottomed flask and dissolved in 30 mL of $CHCl_3$, after 10 min, HOBt (2.00 g, 15.00 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodimide (EDC.HCl) (2.81 g, 15.00 mmol) were added. The reaction mixture was stirred at room temperature for 15 min, then 2-amino-4-phenyl thiazole (**2a**, 1.76 g, 10.00 mmol) and 1.15 mL (11.00 mmol) of triethylamine were added and stirred at room temperature for about 5–6 h. After completion of reaction (monitored by TLC), the reaction mixture was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layer was washed with 5% $NaHCO_3$ solution followed by 5% HCl solution and finally with distilled water and then dried over anhydrous Na_2SO_4. The $CHCl_3$ layer was evaporated under reduced pressure; the crude solid thus obtained was purified by column chromatography using petroleum ether: acetone (8:2) as eluent, which afforded 4-phenyl-*N*-(4-phenyl-thiazol-2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamide (**7a**, 2.05 g) as pale yellow solid in 55% yield, mp 126–128°C. **IR** (KBr cm^{-1}): ν 3114.70 (-NH), 1680 (>C=O), 3059.1 (Ar-CH), 1133 (C–O–C), 1579 (Ar-C=C), 719 (C–S); ^{1}**H NMR** (DMSO-d_6): δ 3.81 (t, 2H, J = 5.2 Hz), 4.42 (t, 2H, J = 5.6 Hz), 6.95–7.42 (m, 5H), 7.52–7.95 (m, 5H), 7.68 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 53.9 (C-6), 62.9 (C-5), 105.8 (C-5′), 113.7 (C-13 and C-9), 121.3 (C-11), 129.7 (C-12 and C-10), 144.1 (C-8), 127.8 (C-8′ and C-12′), 128.9 (C-10′), 129.5 (C-9′ and C-11′), 133.3 (C-7′), 150.4 (C-4′), 150.9 (C-2), 161.2 (C-7), 164.5 (C-2′); **LC-MS**: 365.2 $(M+H)^+$. **Anal. % Calcd for** $C_{19}H_{16}N_4O_2S$: C: 62.66, H: 4.43, N: 15.45. Found: C: 62.60, H: 4.39, N: 15.37. Same procedure was followed for all the derivatives.

2.3a *4-Phenyl-N-(4-(4-chlorophenyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7b**)*: Obtained from **6a** (2.30 g, 11.00 mmol) and **2b** (2.10 g, 10.00 mmol), yield 58% (2.32 g); **IR** (KBr cm^{-1}): ν 3112 (-NH), 1686 (-C=O), 3061 (Ar-CH), 1130 (C-O-C), 1582 (Ar-C=C), 720 (C-S), 2350 (Ar-Cl); ^{1}**H NMR** (DMSO-d_6): δ 3.81 (t, 2H, J = 5.2 Hz), 4.42 (t, 2H, J = 5.6 Hz), 6.93–7.41 (m, 5H), 7.66–8.14 (m, 4H), 7.66 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 53.9 (C-6), 62.8 (C-5), 105.8 (C-5′), 113.9 (C-9 and C-13), 121.3 (C-11), 129.8 (C-10 and C-12), 144.2 (C-8), 129.0 (C-8′ and C-12′), 129.9 (C-9′ and C-11′), 135.1 (C-10′), 131.32 (C-7′), 150.5 (C-4′), 150.9 (C-2), 161.2 (C-7), 164.5 (C-2′); **LC-MS**: 400.1 $(M+H)^+$. **Anal. % Calcd for** $C_{19}H_{15}N_4ClO_2S$: C: 57.21, H: 3.94, N: 14.15; Found: C: 57.17, H: 3.81, N: 14.04.

2.3b *4-Phenyl-N-(4-(4-hydroxyphenyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7c**)*: Obtained from **6a** (2.30 g, 11.00 mmol) and **2c** (1.92 g, 10.00 mmol), yield 56% (2.13 g); **IR** (KBr cm^{-1}): ν 3113 (-NH), 1684 (-C=O), 3058 (Ar-CH), 1135 (C-O-C), 1576 (Ar-C=C), 722 (C-S), 3650 (Ar-OH); ^{1}**H NMR** (DMSO-d_6): δ 3.81 (t, 2H, J = 5.2 Hz), 4.43 (t, 2H, J = 5.6 Hz), 6.91–7.44 (m, 5H), 6.94–7.62 (m, 4H), 5.42 (s, 1H), 7.64 (s, 1H), 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 53.9 (C-6), 62.8 (C-5), 105.8 (C-5′), 113.8 (C-9 and (C-13), 121.3 (C-11), 129.8 (C-10 and C-12), 144.2 (C-8), 129.1 (C-8′ and C-12′), 117.8 (C-9′ and C-11′), 155.8 (C-10′), 126.3 (C-7′), 150.5 (C-4′), 150.9 (C-2), 161.2 (C-7), 164.6 (C-2′). **LC-MS**: 381.4 $(M+H)^+$; **Anal. % Calcd for** $C_{19}H_{16}N_4O_3S$: C: 60.12, H: 4.24, N: 14.73; Found: 59.99, H: 4.20, N: 14.75.

2.3c *4-Phenyl-N-(4-p-tolyl)-thiazol-2-yl)-5,6-dihydro-H-1,3,4-oxadiazine-2-carboxamide (**7d**)*: Obtained from **6a** (2.30 g, 11.00 mmol) and **2d** (1.90 g 10.00 mmol) yield 57% (2.16 g); **IR** (KBr cm^{-1}): ν 3115 (-NH), 1682 (-C=O), 3061 (Ar-CH), 1131 (C-O-C), 1581 (Ar-C=C), 716 (C-S); ^{1}**H NMR** (DMSO-d_6): δ 2.33 (s, 3H), 3.80 (t, 2H, J = 5.2 Hz), 4.43 (t, 2H, J = 5.6 Hz), 6.95–7.42 (m, 5H), 7.40–7.82 (m, 4H), 7.60 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 21.5 (-CH_3), 53.9 (C-6), 62.8 (C-5), 105.8 (C-5'), 113.9 (C-9 and C-13), 121.3 (C-11), 129.8 (C-10 and C-12), 144.2 (C-8), 127.6 (C-8′ and C-12′), 129.9 (C-9′ and C-11′), 137.8 (C-10′), 130.4 (C-7′), 150.4 (C-4′), 150.9 (C-2), 161.2 (C-7), 164.5 (C-2′); **LC-MS**: 379.1 $(M+H)^+$; **Anal. % Calcd for** $C_{20}H_{18}N_4O_2S$: C: 63.51, H: 4.79, N: 14.83; Found: C: 63.47, H: 4.76, N: 14.80.

2.3d *4-(4-Chlorophenyl)-N-(4-(4-chlorophenyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7e**)*: Obtained from **6b** (2.62 g, 11.00 mmol) and **2b** (2.10 g, 10.00 mmol) yield 56.5% (2.45 g); **IR** (KBr cm^{-1}): ν 3112 (-NH), 1690 (-C=O), 3055 (Ar-CH), 1135 (C-O-C), 1575 (Ar- C=C), 715 (C-S), 2350 (Ar-Cl); ^{1}**H NMR** (DMSO-d_6): δ 3.81(t, 2H, J = 5.2 Hz), 4.43 (t, 2H, J = 5.6 Hz), 6.55–7.40 (m, 4H), 7.66–8.14 (m, 4H), 7.67 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 53.9 (C-6), 62.9 (C-5), 105.8 (C-5′), 116.2 (C-9 and C-13), 126.4 (C-11), 129.9 (C-10 and C-12), 141.9 (C-8), 129.0 (C-8′ and C-12′), 129.9 (C-9′ and C-11′), 135.1 (C-10′), 131.3 (C-7′), 150.5 (C-4′), 150.9 (C-2), 161.2 (C-7), 164.5 (C-2′). **LC-MS**: 434.3 $(M+H)^+$; **Anal. % Calc for** $C_{19}H_{14}Cl_2N_4O_2S$: C: 52.67, H: 3.26, N: 12.93; Found: C: 52.59, H: 3.20, N: 12.89.

2.3e *4-(4-Chlorophenyl)-N-(4-(4-hydroxyphenyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-car-*

*boxamide (**7f**)*: Obtained from **6b** (2.62 g, 11.00 mmol) and **2c** (1.92 g, 10.00 mmol), yield 58% (2.40 g); **IR** (KBr cm^{-1}): ν 3114 (-NH), 1690 (-C=O), 3058 (Ar-CH), 1136 (C-O-C), 1578 (Ar-C=C), 722 (C-S); ^{1}**H NMR** (DMSO-d_6): δ 3.81 (t, 2H, J = 5.2 Hz), 4.42 (t, 2H, J = 5.6 Hz), 6.55–7.39 (m, 4H), 6.97–7.65 (m, 4H), 5.43 (s, 1H), 7.66 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 54.0 (C-6), 62.9 (C-5), 105.8 (C-5′), 116.3 (C-9 and C-13), 126.4 (C-11), 129.9 (C-10 and C-12), 142.0 (C-8), 129.1 (C-8′ and C-12′), 117.8 (C-9′ and C-11′), 155.8 (C-10′), 126.3 (C-7′), 150.5 (C-4′), 150.9 (C-2), 161.2 (C-7), 164.5 (C-2′); **LC-MS**: 415.1 $(M+H)^+$; **Anal. % Calc for** $C_{19}H_{15}ClN_4O_3S$: C: 55.10, H: 3.64, N: 13.55; Found: C: 55.01, H: 3.56, N: 13.50.

2.3f *4-(4-Chlorophenyl)-N-(4-(p-tolyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7g**)*: Obtained from **6b** (2.62 g, 11.00 mmol) and **2d** (1.90 g 10.00 mmol), yield 60% (2.48 g); **IR** (KBr cm^{-1}): ν 3112 (-NH), 1688 (-C=O), 3061 (Ar-CH), 1136 (C-O-C), 1583 (Ar- C=C), 722 (C-S), 2230 (Ar-C), 2350 (Ar-Cl); ^{1}**H NMR** (DMSO-d_6): δ 2.34 (s, 3H), 3.80 (t, 2H, J = 5.2 Hz), 4.42 (t, 2H, J = 5.6 Hz), 6.55–7.39 (m, 4H), 7.40–7.82 (m, 4H), 7.68 (s,1H), 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 21.5 (-CH_3), 53.9 (C-6), 62.9 (C-5), 105.8 (C-5′), 116.2 (C-9 and C-13), 126.4 (C-11), 129.9 (C-10 and C-12), 141.9 (C-8), 127.6 (C-8′ and C-12′), 129.9 (C-9′ and C-11′), 137.8 (C-10′), 130.4 (C-7′), 150.4 (C-4′), 150.9 (C-2), 161.3 (C-7), 164.5 (C-2′); **LC-MS**: 413.1 $(M+H)^+$; **Anal. % Calc for** $C_{20}H_{17}ClN_4O_2S$: C: 55.10, H: 3.64, N: 13.55. Found: C: 55.01, H: 3.56, N: 13.50.

2.3g *4-(4-Methoxyphenyl)-N-(4-(4-chlorophenyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7h**)*: Obtained from **6c** (2.68 g, 11.00 mmol) and **2b** (2.10 g, 10.00 mmol) yield 59% (2.52 g); **IR** (KBr cm^{-1}): ν 3114 (-NH), 1680 (-C=O), 3059 (Ar-CH), 1133 (C-O-C), 1579 (Ar-C=C), 719 (C-S), 2350 (Ar-Cl); ^{1}**H NMR** (DMSO-d_6): δ 3.81 (t, 2H, J = 5.2 Hz), 3.90 (s, 3H, -OCH_3), 4.43 (t, 2H, J = 5.6 Hz), 6.55–7.39 (m, 4H), 7.16–7.71 (m, 4H), 7.65 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 54.1 (C-6), 55.9 (-OCH_3), 63.1 (C-5), 105.9 (C-5′), 114.8 (C-9 and C-13), 115.3 (C-10 and C-12), 129.1 (C-8′ and C-12′), 130.0 (C-9′ and C-11′), 131.3 (C-7′), 135.1 (C-10′), 136.4 (C-8), 150.5 (C-4′), 150.9 (C-2), 152.4 (C-11), 161.6 (C-7), 165.3 (C-2′); **LC-MS**: 428.6 $(M+H)^+$; **Anal. % Calc for** $C_{20}H_{17}ClN_4O_3S$: C: 56.11, H: 4.00, N: 13.16; Found: C: 56.01, H: 3.87, N: 13.06.

2.3h *4-(4-Methoxyphenyl)-N-(4-(4-hydroxyphenyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7i**)*: Obtained from **6c** (2.68 g, 11.00 mmol) and **2c** (1.92 g, 10.00 mmol), yield 59.5% (2.44 g) **IR** (KBr cm^{-1}): ν 3113 (-NH), 1686 (-C=O), 3063 (Ar-CH), 1132 (C-O-C), 1584 (Ar-C=C), 723 (C-S), 3650 (Ar-OH); ^{1}**H NMR** (DMSO-d_6): δ 3.81 (t, 2H, J = 5.2 Hz), 3.88 (s, 3H, -OCH_3), 4.43 (t, 2H, J = 5.6 Hz), 6.64–7.10 (m, 4H), 6.97–7.65 (m, 4H), 5.47 (s, 1H), 7.67 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 54.1 (C-6), 55.9 (-OCH_3), 63.1(C-5), 105.8 (C-5′), 114.8 (C-9 and C-13), 115.3 (C-10 and C-12), 117.8 (C-9′ and C-11′), 129.1 (C-8′ and C-12′), 155.8 (C-10′), 126.3 (C-7′), 136.4 (C-8), 150.5 (C-4′), 150.9 (C-2), 152.4 (C-11), 161.2 (C-7), 164.5 (C-2′); **LC-MS**: 411.1 $(M+H)^+$; **Anal. % Calc for** $C_{20}H_{18}N_4O_4S$: C: 58.58, H: 4.42, N: 13.65; Found: C: 58.53, H: 4.38, N: 13.61.

2.3i *4-(4-Methoxyphenyl)-N-(4-(p-tolyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7j**)*: Obtained from **6c** (2.68 g, 11.00 mmol) and **2d** (1.90 g 10.00 mmol), yield 59% (2.40 g); **IR** (KBr cm^{-1}): ν 3116 (-NH), 1695 (-C=O), 3062 (Ar-CH), 1137 (C-O-C), 1582 (Ar-C=C), 722 (C-S), 3654 (Ar-C); ^{1}H NMR (DMSO-d_6) : δ 2.35 (s, 3H, CH_3), 3.81 (t, 2H, J = 5.2 Hz), 3.93 (s, 3H, -OCH_3), 4.42 (t, 2H, J = 5.6 Hz), 6.64–7.14 (m, 4H), 7.40–7.84 (m, 4H), 7.69 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 21.5 (-CH_3), 54.1 (C-6), 55.9 (-OCH_3), 63.1 (C-5), 105.9 (C-5′), 114.8 (C-9 and C-13), 115.3 (C-10 and C-12), 127.6 (C-8′ and C-12′), 129.9 (C-9′ and C-11′), 137.8 (C-10′), 130.4 (C-7′), 136.4 (C-8), 150.5 (C-4′), 150.9 (C-2), 152.4 (C-11), 161.6 (C-7), 164.5 (C-2′); **LC-MS**: 409.2 $(M+H)^+$; **Anal. % Calc for** $C_{21}H_{20}N_4O_3S$: C: 61.75, H: 4.94, N: 13.79; Found: C: 61.69, H: 4.89, N: 13.72.

2.3j *4-(4-Nitrophenyl)-N-(4-(4-chlorophenyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7k**)*: Obtained from **6d** (2.72 g, 11.00 mmol) and **2b** (2.10 g, 10.00 mmol) yield 57% (2.41 g); **IR** (KBr cm^{-1}): ν 3119 (-NH), 1698 (-C=O), 3062 (Ar-CH), 1135 (C-O-C), 1589 (Ar-C=C), 724 (C-S), 2350 (Ar-Cl), 1545 and 1350 (NO_2); ^{1}**H NMR** (DMSO-d_6): δ 3.82 (t, 2H, J = 5.2 Hz), 4.43 (t, 2H, J = 5.6 Hz), 7.32–8.21 (m, 4H), 7.16–7.72 (m, 4H), 7.66 (s, 1H) 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 54.2 (C-6), 63.2 (C-5), 105.9 (C-5′), 114.7 (C-9 and C-13), 124.9 (C-10 and C-12), 129.1 (C-8′ and C-12′), 130.0 (C-9′ and C-11′), 135.1 (C-10′), 131.3 (C-7′), 141.3 (C-11), 150.1 (C-8), 150.5 (C-4′), 150.9 (C-2), 166.3 (C-7),

164.6 (C-2′); **LC-MS**: 424.4 $(M+H)^+$; **Anal. % Calc for** $C_{19}H_{14}ClN_5O_4S$: C: 51.48, H: 3.18, N: 15.78; Found: C: 51.41, H: 3.15, N: 15.70.

2.3k *4-(4-Nitrophenyl)-N-(4-(p-tolyl)-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamide (**7l**)*: Obtained from **6d** (2.72 g 11.00 mmol) and **2d** (1.90 g 10.00 mmol), yield 58% (2.45 g); **IR** (KBr cm^{-1}): ν 3114 (-NH), 1683 (-C=O), 3062 (Ar-CH), 1135 (C-O-C), 1581 (Ar-C=C), 721 (C-S), 2232 (Ar-C), 1545 and 1350 ($-NO_2$); ^{1}**H NMR** (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 3.81 (t, 2H, $J = 5.2$ Hz), 4.43 (t, 2H, $J = 5.6$ Hz), 7.32–8.21 (m, 4H), 7.40–7.84 (m, 4H), 7.68 (s, 1H), 12.26 (s, 1H); ^{13}**C NMR** (DMSO-d_6): δ 21.5 ($-CH_3$), 54.3 (C-6), 63.1 (C-5), 105.8 (C-5′), 114.7 (C-9 and C-13), 124.9 (C-10 and C-12), 141.3 (C-11), 150.1 (C-8), 127.6 (C-8′ and C-12′), 130.0 (C-9′ and C-11′), 137.8 (C-10′), 130.44 (C-7′), 150.4 (C-4′), 150.9 (C-2), 161.2 (C-7), 164.5 (C-2′); **LC-MS**: 424.5 $(M+H)^+$; **Anal. % Calc for** $C_{20}H_{17}N_5O_4S$: C: 56.76, H: 4.06, N: 16.54; Found: C: 56.70, H: 4.02, N: 16.47.

2.4 *Biological studies of 4-Aryl-N-(4-aryl-thiazol-2-yl)-5,6-dihydro-4H-1,3,4-oxadiazine-2-carboxamides 7a–l*

2.4a *Antioxidant studies*:

2.4b *DPPH radical scavenging assay*: Antioxidant activity of the synthesized 4-aryl-*N*-(4-aryl-thiazol-2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamides **7a-l** in comparison to standard antioxidant butylated hydroxyl toluene (BHT) on DPPH radical was estimated according to the method of Lai *et al.*[25] Samples **7a-l** (10, 50 and 100 μg/mL) and BHT (0–5 μg/mL) were taken in methanol in 200 μL aliquot and mixed with 100 mM Tris-HCl buffer (800 μL, pH 7.4) and then 1 mL of 500 μM DPPH was added in ethanol (final concentration of 250 μM). The mixture was shaken vigorously and left to settle for 20 min at room temperature in the dark. Absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The capability to scavenge DPPH radical was calculated using eq. (1).

$$\text{DPPH Scavenging activity } (\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \times 100 \quad (1)$$

2.4c *Measurement of reducing power*: The reducing power of synthesized 4-aryl-*N*-(4-aryl-thiazol-2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamides **7a-l** was determined according to the method of Yen and Chen.[26] The samples **7a-l** (10, 50 and 100 μg/mL) were mixed with equal volumes of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, an equal volume of 10% trichloroacetic acid was added to the mixture and then centrifuged at 5000 rpm for 10 min. The upper layer of solution was mixed with distilled water and 0.1% ferric chloride at a ratio of 1:1:2 and the absorbance were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.4d *DNA damage inhibition assay*: DNA damage inhibition ability of synthesized 4-aryl-*N*-(4-aryl-thiazol- 2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamides **7a-l** was performed using lambda phage DNA.[27,28] Briefly, lambda phage DNA (0.6 μg) was subjected to oxidation using Fenton's reagent (0.3 mM hydrogen peroxide, 0.5 μM ascorbic acid and 0.8 μM ferric chloride) in presence and absence of the sample (0.2 mg) for 2 h at 37°C. The samples **7a-l** were subjected to electrophoresis (Bio-rad Gel electrophoresis unit) on 1% agarose for 2 h at 50 volts DC. Gels were stained with ethidium bromide (0.5 μg/mL) and documented (Bio-rad gel documentation unit).

2.4e *Statistical analysis*: All the experiments were carried out in triplicates ($n = 3$) and the results were expressed as mean ± standard deviation (SD) as shown in table 1. Graphical representation of DPPH radical scavenging activity of the synthesized 4-aryl-*N*-(4-aryl-thiazol-2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamides **7a-l** is as shown in figures 1–3. Pictorial representation of DNA damage inhibition activity is shown in figure 4.

3. Results and Discussion

Derivatives of 4-aryl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylic acid **6a-d** were obtained from 4-aryl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylates **5a-d** on treating with 5% NaOH solution using methanol as a solvent for about 24 h. The obtained compounds **6a-d** were soluble in 5% $NaHCO_3$ and re-precipitated on acidification, which indicated the formation of 4-phenyl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylic acid. Structural proof for the compounds **6a-d** was confirmed by spectral studies. For instance, in IR spectra, signal at 1725–1736 cm^{-1} of >C=O is shifted to 1680–1695 cm^{-1} and a signal appeared at 2733–2745 cm^{-1} due to -OH group. In ^{1}H NMR

Table 1. DPPH radical scavenging activity and reducing power of samples **7a–l** compared with standard antioxidant BHT.

Compounds	% DPPH radical scavenging activity*			% Reducing power determination*		
	10 (μg/mL)	50 (μg/mL)	100 (μg/mL)	10 (μg/mL)	50 (μg/mL)	100 (μg/mL)
7a	16 ± 0.022	28 ± 0.091	51 ± 0.099	06 ± 0.091	12 ± 0.082	56 ± 0.088
7b	18 ± 0.092	33 ± 0.013	55 ± 0.066	07 ± 0.019	22 ± 0.027	62 ± 0.082
7c	24 ± 0.016	41 ± 0.086	76 ± 0.055	11 ± 0.036	34 ± 0.011	79 ± 0.045
7d	23 ± 0.022	42 ± 0.012	67 ± 0.079	08 ± 0.086	22 ± 0.092	65 ± 0.012
7e	29 ± 0.013	39 ± 0.032	69 ± 0.086	12 ± 0.083	28 ± 0.016	68 ± 0.023
7f	27 ± 0.191	56 ± 0.111	76 ± 0.093	13 ± 0.019	29 ± 0.016	69 ± 0.061
7g	23 ± 0.226	40 ± 0.183	68 ± 0.082	10 ± 0.086	26 ± 0.092	62 ± 0.012
7h	29 ± 0.10	53 ± 0.099	82 ± 0.093	14 ± 0.016	32 ± 0.082	82 ± 0.082
7i	25 ± 0.012	42 ± 0.082	44 ± 0.016	07 ± 0.016	27 ± 0.082	64 ± 0.082
7j	18 ± 0.076	28 ± 0.036	39 ± 0.032	08 ± 0.019	16 ± 0.016	58 ± 0.061
7k	15 ± 0.067	29 ± 0.036	41 ± 0.034	06 ± 0.024	15 ± 0.026	57 ± 0.053
7l	17 ± 0.421	27 ± 0.136	38 ± 0.133	05 ± 0.213	17 ± 0.417	56 ± 0.396
(BHT) Standard	32 ± 0.018	57 ± 0.083	94 ± 0.012	16 ± 0.183	32 ± 112	86 ± 0.163

* Values are expressed as mean ± standard deviation ($n = 3$)

spectra, the absence of quartet in the region δ 4.12–4.31 ppm, (2H for -OCH_2 group) and triplet in the region δ 1.18–1.30 ppm, (3H for -OCH_2-CH_3 group) and the presence of carboxylic acid peak at δ 11.82–11.85 ppm confirmed the formation of product **6a-d**. In ^{13}C NMR, peak due to -CH_3 at δ 13.7–13.9 ppm and -OCH_2 at δ 60.9–61.4 ppm disappeared and the peak, due to -C=O shifted to δ 168.1–168.2 ppm from δ 158.4–158.6 ppm. All the synthesized 4-aryl-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxylic acids showed $(M+H)^+$ peak which was consistent with the assigned structure in the mass spectra.

The obtained compounds **6a-d** were condensed with 2-amino-4-aryl-thiazole derivatives **2a-d** to give 4-aryl-*N*-(4-aryl-thiazol-2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamides **7a-l**. The obtained samples **7a-l** were confirmed by IR, ^{1}H NMR, ^{13}C NMR and MS studies. The IR spectra shows the amide -NH frequency in the region 3112.4–3120.9 cm^{-1} and -C=O frequency region 1670.2–1690.5 cm^{-1}. ^{1}H NMR spectra shows, the absence of carboxylic acid peak at δ 11.82–11.85 ppm and the appearance of single small peak due to -CONH- protons in the region δ 12.24–12.31 ppm. The peaks of aromatic protons due to substituted thiazole moiety appear at δ 7.4–8.12 ppm, and in all the derivatives, one singlet peak of thiazole -CH- proton appeared at δ 7.65–7.69 ppm. In ^{13}C NMR, in addition to aromatic carbon peaks at δ 113–154 ppm, a single peak at δ 105.4–105.9 ppm appeared in all the derivatives. All the 4-aryl-*N*-(4-aryl-thiazol-2-yl)-5,6-dihydro-4*H*-1,3,4-oxadiazine-2-carboxamides **7a-l** showed $(M+H)^+$ peak in the mass spectra. The above spectral evidence confirms the formation of products.

3.1 *Antioxidant activity of samples 7a–l*

3.1a *DPPH radical scavenging assay*: The free radical scavenging ability of samples **7a-l** was evaluated

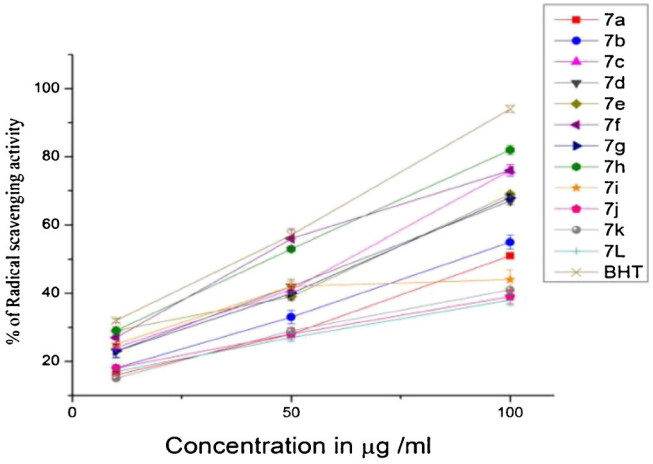


Figure 1. DDPH radical scavenging assay of **7a-l**.

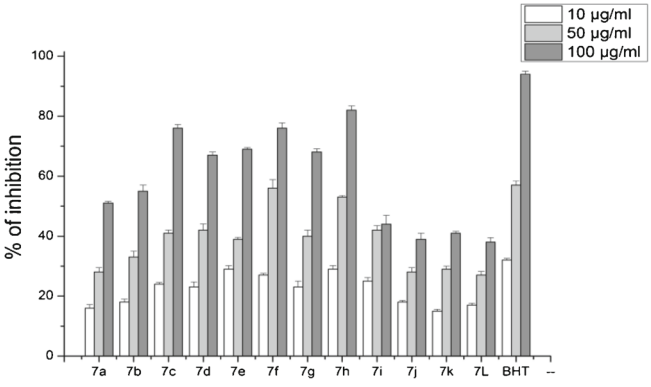


Figure 2. Bar graph representation of DDPH radical scavenging assay of **7a-l**.

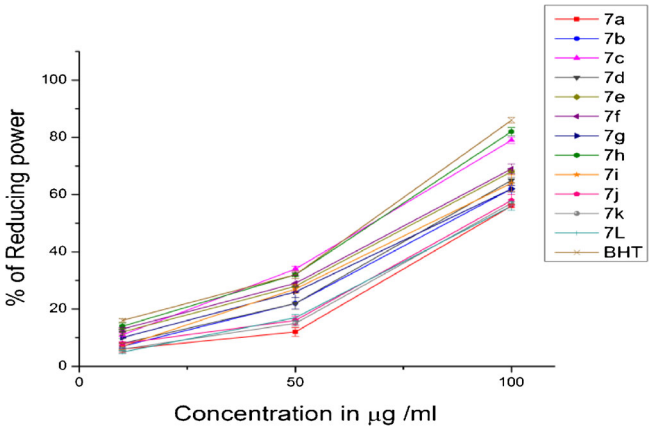


Figure 3. Graphical representation of reducing power of **7a-l**.

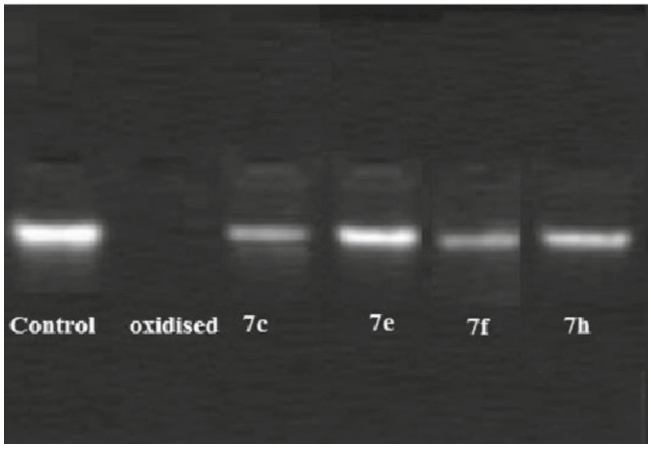


Figure 4. Electrophoretic mobility of DNA in treated samples.

by DPPH scavenging model system using eq. (1). All the samples **7a-l** showed free radical scavenging ability. The data from table 1 reveals that compounds **7a-l** of synthesized derivatives **7a-l** exhibit good DPPH free radical scavenging activity compared to standard antioxidant (BHT). These results indicate the potential electron donating ability of the samples. The DDPH radical scavenging assays of **7a-l** are shown graphically in figures 1 and 2.

3.1b *Reducing ability study of **7a-l***: Reducing power of samples **7a–l** was also evaluated (table 1) for their ability to reduce ferric chloride and potassium ferricyanide complex. At the initial concentrations (10 μg/mL), no significant differences in the activity were observed. However, as the concentration was increased (50–100 μg/mL), reducing power also increased. Increased absorbance at 700 nm indicated the reducing ability of the synthesized compounds. The enhanced reducing power of **7h** and **7c** may be due to the presence of electron donating groups at para position of the phenyl ring. Graphical representation of reducing power is shown in figure 3.

3.1c *DNA damage inhibition studies*: DNA damage inhibition ability of samples **7a-l** was evaluated on lambda phage DNA oxidation. The hydroxyl radical generated by Fenton's reagent caused DNA fragmentation with increase in its electrophoresis mobility and thereby the fragments had run out of the gel (lane 2). Treatment of samples **7a-l** prior to oxidation minimized the extent of DNA damage. As evidenced by gel documentation analysis, compared to negative DNA (figure 4), out of all the samples compounds having electron donating groups at para-position of the aryl group, **7e**, **7h**, **7c** and **7f**, showed better DNA damage inhibition. Electrophoretic mobility of DNA is shown in figure 4.

4. Conclusions

We have synthesized a new series of compounds **7a–l** and evaluated them for their DNA damage inhibition and antioxidant activities. Compounds **7e**, **7h**, **7c** and **7f** having electron donating groups at para position of aryl moiety demonstrated potent antioxidant activity and DNA damage inhibition compared to the standard. These compounds might be blocking the process of oxidation by neutralizing free radicals via interaction of the lone pair of electrons (on -Cl, -OCH_3 and -OH) with DPPH radical.

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