Original Research Article

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF NOVEL 4,8-DISUBSTITUTED-3,4-DIHYDRO-6-METHYL-IMIDAZO[1,5-*b*][1,2,4]TRIAZIN-2(8*H*)-ONE DERIVATIVES.

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ABSTRACT

Purpose: The main objective of the present research study is to synthesize series of novel 4,8-disubstituted disubstituted-3,4-dihydro-6-methyl-imidazo[1,5-*b*][1,2,4]triazin-2(8*H*)-one derivatives (5a-5f) and evaluate them for their antioxidant effect.
Methods: The said compounds were synthesized in total three steps viz Earlenmeyer-Azlactone synthesis, followed by reaction with substituted and unsubstituted 2, 4-dinitrophenylhydrazine and lastly reaction with chloracetamide. In vitro antioxidant study was performed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay using various concentrations such as 10, 20, 30, 40 and 50 µg/mL. Synthesis of compounds was confirmed by melting point, HPTLC, FTIR, ^{1}H NMR, ^{13}C NMR and LC-MS. The obtained results (IC_{50} values) were compared with standard antioxidant agent ascorbic acid.
Results: The IC_{50} values were compared with standard antioxidant ascorbic acid. Compound **5a, 5b** and **5c** showed very higher significant activity (p<0.01), **5d** showed significant activity while compound **5e** and **5f** showed marginally significant activity.
Conclusion: Compounds bearing electron donating group showed higher antioxidant effect as compared to the compounds with electron withdrawing groups.

Keywords*:* Earlenmeyer-Azlactone synthesis, DPPH, Radical scavenging assay, Ascorbic acid.

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Running Title-Synthesis and antioxidant activity of some novel heterocycles.

INTRODUCTION

Imidazole and triazine heterocycles are important building blocks for development of novel medicinal, pharmaceutical and agricultural agents. Most classes of heterocyclic medicinal compounds contain imidazole ring in their chemical structure such as antioxidant agents [1], anticancer agents [2], antifungal [3], antibacterial [4], antiprotozoal [5]. Coming to the triazine nucleus, it has very importance because of its effectiveness in most of chemical compounds such as drugs, polymers, resins, agricultures and optics [6-10]. Triazine based complexes of copper

has superoxide radical scavenging effect in pathological cascade [11]. Various novel fused 1,2,4-triazine aryl analogues antitumor effect [12], ethanol induced antistress effect in mouse brain [13], and adenosine receptor antagonist [14]. The literature survey revealed that when dynamically active 2 hetocycles couples with each other, new molecule development occurs and which can display synergistic effect on biological systems. This encouraged us to synthesize imidazole ring clamped with triazine ring could give entrance to novel antioxidant agents. Therefore an attempt has been made to synthesize newer 4,8-disubstituted-3,4-dihydro-6-methyl-imidazo[1,5-*b*][1,2,4]triazin-2(8*H*)-one derivatives as antioxidants.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), capable of causing damage to DNA, has been associated with carcinogenesis, coronary heart disease, and many other health problems related to advancing age. In low concentrations, synthetic antioxidants are also in use for many industrial processes e.g. inhibition of radical formation for preventing premature polymerization during processing, storage and transportation of unsaturated monomers. They exert their effects by scavenging or preventing the generation of ROS which can protect the formation of free radicals and retard the progress of many chronic diseases including cancer, neurodegenerative, inflammation and cardiovascular diseases [15-17]. Hence the interest for the protective role of antioxidant drugs has been growing over last 14 years. They are considered as potential medicines because of their ability to reduce or stop free radical reactions initiated by ROS/RNS.

MATERIALS AND METHODS

General:

All reagents were used as purchased from E. Merck, Mumbai and used without further purification. Melting points were determined by using a Remi digital melting point determination apparatus and are uncorrected. Purity of compounds were checked by High Performance Thin-layer chromatography (HPTLC) and was performed on CAMAG twin with applicator Linomat-IV and plate specifications are Merck precoated silica gel 60 F_{254} with 0.2 mm thickness. Spectroscopic data were recorded by using FT-IR (Shimadzu spectrophotometer 8400 using KBr), ^{1}H NMR (Varian Mercury 400, Model- Unity AS400, serial- S0121719, frequency 400 MHz using DMSO as a solvent and tetramethylsilane (TMS) as an internal standard and chemical shifts were expressed as δ values in ppm), ^{13}C NMR (INOVA-300 with 75 MHz frequency DMSO as a solvent and tetramethylsilane (TMS) as an internal standard), LC-MS (Benchtop Agilent 1100 series LC–MSD (Agilent Technologies, Waldbronn, Germany), Column: C18, preparation on ODS (octadecylsilica) Hypersil column (Agilent Technologies), Flow-rate was 0.25 mL/min to 0.50 mL/min). Absorbance was recorder using UV Jasco spectrophotometer model V-630. Antioxidant activity was performed by using DPPH radical scavenging assay.

Experimental:

a.Typical procedure for synthesis of compounds (3a-3f) by Erlenmeyer-Azlactone synthesis [18].

Warm a mixture of 29 g (0.25 mol) of *N*-acetylglycine, 37.5 ml (0.37 mol) of aromatic aldehydes **(1a-1g)**, 15 gm (0.183 mol) of anhydrous sodium acetate and 59 mL (0.62 mol) of acetic anhydride in 500 mL flask equipped with a reflux condenser, on water bath with occasional shaking until solution is complete (10-20 min). Boil the resulting solution for 1 h, cool and leave

in a refrigerator overnight. Stir the solid mass of yellow crystals with 60 mL of cold water, transfer to a Buchner funnel and wash well with cold water. Wash with a little ether. Crystallized from carbon tetrachloride and used for next step of synthesis.

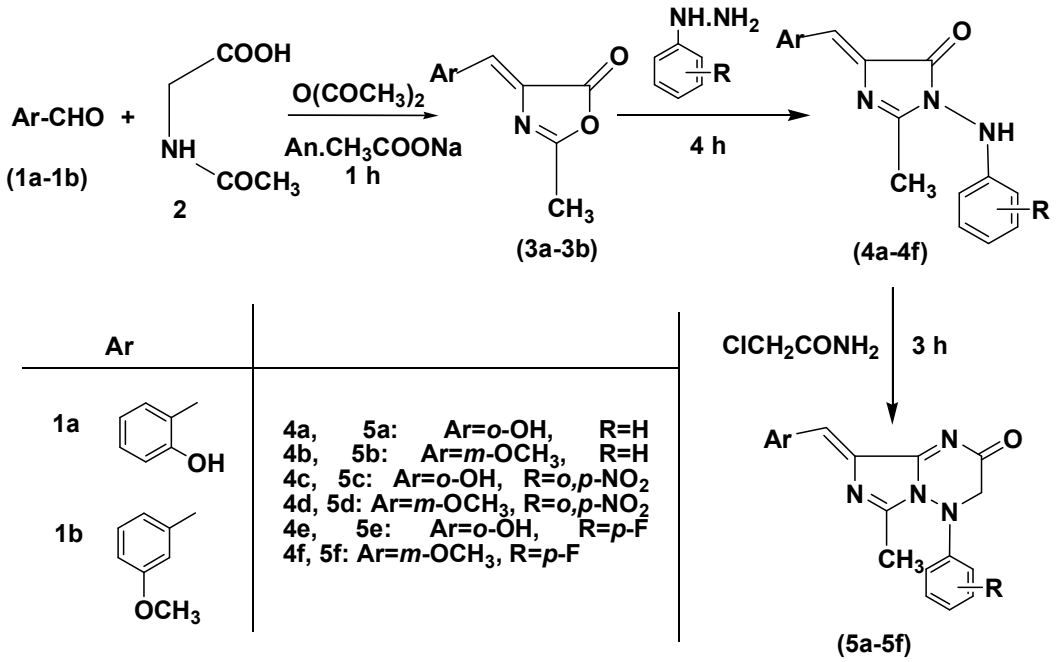

Figure 1-Synthetic route.

b. Typical procedure for synthesis of compounds (4a-4f)

A solution of **3a-3f** (6 mmole) in dry benzene (30 mL) and 2,4-dinitro phenylhydrazine (5 mmole) was heated under reflux for 4 h. Then the mixture was poured upon water. The precipitated solid was filtered off, dried and crystallized from ethanol to get the desired compounds.

c. Typical procedure for synthesis of compounds (5a-5f)

A solution of **4a-4f** (8 mmole) and chloroacetamide (8 mmole) was refluxed for 3 h in boiling *N, N*-dimethylformamide (30 mL). Then the mixture was poured into water. The precipitated solid was filtered off, dried and crystallized from ethanol to get the desired compounds.

Antioxidant activity by DPPH free radical scavenging assay [19]:

Antioxidant activity of all novel synthesized compounds **(5a-5f)** was done by DPPH free radical scavenging assay as described by Philip Molyneux. Ascorbic acid and the synthesized compounds of different concentrations were prepared in distilled ethanol. 3 mL of each compound solution having different concentrations (10, 20, 30, 40 and 50 µg/mL) were taken in different test tubes of 0.004% DPPH in ethanol was added in required to obtain above mentioned five concentrations and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Decrease in the absorbance at 517 nm was measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated

on the basis of the observed decrease in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

Radical scavenging activity (%) = $[(A_o-A_1)/A_o \times 100]$

Where A_o is the absorbance of the control (blank) and A_1 is the absorbance of the compound.

RESULTS

Novel compound of each series were identified by determining their melting points, % yield. They were also confirmed spectrophotometrically using HPTLC, IR, ^{1}H NMR, ^{13}C NMR, LC-MS etc.

4-(2-hydroxybenzylidene)-2-methyloxazol-5(4H)-one (3a)

Mol. Form. $C_{11}H_9NO_3$; Ar = (-C_6H_5-*o*-OH); mp 305-307 ºC; yield: 89%; IR (KBr, ν_{max}, cm $^{-1}$): 855 (C-H bend), 1294 (C-O str), 1355 (C-N str), 1520 (C=C str), 1605 (C=N str), 1671 (C=O str), 2970 (CH_3 str), 3041 (C-H str), 3324 (O-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 1.42 (s, 1H, CH_3), 6.68 (s, 1H, CH), 6.71 (d, 1H, ArH), 6.77 (t, 1H, ArH), 6.97 (t, 1H, ArH), 7.13 (d, 1H, ArH), 11.32 (s, 1H, OH).

4-(3-methoxybenzylidene)-2-methyloxazol-5(4H)-one (3b)

Mol. Form. $C_{12}H_{11}NO_3$; Ar = (-C_6H_5-*m*-OCH_3); mp 195-197 ºC; yield: 88%; IR (KBr, ν_{max}, cm $^{-1}$): 881 (C-H bend), 1170 (C-O str), 1300 (C-N str), 1509 (C=C str), 1680 (C=N str), 1772 (C=O str), 2902 (CH_3 str), 3068 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.34 (s, 1H, CH_3), 3.73 (s, 1H, OCH_3), 6.75 (d, 1H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.10 (t, 1H, ArH), 7.64 (s, 1H, CH).

5-(2-hydroxybenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one (4a)

Mol. Form. $C_{17}H_{15}N_3O_2$; Ar = (-C_6H_5-*o*-OH); mp 90-92 ºC, yield: 72%; HPTLC: R_f 0.62, Chloroform: methanol: water (6:2:2); IR (KBr, ν_{max}, cm $^{-1}$): 884 (C-H bend), 1133 (C-O str), 1231 (C-N str), 1617 (C=C str), 1637 (C=N str), 1747 (C=O str), 2971 (CH_3 str), 3128 (C-H str), 3431 (N-H str), 3674 (O-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.13 (s, 3H, CH_3), 6.21 (s, 1H, NH), 6.32 (s, 1H, CH), 6.64 (d, 3H, ArH), 6.74 (t, 2H, ArH), 6.96 (t, 1H, ArH), 7.17 (d, 1H, ArH), 7.19 (t, 2H, ArH), 11.71 (s, 1H, OH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.31, 108.55, 113.31, 115.86, 116.12, 119.21, 121.23, 127.81, 129.65, 130.61, 144.72, 151.81, 158.91, 166.13; LC-MS (m/z): 294.37 [M^++1].

5-(3-methoxybenzylidene)-2-methyl-3-(phenylamino)-3,5-dihydro-4H-imidazol-4-one (4b)

Mol. Form. $C_{18}H_{17}N_3O_2$; Ar = (-C_6H_5-*m*-OCH_3); mp 176-178 ºC, yield: 83%; HPTLC: R_f 0.61, Chloroform: methanol: water (8:1:1); IR (KBr, ν_{max}, cm $^{-1}$): 851 (C-H bend), 1334 (C-N str), 1519 (C=C str), 1634 (C=N str), 1723 (C=O str), 3032 (CH_3 str), 3134 (C-H str), 3485 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 6.65 (d, 1H, ArH), 6.66 (t, 1H, ArH), 6.71 (d, 2H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.10 (t, 1H, ArH), 7.19 (t, 2H, ArH), 7.56 (s, 1H, CH), 7.94 (s, 1H, NH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 20.87, 55.09, 108.04, 112.67, 113.02, 113.85, 118.45, 119.54, 129.63, 129.73, 130.40, 137.54, 144.76, 161.89, 166.43; LC-MS (m/z): 308.92 [M^++1].

1-(2,4-dinitrophenylamino)-4-(2-hydroxybenzylidene)-2-methyl-1H-imidazol-5(4H)-one (4c)

Mol. Form. $C_{17}H_{13}N_5O_6$; Ar = (-C_6H_5-*o*-OH); mp 190-193 ºC, yield: 67%; HPTLC: R_f 0.58, Toluene: ethyl acetate (8:2); IR (KBr, ν_{max}, cm $^{-1}$): 849 (C-H bend), 1261 (C-O str), 1324 (C-N str), 1672 (C=C str), 1672 (C=N str), 1763 (C=O str), 2966 (CH_3 str), 3041 (C-H str), 3476 (N-H str), 3623 (OH str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.54 (s, 3H, CH_3), 6.43 (s, 1H, CH), 6.64 (s, 2H, NH), 6.69 (d, 1H, ArH), 6.74 (t, 1H, ArH), 6.94 (t, 1H, ArH), 7.18 (d, 1H, ArH), 7.21 (d, 1H, ArH), 8.51 (d, 1H, ArH), 9.03 (s, 1H, ArH), 11.72 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.93, 40.87, 108.98, 113.93, 114.65, 119.45, 124.65, 127.35, 129.46, 130.76, 144.24, 149.67, 151.87, 166.13; LC-MS (m/z): 383.31 [M^++1].

1-(2,4-dinitrophenylamino)-4-(3-methoxybenzylidene)-2-methyl-1H-imidazol-5(4H)-one (4d)

Mol. Form. $C_{18}H_{15}N_5O_6$; Ar = (-C_6H_5-*m*-OCH_3); mp 90-91ºC, yield: 57%; HPTLC: R_f 0.73, Toluene: ethyl acetate (7:3); IR (KBr, ν_{max}, cm $^{-1}$): 842 (C-H bend), 1167 (C-O str), 1323 (C-N str), 1534 (C=C str), 1624 (C=N str), 1742 (C=O str), 2965 (CH_3 str), 3097 (C-H str), 3454 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 6.65 (d, 1H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.11 (t, 1H, ArH), 7.19 (d, 1H, ArH), 7.45 (s, 1H, CH), 8.50 (d, 1H, ArH), 9.05 (s, 1H, ArH), 9.12 (s, 1H, NH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.87, 55.09, 108.04, 112.67, 113.02, 113.85, 118.45, 119.54, 129.63, 129.73, 130.40, 137.54, 144.76, 161.89, 166.43; LC-MS (m/z): 397.34 [M^++1].

4-(2-hydroxybenzylidene)-1-(4-fluorophenylamino)-2-methyl-1H-imidazol-5(4H)-one (4e)

Mol. Form. $C_{17}H_{14}FN_3O_2$; Ar = (C_6H_5-*o*-OH); mp 105-106ºC, yield: 71%; HPTLC: R_f 0.61, Toluene: ethyl acetate: formic acid (8:1:1); IR (KBr, ν_{max}, cm $^{-1}$): 726 (C-H bend), 1080 (C-F str), 1183 (C-O str), 1364 (C-N str), 1483 (C=C str), 1683 (C=N str), 1764 (C=O str), 2965 (CH_3 str), 3069 (C-H str), 3644 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.61 (s, 3H, CH_3), 6.33 (s, 1H, CH), 6.61 (d, 2H, ArH), 6.64 (d, 1H, ArH), 6.74 (t, 1H, ArH), 6.79 (s, 1H, NH), 6.83 (d, 2H, ArH), 6.94 (t, 1H, ArH), 7.18 (d, 1H, ArH), 11.32 (s, 1H, OH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.71, 108.65, 114.81, 115.51, 116.11, 116.89, 121.31, 127.76, 129.76, 130.76, 144.78, 146.56, 153.31, 158.98, 166.34.

4-(3-methoxybenzylidene)-1-(4-fluorophenylamino)-2-methyl-1H-imidazol-5(4H)-one (4f)

Mol. Form. $C_{18}H_{16}FN_3O_2$; Ar = (-C_6H_5-*m*-OCH_3); mp 139-141ºC, yield: 67%; HPTLC: R_f 0.47, Toluene: ethyl acetate: formic acid (7:2:1); IR (KBr, ν_{max}, cm $^{-1}$): 768 (C-H bend), 1057 (C-F str), 1186 (C-O str), 1374 (C-N str), 1468 (C=C str), 1663 (C=N str), 1775 (C=O str), 2971 (CH_3 str), 3044 (C-H str), 3468 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 6.63 (d, 2H, ArH), 6.66 (d, 1H, ArH), 6.81 (s, 1H, ArH), 6.87 (d, 1H, ArH), 6.89 (d, 2H, ArH), 7.11 (t, 1H, ArH), 7.58 (s, 1H, CH), 7.94 (s, 1H, NH) ; ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.09, 55.90, 108.86, 110.65, 112.87, 114.87, 116.65, 118.86, 129.54, 130.06, 136.14, 144.65, 146.43, 153.65, 161.76, 166.18

8-(2-hydroxybenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one (5a)

Mol. Form. $C_{19}H_{16}N_4O_2$; Ar = (-C_6H_5-*o*-OH); m.p: 82-83 ºC; yield: 79%; HPTLC: R_f 0.63, Chloroform: methanol: water (7:1:2); IR (KBr, ν_{max}, cm $^{-1}$): 885 (C-H bend), 1110 (C-O str), 1223 (C-N str), 1617 (C=C str), 1631 (C=N str), 1764 (C=O str), 2928 (=CH_2 str, sym), 2851 (=CH_2 str, asym), 3074 (CH_3 str), 3164 (C-H str), 3613 (O-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.82 (s, 3H, CH_3), 4.23(s, 2H, CH_2), 6.64 (d, 3H, ArH), 6.78 (t, 1H, ArH), 6.89 (t,

1H, CH & 2H, ArH), 6.93 (t, 1H, ArH), 7.18 (d,1H, ArH), 7.22 (d, 1H, ArH), 11.72 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 21.38, 62.55, 102.66, 113.21, 116.12, 115.81, 119.23, 121.33, 127.11, 127.98, 129.21, 129.99, 144.72, 151.44, 158.31, 164.56, 200.21; LC-MS (m/z): 333.16 [M^++1].

8-(3-methoxybenzylidene)-3,4-dihydro-6-methyl-4-phenylimidazo[1,5-b][1,2,4]triazin-2(8H)-one (5b)

Mol. Form. $C_{20}H_{18}N_4O_2$; Ar = (-C_6H_5-*m*-OCH_3); mp 120-122 ºC; yield: 90%; HPTLC: R_f 0.61, Chloroform: methanol: water (8:1:1); IR (KBr, ν_{max}, cm $^{-1}$): 876 (C-H bend), 1365 (C-N str), 1523 (C=C str), 1633 (C=N str), 1745 (C=O str), 2872 (=CH_2 str, sym), 2902 (=CH_2 str, asym), 3054 (CH_3 str), 3121 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 6.62 (s, 1H, CH), 6.66 (d, 1H, ArH), 6.67 (d, 2H, ArH), 6.72 (t, 1H, ArH), 6.80 (s, 1H, ArH), 6.85 (d,1H, ArH), 7.11 (t, 1H, ArH), 7.19 (t, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz): 21.30, 56.65, 62.40, 102.62, 113.65, 119.65, 123.26, 126.86, 127.70, 128.26, 128.62, 129.43, 136.20, 138.26, 144.70, 151.42, 164.86, 200.56; LC-MS (m/z): 347.64 [M^++1].

8-(2-hydroxybenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one (5c)

Mol. Form. $C_{19}H_{14}N_6O_6$; Ar = (-C_6H_5-*o*-OH); mp 82-83 ºC, yield: 89%; HPTLC: R_f 0.77, Toluene: ethyl acetate (9:1); IR (KBr, ν_{max}, cm $^{-1}$): 861 (C-H bend), 1274 (C-O str), 1372 (C-N str), 1561 (C=C str), 1692 (C=N str), 1762 (C=O str), 2837 (=CH_2 str, sym), 2915 (=CH_2 str, asym), 2972 (CH_3 str), 3061 (C-H str), 3634 (OH str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 6.67 (d, 1H, ArH), 6.77 (t, 1H, ArH), 6.81 (s, 1H, CH), 6.92 (t, 1H, , ArH), 7.17 (d, 1H, ArH), 7.21 (d, 1H, ArH), 8.51 (d, 1H, ArH), 9.04 (s, 1H, ArH), 11.79 (s, 1H, OH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.65, 61.44, 102.75, 116.98, 117.54, 119.21, 121.24, 127.11, 127.89, 129.88, 132.65, 139.98, 143.23, 144.28, 158.87, 166.43, 164.56, 200.45; LC-MS (m/z): 422.35 [M^++1].

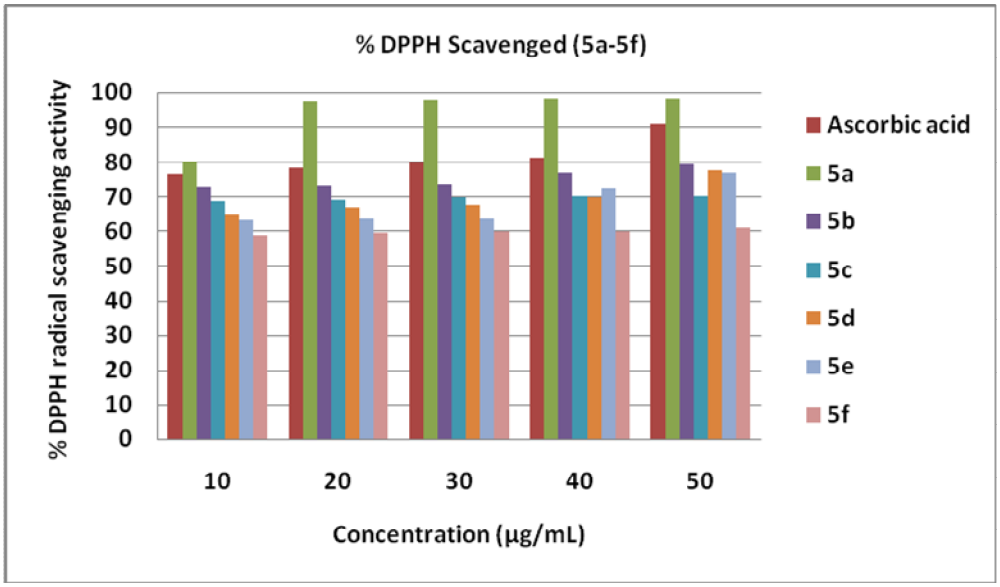

Figure 2. % DPPH radical scavenging effect.

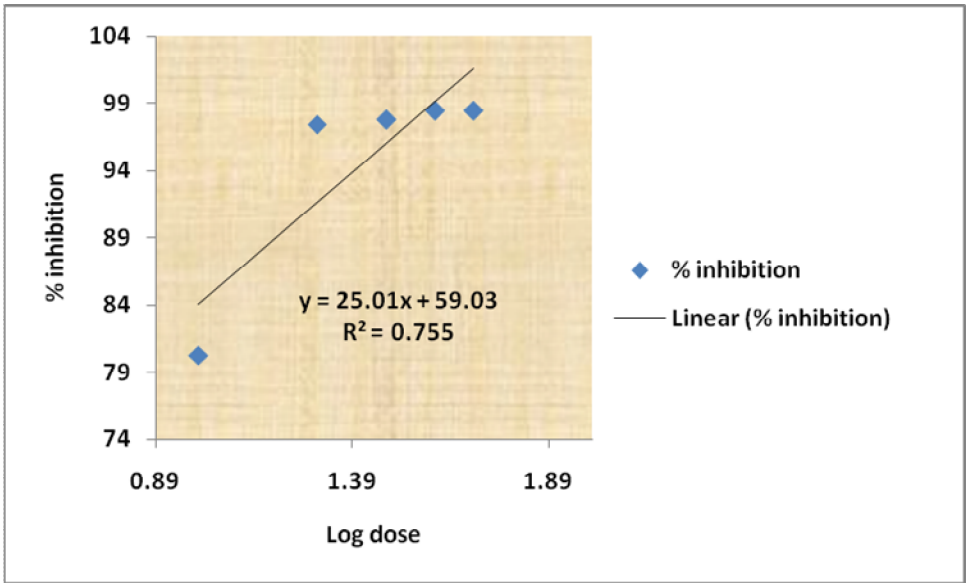

Figure 3. IC_{50} value of compound 5a.

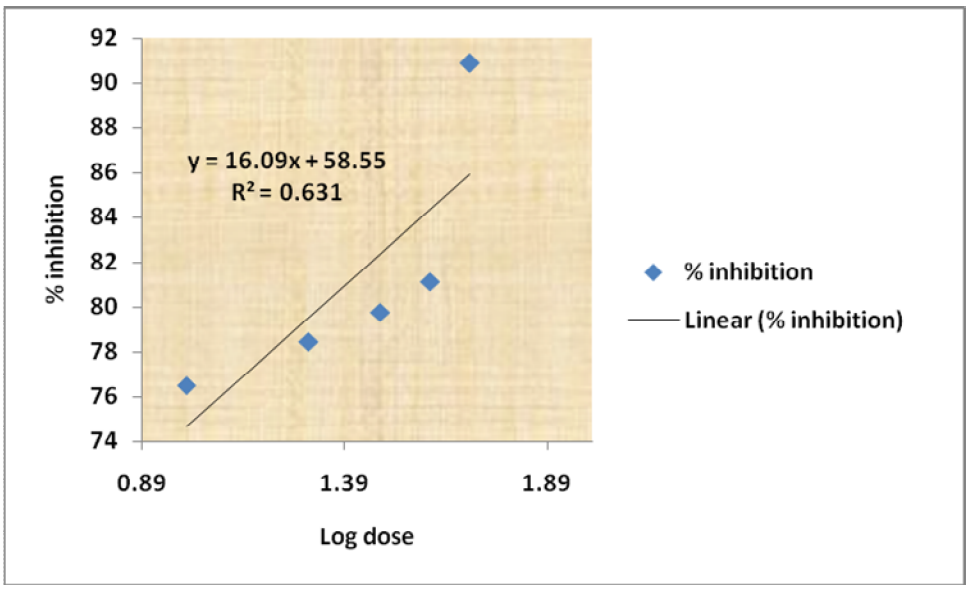

Figure 4. IC_{50} value of ascorbic acid.

8-(3-methoxybenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one (5d)

Mol. Form. $C_{20}H_{16}N_6O_6$; Ar = (-C_6H_5-*o*-OCH_3); mp 105-108 ºC, yield: 77%; HPTLC: R_f 0.73, Toluene: ethyl acetate (7:3); IR (KBr, ν_{max}, cm $^{-1}$): 843 (C-H bend), 1133 (C-O str), 1375 (C-N str), 1512 (C=C str), 1645 (C=N str), 1734 (C=O str), 2848 (=CH_2 str, sym), 2925 (=CH_2 str, asym), 2971 (CH_3 str), 3069 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 6.60 (s, 1H, CH), 6.66 (s, 1H, ArH), 6.81 (d, 1H, ArH), 6.86 (d, 1H, ArH), 7.11 (t, 1H, ArH), 7.19 (d, 1H, ArH), 8.51 (d,1H, ArH), 9.05 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.98, 55.90, 61.86, 102.56, 110.85, 113.56,

115.76, 118.76, 119.54, 127.65, 128.05, 131.56, 134.87, 138.87, 141.65, 143.54, 144.12, 161.45, 165.25, 200.65; LC-MS (m/z): 436.37 [M^++1].

8-(2-hydroxybenzylidene)-4-(4-fluorophenyl)-3,4-dihydro-6-methylimidazo[1,5-b][1,2,4]triazin-2(8H)-one (5e)

Mol. Form. $C_{19}H_{15}FN_4O_2$; Ar = (-C_6H_5-*o*-OH); mp 97-99ºC, yield: 71%; HPTLC: R_f 0.62, Toluene: ethyl acetate: formic acid (6:2:2); IR (KBr, ν_{max}, cm $^{-1}$): 784 (C-H bend), 1050 (C-F str), 1178 (C-O str), 1376 (C-N str), 1465 (C=C str), 1653 (C=N str), 1798 (C=O str), 2851 (CH_2 str, sym), 2921 (CH_2 str, asym), 2970 (CH_3 str), 3041 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.69 (s, 3H, CH_3), 4.20 (s, 2 H, CH_2), 6.61 (t, 2H, ArH), 6.64 (d, 1H, ArH), 6.74 (t, 1H, ArH), 6.79 (s, 1H, CH), 6.83 (t, 2H, ArH), 6.94 (t, 1H, ArH), 7.18 (d, 1H, ArH), 11.58 (s, 1H, OH) ; ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.31, 62.44, 102.66, 114.98, 115.08, 116.54, 116.99, 121.34, 127.12, 127.98, 129.65, 144.72, 147.98, 153.25, 158.34, 164.24, 200.13

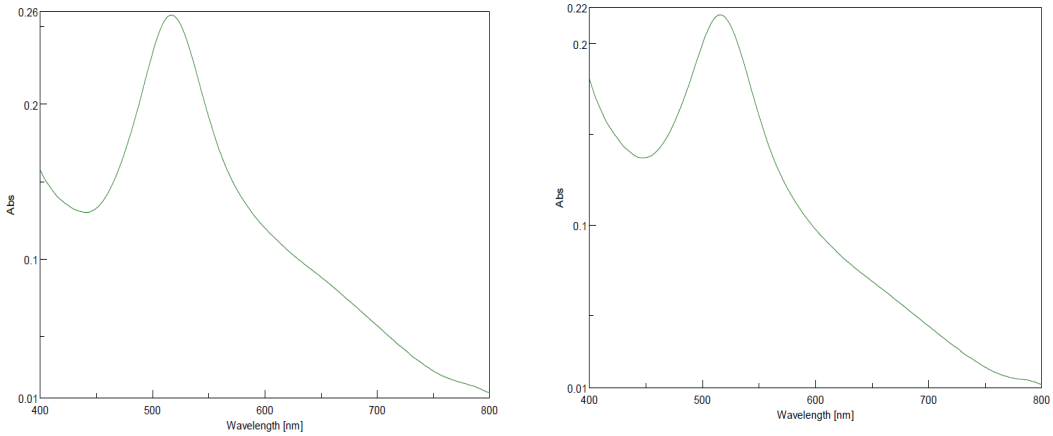

Figure 5. UV absorption plots for compound 5a at conc of 10 and 20 µg/mL.

8-(3-methoxybenzylidene)-4-(4-fluorophenyl)-3,4-dihydro-6-methylimidazo[1,5-b][1,2,4]triazin-2(8H)-one (5f)

Mol. Form. $C_{20}H_{17}FN_4O_2$; Ar = (-C_6H_5-*m*-OCH_3); mp 109-111ºC, yield: 65%; HPTLC: R_f 0.47, Toluene: ethyl acetate: formic acid (7:2:1); IR (KBr, ν_{max}, cm $^{-1}$): 771 (C-H bend), 1049 (C-F str), 1178 (C-O str), 1354 (C-N str), 1459 (C=C str), 1661 (C=N str), 1770 (C=O str), 2871 (CH_2 str, sym), 2915 (CH_2 str, asym), 2955 (CH_3 str), 3039 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.33 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2), 6.61 (s, 1H, CH), 6.62 (d, 2H, ArH), 6.64 (d, 1H, ArH), 6.78 (s, 1H, ArH), 6.85 (d, 1H, ArH), 6.89 (d, 2H, ArH), 7.13 (t, 1H, ArH) ; ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.56, 55.96, 62.56, 103.35, 111.05, 113.45, 114.27, 116.12, 118.74, 127.76, 130.27, 136.26, 144.65, 147.98, 153.98, 160.62, 164.26, 200.12

Antioxidant activity of the synthesized compounds is depicted in tabular and graphical form as follows.

Table 1. % DPPH radical scavenging effect.

Conc. (µg/mL)	% DPPH radical scavenged						
	Ascorbic acid	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**
10	76.52	80.27	72.61	68.81	64.62	63.30	58.94
20	78.45	97.41	73.30	69.11	67.04	63.42	59.38
30	79.76	97.76	73.61	70.02	67.45	63.54	59.71
40	81.15	98.4	76.99	70.15	69.83	72.30	59.98
50	90.88	98.43	79.47	70.26	77.76	76.95	61.01

STATISTICAL ANALYSIS

Experimental results were expressed as mean ± SD of 3 parallel readings. Results were analyzed using ANOVA followed by Student's'‘t’ test analysis.

DISCUSSION

All synthesized compounds **(5a-5d)** were evaluated using physical data such as melting point and R_f values as well as spectroscopic methods IR, ^{1}H NMR, ^{13}C NMR, LC-MS etc and showed best correlation with the same. All these novel compounds were screened for in vitro antioxidant activity by DPPH radical scavenging effect at various concentrations of 10, 20, 30, 40 and 50 µg/mL and IC_{50} values had been determined for each compound and compared with standard antioxidant. Compound **5a** is more active than that of ascorbic acid, compound **5b** showed comparable activity as that of ascorbic acid while other compounds such as **5c, 5d, 5e** and **5f** are less active.

CONCLUSION

IC_{50} value of compound **5a** was found to be smaller than that of ascorbic acid which shows that compound **5a** is strong DPPH radical scavenger than ascorbic acid, compound **5b** is comparable DPPH radical scavenger while all other are less DPPH radical scavenger than ascorbic acid. Stronger DPPH scavenging effect of compound **5a** and **5b** is owing to presence of electron donating groups viz hydroxyl and methoxy in their structure, which increases stability of phenoxy radical. In comparison, compound **5a** is more active that compound **5b** as former contain hydroxyl group at ortho position of benzylidene moiety while later contain methoxy group at meta position of benzylidene moiety and o-hydroxyl group shows resonating effect while m-methoxy group shows +I effect. Former is stronger than later, hence compound **5a** is more active than compound **5b** and ascorbic acid. Weaker DPPH scavenging effect of remaining compounds is owing to presence of electron withdrawing groups such as nitro and fluoro.

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