



Research Article

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Discovery of Some Emerging Free Radical Scavenging Candidates Bearing 2-(*p*-hydroxyphenyl)-4-(substitutedphenyl)-1*H*-1,5-Benzodiazepines Pharmacophore

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ABSTRACT

In the present investigation, the synthesis of few novel leads bearing 2-(*p*-hydroxyphenyl)-4-(substitutedphenyl)-1*H*-1,5-benzodiazepine pharmacophore is described. The substituted chalcone and their derivatives 3(a-j) were synthesized by base catalyzed Claisen-Schmidt condensation between *p*-hydroxyacetophenone and appropriate aldehydes. The dibromostyryl ketones 4(a-j) were obtained by the reaction of the chalcone with bromine in acetic acid. The dibromostyryl ketone were reacted with methanol in presence of sodium methoxide followed by acidic hydrolysis give 1-(4-hydroxyphenyl)-3-(substitutedphenyl)-1,3-propanediones. The targeted compounds; the substituted 1,5-benzodiazepines were synthesized with *o*-phenylenediamine and synthesized 1,3-propanediones. The structures of synthesized compounds were confirmed by spectroscopic and analytical techniques (IR, ¹H-NMR, and MS). The free radical scavenging activity of the synthesized analogs was monitored by *in vitro* antioxidant activity protocol. The derivatives 6f, 6g, 6i, and 6j were found to exhibit good antioxidant activity with 59.07%, 41.33%, 68.3% and 60.4% scavenging activity respectively as compared to standard ascorbic acid which demonstrated 79.73% activity. The current research revealed the potential of 2-(*p*-hydroxyphenyl)-4-(substituted-phenyl)-1*H*-1,5-benzodiazepine as emerging free radical scavengers. The study helped to establish a structure-activity relationship (SAR) where the substitution on the phenyl moiety of the 1,5-benzodiazepine was found to play profound role and influence over biological activity. The research will open new avenues for the development of antioxidant moieties having perspectives in cancer, inflammation, and several other ailments.

Keywords: 1,5-benzodiazepine; chalcone; synthesis; antioxidant; free radical; scavenging.

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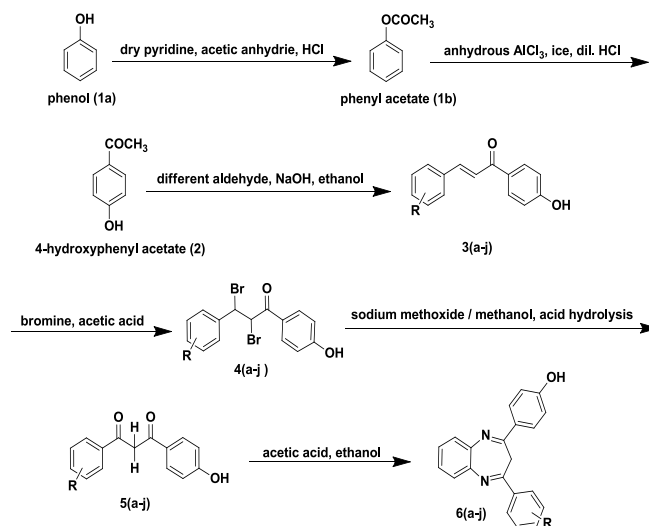
INTRODUCTION

In healthy organisms, production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is approximately balanced by antioxidant defense systems. However, an organism can be suffering from so-called 'oxidative stress' while it is experiencing disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage. Reactive oxygen species (ROS) produced in cells include hydrogen peroxide (H_2O_2), free radicals such as the hydroxyl radical ($\cdot OH$), the superoxide anion ($O_2^{\cdot -}$) and reactive nitrogen species (RNS) are highly reactive and potentially damaging transient chemical species formed in aerobic life. [1] Free radicals are produced in normal and/or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis, and arteriosclerosis as well as in degenerative processes associated with aging. Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. The awareness of the potential benefits of antioxidant nutrients in health maintenance is growing. [2]

With the discovery of benzodiazepine, the scaffold achieved a milestone of the medicinal chemistry due to its invaluable therapeutic and pharmacological properties. It possesses a wide spectrum of physiological properties. Intense competitive researches have been initiated due to the importance of benzodiazepine as an anxiolytic and tranquilizing agent into the synthesis of new analogs of this class of drugs. [3] Naturally occurring pyrrolo[2,1-c] [1,4] benzodiazepines have attracted the attention of many scientists because of their anticancer activity exhibited in most of the compounds with this ring system. [4] Later, various substituted 7,12-dihydroindole[3,2-b][1,5]-benzodiazepine have been synthesized which have tremendous potential to kill few strains of bacteria like *E. coli* and *S. aureus*. [5] Several 1,5-benzodiazepine molecules have the ability to scavenge free radical, which provided a basis for designing novel molecules with pronounced antioxidant activity. [6]

Preceding discussion through light on the importance of benzodiazepines as antioxidant agents, the present work few novel importance 2-(*p*-hydroxyphenyl)-4-(substitutedphenyl)-1*H*-1,5-benzodiazepine obtained by the synthetic protocol described in the synthetic Scheme 1, in the search of novel effective antioxidant

agent. The main objective of the study is to establish a structure-activity relationship (SAR) of 1,5-benzodiazepine scaffold for exhibiting antioxidant activity.



Scheme 1: Synthetic protocol for target compounds

MATERIALS AND METHODS

Chemical and Instrumentation

Solvents and reagents obtained from commercial sources were used with purification and drying wherever required. The characterizations of the synthesized compounds are mentioned in the individual description. The melting points of the products reported here were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The analytical TLC was performed by using pre-coated Silica gel G plate (Merck). The percentage yields are based upon products obtained after purification. The IR spectra were undertaken on a Jasco infrared spectrometer at M.G.V.'s Pharmacy College, Nashik. The absorption frequencies were reported in cm^{-1} . The 1H NMR was recorded at Sophisticated Analytical Instrument Facility (SAIF), Chandigarh using Bruker Avance-II instrument where the chemical shifts were given in ppm relative to tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on MICROMASS Q-TOF instrument. The CHN analysis was obtained using Elemental Analyzer of Perkin-Elmer 2400 model.

Synthesis of Target Compounds

The synthesis of target compounds was accomplished by the reaction sequence illustrated in Scheme 1. The substituted chalcone and their derivatives 3(a-j) were synthesized by base catalyzed Claisen-Schmidt condensation between *p*-hydroxy-acetophenone and appropriate aldehydes. The dibromostyryl ketones 4(a-j) were obtained by reacting the chalcone with bromine

in acetic acid. The dibromostyryl ketone were reacted with methanol in presence of sodium methoxide followed by acidic hydrolysis give 1-(4'-hydroxyphenyl)-3-(substitutedphenyl)-1,3-propanediones 5(a-j). The targeted compounds; substituted 1,5-benzodiazepines 6(a-j) were synthesized with *o*-phenylenediamine and synthesized 1,3-propanediones.

Synthetic protocol for the synthesis of phenyl acetate; (1b)

A mixture of phenol (94 g; 1 mol) (1a) and dry pyridine (10 mL) in 500 mL beaker was placed in ice bath. To the resultant mixture, previously cooled acetic anhydride (127 mL; 1.25 mol) was added drop wise with stirring. The resultant reaction mixture was kept standing overnight at room temperature, then it was poured over the mixture of ice and concentrated hydrochloric acid (50 mL), extracted with carbon tetrachloride (100 mL). The extract was washed successively with water, 10% sodium hydroxide solution and dried over calcium chloride. The solvent was removed by distillation and phenylacetate was collected at 194-197°C as a colorless oily liquid, b.p. 196°C, yield 90%.

Synthetic protocol for the synthesis of *p*-Hydroxyacetophenone; (2)

Phenyl acetate (13.6 g; 1 mol) (1b) was poured in 1 L round bottomed flask containing finely powdered anhydrous AlCl₃ (16.3 g; 0.125 mol). The reaction mixture was heated on an oil bath at 60-70°C for two hours. Upon cooling, the mixture of crushed ice (80 g) and cold conc. hydrochloric acid (20 mL) was added to the flask to form the mixture of *o*-hydroxyacetophenone and *p*-hydroxyacetophenone. *o*-Hydroxyacetophenone was removed by steam distillation. Crude *p*-hydroxyacetophenone was filtered, air dried, and recrystallized from ethanol, yield 62%, m.p. 107°C.

Synthetic protocol for the synthesis of various 4-hydroxy substituted benzalacetophenone derivatives; 3(a-j)

p-Hydroxyacetophenone (13.6 g 0.1 mol) (2), different aromatic aldehydes (11.6 mL; 0.12 mol) and ethanol 100 ml were taken. To this solution, aqueous NaOH (40%, 30 mL) were added dropwise with constant stirring at room temperature to produce dark yellow mass. The reaction mixture was allowed to stand overnight and acidified with 10% dilute solution of hydrochloric acid solution. The solid obtained were filtered, washed with 5 mL portions of cold water and dried.

Synthetic protocol for the synthesis of various 4-hydroxy- α,β -dibromo benzalacetophenone derivatives; 4(a-j)

To different prepared chalcone derivatives 3(a-j) (0.05 mol) about 20 mL glacial acetic acid added stirred to affect solution. To this resultant solution, molecular bromine in acetic acid (4:10 mL or 40% solution in acetic acid) was added drop wise with constant stirring. The reaction mixture was poured into ice cold water with vigorous stirring. The solid was filtered, washed with water and sodium metabisulphite solution and again with water and dried and crystallized.

Synthetic protocol for the synthesis of various 1-(*p*-hydroxyphenyl)-3-(substitutedphenyl) propane-1,3-dione derivatives; 5(a-j)

Compounds 4(a-j) (0.025 mol) and absolute methanol (super dry 8 mL) were taken in 250 mL round bottomed flask. A solution of sodium methoxide prepared from dry sodium (1.5 g) and methanol (15 mL) was added rapidly to the flask and the reaction mixture was refluxed on a water bath for 2 hours. The reaction mixture was neutralized with concentrated hydrochloric acid, was allowed to cool and diluted with crushed ice. The solids obtained were filtered, dried, and crystallized from ethanol. Table 1 depicted the physicochemical properties of the synthesized derivatives.

Synthetic protocol for synthesis of 2-(*p*-hydroxyphenyl)-4-substitutedphenyl-1*H*-1,5-benzodiazepines; 6 (a-j)

A mixture of different 1-(*p*-hydroxyphenyl)-3-(substitutedphenyl) propane 1,3-dione derivatives (2.4 g; 0.01 mole) 5(a-j), *o*-phenylenediamine (1.08 g; 0.01 mole), and 20 mL ethanol with glacial acetic acid (3-5 drops) were taken into three necked round bottomed flask. A nitrogen atmosphere was created by using balloon technique through nitrogen cylinder. The resultant reaction mixture was refluxed on boiling water bath 12 hours. Reaction mixture was allowed to cool and solid was formed. Then, the crude product was subjected to column chromatography using silica gel (hexane: ethylacetate; 8:2) to yield pure product. [7] Then, the solid was recrystallized from ethanol. Table 1 depicted the physicochemical properties of the synthesized derivatives.

2-(4'-hydroxyphenyl)-4-(phenyl)-1*H*-1,5-benzodiazepine (6a)

IR (KBr): 3408 cm⁻¹ (ν_{NH}), 1610 cm⁻¹ ($\nu_{\text{C=N}}$), 3330 cm⁻¹ (ν_{OH}); ¹H-NMR (DMSO-*d*₆): δ_{H} 7.95 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.34 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.72 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.78 (s, 1H, -C=CH-) 3.71 (s, 1H, NH), 7.73 (m, 4H, Har), 7.76 (m, 1H, Har), 7.34 (m, 2H, Har), 7.44 (m, 2H, Har), 10.31 (br, 1H, OH); ¹³C-NMR (DMSO-*d*₆): δ_{C} 152.1 (C-2), 131.2 (C-3), 164.3 (C-4), 134.2 (C-5a), 116.8 (C-6), 135.2 (C-7), 127.4 (C-8), 129.9 (C-9), 128.9 (C-9a), 125.6 (C'-1), 138.9 (C'-2), 128.5 (C'-3), 126.1 (C'-4), 130.2 (C'-5), 139.2 (C'-6), 133.3 (C''-1), 129.2 (C''-2), 128.9 (C''-3), 131.2 (C''-4), 128.9 (C''-5), 129.2 (C''-6), 133.1 (C''-1), 129.4 (C''-2), 128.5 (C''-3), 131.3 (C''-4), 128.7 (C''-5), 129.5 (C''-6); MS (EI): *m/z* (%) 313 (24.49, M+1), 312 (100, M⁺), 236 (35), 195 (26), 194 (13), 103 (24).

2-(4'-hydroxyphenyl)-4-(2''-chlorophenyl)-1*H*-1,5-benzodiazepine (6b)

IR (KBr): 3410 cm⁻¹ (ν_{NH}), 1605 cm⁻¹ ($\nu_{\text{C=N}}$), 3325 cm⁻¹ (ν_{OH}); ¹H-NMR (DMSO-*d*₆): δ_{H} 7.92 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.31 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.75 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.70 (s, 1H, -C=CH-), 3.65 (s, 1H, NH), 7.62 (m, 1H, Har), 7.50 (m, 1H, Har), 7.42 (m, 2H, Har), 7.50 (m, 2H, Har); 7.54 (m, 2H, Har), 10.15 (br, 1H, OH); ¹³C-NMR (DMSO-*d*₆): δ_{C} 152.3 (C-2), 131.1 (C-

3), 164.2 (C-4), 134.3 (C-5a), 116.6 (C-6), 135.3 (C-7), 127.5 (C-8), 129.7 (C-9), 128.6 (C-9a), 125.8 (C'-1), 138.7 (C'-2), 128.6 (C'-3), 126.3 (C'-4), 130.4 (C'-5), 139.3 (C'-6), 128.7 (C''-1), 134.9 (C''-2), 28.8 (C''-3), 129.6 (C''-4), 4 (C''-5), 128.6 (C''-6); MS (EI): *m/z* (%) 348 (38.29, M+2), 346 (100, M+), 255 (13), 236 (26), 195 (21), 194 (16), 103 (22).

2-(4'-hydroxyphenyl)-4-(3''-chlorophenyl)-1H-1,5-benzodiazepine (6c)

IR (KBr): 3408 cm^{-1} (ν_{NH}), 1616 cm^{-1} ($\nu_{\text{C=N}}$), 3315 cm^{-1} (ν_{OH}); $^1\text{H-NMR}$ (DMSO- d_6): δ_{H} 7.91 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.33 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.71 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.72 (s, 1H, -C=CH-), 3.58 (s, 1H, NH), 7.63 (m, 1H, Har), 7.70 (m, 2H, Har), 7.41 (m, 1H, Har), 7.45 (m, 2H, Har), 7.55 (m, 2H, Har), 10.2 (br, 1H, OH); $^{13}\text{C-NMR}$ (DMSO- d_6): δ_{C} 151.9 (C-2), 131.4 (C-3), 163.8 (C-4), 134.4 (C-5a), 117.1 (C-6), 135.5 (C-7), 127.2 (C-8), 130.1 (C-9), 128.7 (C-9a), 125.8 (C'-1), 138.6 (C'-2), 128.9 (C'-3), 125.8 (C'-4), 130.3 (C'-5), 139.3 (C'-6), 129.6 (C''-1), 128.7 (C''-2), 134.9 (C''-3), 128.6 (C''-4), 129.5 (C''-5), 126.5 (C''-6); MS (EI): *m/z* (%) 348 (30.29, M+2), 346 (100, M+), 255 (17), 236 (22), 195 (24), 194 (13), 103 (26).

2-(4'-hydroxyphenyl)-4-(4''-chlorophenyl)-1H-1,5-benzodiazepine (6d)

IR (KBr): 3412 cm^{-1} (ν_{NH}), 1615 cm^{-1} ($\nu_{\text{C=N}}$), 3345 cm^{-1} (ν_{OH}); $^1\text{H-NMR}$ (DMSO- d_6): δ_{H} 7.95 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.32 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.75 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.70 (s, 1H, -C=CH-), 3.68 (s, 1H, NH), 7.65 (m, 2H, Har), 7.75 (m, 2H, Har), 7.44 (m, 2H, Har), 7.59 (m, 2H, Har), 10.1 (br, 1H, OH); $^{13}\text{C-NMR}$ (DMSO- d_6): δ_{C} 152.3 (C-2), 131.5 (C-3), 164.6 (C-4), 134.4 (C-5a), 116.7 (C-6), 135.4 (C-7), 127.6 (C-8), 129.9 (C-9), 128.7 (C-9a), 125.8 (C'-1), 138.7 (C'-2), 128.4 (C'-3), 126.3 (C'-4), 130.4 (C'-5), 139.5 (C'-6), 126.6 (C''-1), 129.4 (C''-2), 128.7 (C''-3), 134.9 (C''-4), 128.8 (C''-5), 129.6 (C''-6); MS (EI): *m/z* (%) 348 (32.29, M+2), 346 (100, M+), 255 (18), 236 (26), 195 (21), 194 (14), 103 (22).

2-(4'-hydroxyphenyl)-4-(4''-fluorophenyl)-1H-1,5-benzodiazepine (6e)

IR (KBr): 3405 cm^{-1} (ν_{NH}), 1612 cm^{-1} ($\nu_{\text{C=N}}$), 3423 cm^{-1} (ν_{OH}); $^1\text{H-NMR}$ (DMSO- d_6): δ_{H} 7.94 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.36 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.74 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.72 (s, 1H, -C=CH-), 3.71 (s, 1H, NH), 7.5 (m, 2H, Har), 7.57 (m, 2H, Har), 7.39 (m, 2H, Har), 7.44 (m, 2H, Har), 10.1 (br, 1H, OH); $^{13}\text{C-NMR}$ (DMSO- d_6): δ_{C} 152.4 (C-2), 131.5 (C-3), 164.2 (C-4), 134.4 (C-5a), 116.7 (C-6), 135.4 (C-7), 127.6 (C-8), 130.1 (C-9), 128.7 (C-9a), 126.2 (C'-1), 138.7 (C'-2), 128.1 (C'-3), 126.3 (C'-4), 130.4 (C'-5), 139.3 (C'-6), 125.1 (C''-1), 129.4 (C''-2), 114.3 (C''-3), 163.6 (C''-4), 129.4 (C''-5), 114.5 (C''-6); MS (EI): *m/z* (%) 331 (21.65, M+1), 330 (100, M+), 238 (28.25), 236 (20), 195 (13), 103 (22).

2-(4'-hydroxyphenyl)-4-(3''-methoxyphenyl)-1H-1,5-benzodiazepine (6f)

IR (KBr): 3415 cm^{-1} (ν_{NH}), 1615 cm^{-1} ($\nu_{\text{C=N}}$), 3333 cm^{-1} (ν_{OH}); $^1\text{H-NMR}$ (DMSO- d_6): δ_{H} 7.96 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.33 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.75 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.65 (s, 1H, -C=CH-), 3.72 (s,

1H, NH), 7.37 (m, 1H, Har), 7.42 (m, 2H, Har), 7.57 (m, 1H, Har), 7.47 (m, 2H, Har), 7.60 (m, 2H, Har), 10.1 (br, 1H, OH), 3.80 (s, 3H, -CH₃); $^{13}\text{C-NMR}$ (DMSO- d_6): δ_{C} 151.8 (C-2), 130.9 (C-3), 164.5 (C-4), 133.9 (C-5a), 116.7 (C-6), 134.8 (C-7), 127.5 (C-8), 129.9 (C-9), 129.1 (C-9a), 125.4 (C'-1), 139.2 (C'-2), 128.7 (C'-3), 126.3 (C'-4), 130.3 (C'-5), 139.1 (C'-6), 127.5 (C''-1), 114.2 (C''-2), 159.9 (C''-3), 114.1 (C''-4), 127.6 (C''-5), 120.9 (C''-6), 31.6 (C-methoxy); MS (EI): *m/z* (%) 343 (25.3, M+1), 342 (100, M+), 249 (30), 235 (21), 195 (13), 103 (21).

2-(4'-hydroxyphenyl)-4-(4''-methoxyphenyl)-1H-1,5-benzodiazepine (6g)

IR (KBr): 3413 cm^{-1} (ν_{NH}), 1619 cm^{-1} ($\nu_{\text{C=N}}$), 3348 cm^{-1} (ν_{OH}); $^1\text{H-NMR}$ (DMSO- d_6): δ_{H} 7.93 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.35 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.74 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.75 (s, 1H, -C=CH-), 3.65 (s, 1H, NH), 7.31 (m, 2H, Har), 7.40 (m, 2H, Har), 7.52 (m, 2H, Har), 7.44 (m, 2H, Har), 10.16 (br, 1H, OH), 3.86 (s, 3H, -CH₃); $^{13}\text{C-NMR}$ (DMSO- d_6): δ_{C} 152.2 (C-2), 131.3 (C-3), 164.5 (C-4), 134.4 (C-5a), 116.7 (C-6), 135.5 (C-7), 127.8 (C-8), 130.2 (C-9), 128.8 (C-9a), 125.5 (C'-1), 138.7 (C'-2), 128.7 (C'-3), 126.2 (C'-4), 129.8 (C'-5), 139.1 (C'-6), 120.9 (C''-1), 127.6 (C''-2), 114.2 (C''-3), 159.8 (C''-4), 114.3 (C''-5), 127.4 (C''-6), 31.5 (C-methoxy); MS (EI): *m/z* (%) 343 (26, M+1), 342 (100, M+), 249 (28), 235 (24), 195 (13), 103 (21).

2-(4'-hydroxy phenyl)-4-(3'',4''-dimethoxyphenyl)-1H-1,5-benzodiazepine (6h)

IR (KBr): 3404 cm^{-1} (ν_{NH}), 1617 cm^{-1} ($\nu_{\text{C=N}}$), 3325 cm^{-1} (ν_{OH}); $^1\text{H-NMR}$ (DMSO- d_6): δ_{H} 7.97 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.34 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.75 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.70 (s, 1H, -C=CH-), 3.75 (s, 1H, NH), 7.34 (m, 1H, Har), 7.40 (m, 1H, Har), 7.44 (m, 1H, Har), 7.60 (m, 2H, Har), 7.65 (m, 2H, Har), 10.16 (br, 1H, OH), 3.86 (s, 6H, -2CH₃); $^{13}\text{C-NMR}$ (DMSO- d_6): δ_{C} 151.9 (C-2), 131.1 (C-3), 164.2 (C-4), 134.4 (C-5a), 116.9 (C-6), 135.5 (C-7), 127.6 (C-8), 129.8 (C-9), 128.7 (C-9a), 125.9 (C'-1), 138.7 (C'-2), 128.8 (C'-3), 126.2 (C'-4), 130.4 (C'-5), 139.6 (C'-6), 121.8 (C''-1), 115.2 (C''-2), 145.6 (C''-3), 145.5 (C''-4), 115.2 (C''-5), 128.9 (C''-6), 31.4 (C-methoxy), 31.6 (C-methoxy); MS (EI): *m/z* (%) 373 (25, M+1), 372 (100, M+), 279 (30), 235 (18), 195 (11), 103 (23).

2-(4'-hydroxyphenyl)-4-(2''-nitrophenyl)-1H-1,5-benzodiazepine (6i)

IR (KBr): 3409 cm^{-1} (ν_{NH}), 1614 cm^{-1} ($\nu_{\text{C=N}}$), 3340 cm^{-1} (ν_{OH}), 1545 and 1340 cm^{-1} (ν_{NO_2}); $^1\text{H-NMR}$ (DMSO- d_6): δ_{H} 7.94 (dd, 2H, $J_{13} = 2.1$, $J_{12} = 6.8$ Hz), 7.36 (dd, 1H, $J_{13} = 1.8$, $J_{12} = 7.6$ Hz), 6.71 (dd, 1H, $J_{13} = 1.7$, $J_{12} = 7.5$ Hz), 4.72 (s, 1H, -C=CH-), 3.70 (s, 1H, NH), 7.82 (m, 1H, Har), 7.64 (m, 3H, Har), 7.52 (m, 2H, Har), 7.45 (m, 2H, Har), 10.16 (br, 1H, OH); $^{13}\text{C-NMR}$ (DMSO- d_6): δ_{C} 152.3 (C-2), 131.4 (C-3), 164.5 (C-4), 134.1 (C-5a), 116.9 (C-6), 135.4 (C-7), 127.6 (C-8), 129.7 (C-9), 128.8 (C-9a), 125.8 (C'-1), 138.5 (C'-2), 128.6 (C'-3), 126.3 (C'-4), 130.5 (C'-5), 139.6 (C'-6), 123.2 (C''-1), 148.1 (C''-2), 123.3 (C''-3), 129.4 (C''-4), 134.5 (C''-5), 129.5 (C''-6); MS (EI): *m/z* (%) 358 (22, M+1), 357 (100, M+), 312 (21), 264 (21), 235 (31), 195 (22), 194 (15), 103 (24).

Table 1: Physicochemical characterization of the synthesized compounds

Code	Substituent	Yield (%)	M. P. (°C)	Calculated (found)		
				G	H	N
5a	C ₆ H ₅	52	141	74.99 (75.20)	5.03 (5.13)	-
5b	2-Cl C ₆ H ₄	56	156	65.58 (65.76)	4.04 (4.17)	-
5c	3-Cl C ₆ H ₄	48	152	65.58 (65.87)	4.04 (4.15)	-
5d	4-Cl C ₆ H ₄	58	147	65.58 (65.72)	4.04 (4.11)	-
5e	2-F C ₆ H ₄	50	161	69.76 (69.92)	4.29 (4.36)	-
5f	3-OCH ₃ C ₆ H ₄	53	166	71.10 (71.54)	5.22 (5.25)	-
5g	4-OCH ₃ C ₆ H ₄	55	176	71.10 (71.42)	5.22 (5.24)	-
5h	3,4(OCH ₃) ₂ C ₆ H ₃	59	172	67.99 (68.07)	5.37 (5.35)	-
5i	3-NO ₂ C ₆ H ₄	52	145	63.16 (63.43)	3.89 (3.95)	4.91 (4.98)
5j	4-NO ₂ C ₆ H ₄	54	148	63.16 (63.29)	3.89 (3.92)	4.91 (4.95)
6a	C ₆ H ₅	65	204	80.75 (80.84)	5.16 (5.20)	8.97 (8.99)
6b	2-Cl C ₆ H ₄	58	124	72.73 (72.86)	4.36 (4.42)	8.08 (8.11)
6c	3-Cl C ₆ H ₄	56	156	72.73 (72.84)	4.36 (4.40)	8.08 (8.13)
6d	4-Cl C ₆ H ₄	59	214	72.73 (72.77)	4.36 (4.41)	8.08 (8.14)
6e	2-F C ₆ H ₄	54	126	76.35 (76.42)	4.58 (4.63)	8.48 (8.51)
6f	3-OCH ₃ C ₆ H ₄	62	112	77.17 (77.24)	5.30 (5.32)	8.18 (8.21)
6g	4-OCH ₃ C ₆ H ₄	60	133	77.17 (77.19)	5.30 (5.35)	8.18 (8.23)
6h	3,4(OCH ₃) ₂ C ₆ H ₃	63	145	74.18 (74.26)	5.41 (5.44)	7.52 (7.58)
6i	3-NO ₂ C ₆ H ₄	49	109	70.58 (70.60)	4.23 (4.26)	11.76 (11.81)
6j	4-NO ₂ C ₆ H ₄	51	134	70.58 (70.63)	4.23 (4.24)	11.76 (11.83)

2-(4'-hydroxyphenyl)-4-(3''-nitrophenyl)-1H-1,5-benzodiazepine (6j)

IR (KBr): 3412 cm⁻¹ (ν_{NH}), 1617 cm⁻¹ (ν_{C=N}), 3335 cm⁻¹ (ν_{OH}), 1540 and 1334 cm⁻¹ (ν_{NO2}); ¹H-NMR (DMSO-d₆): δ_H 7.95 (dd, 2H, J₁₃ = 2.1, J₁₂ = 6.8 Hz), 7.36 (dd, 1H, J₁₃ = 1.8, J₁₂ = 7.6 Hz), 6.75 (dd, 1H, J₁₃ = 1.7, J₁₂ = 7.5 Hz), 4.78 (s, 1H, -C=CH-), 3.70 (s, 1H, NH), 7.82 (m, 2H, Har), 7.75 (m, 1H, Har), 7.78 (m, 1H, Har), 7.48 (m, 2H, Har), 7.59 (m, 2H, Har), 10.16 (br, 1H, OH); ¹³C-NMR (DMSO-d₆ δ_C: 151.9 (C-2), 131.5 (C-3), 164.4 (C-4), 134.5 (C-5a), 116.9 (C-6), 135.4 (C-7), 127.7 (C-8), 129.8 (C-9), 128.9 (C-9a), 125.6 (C'-1), 139.3 (C'-2), 128.4 (C'-3), 126.3 (C'-4), 130.5 (C'-5), 139.4 (C'-6), 129.4 (C''-1), 123.2 (C''-2), 148.1 (C''-3), 123.3 (C''-4), 129.3 (C''-5), 134.5 (C''-6); MS (EI): m/z (%) 358 (24, M+1), 357 (100, M⁺), 312 (23), 264 (20), 235 (32), 195 (21), 194 (13), 103 (22).

Antioxidant activity

The free radicals scavenging activity of the synthesized compounds was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. [8] Various concentrations of methanolic solution (0.3 mL) of tested compounds were mixed with a methanolic solution containing DPPH radicals (1 mM, 2.7 mL). The mixture was shaken vigorously and left to stand for 2 h in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. Ascorbic acid was used as the standard. Antioxidant activity of the compounds was expressed as the percentage (%). The Radical Scavenging Activity (RSA) was calculated as a percentage of DPPH decoloration using the following equation. The mean values from three independent samples were calculated for each compound.

$$\text{Scavenging Effect (\%)} = 1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Sample}} \times 100$$

RESULTS AND DISCUSSION

Chemistry

All the synthesized compounds were characterized by their physical and spectral data (IR, NMR, MS) and confirmed the structures of the novel compounds. The IR spectra of the chalcones showed the characteristic band for conjugated C=O at 1639-1664 cm⁻¹. In NMR spectra of 3(a-f), a pair of the trans-olefinic proton doublets appeared at 7.54-7.69 ppm and 7.79-7.89 ppm, respectively; with a J value of 14.8-15.6 Hz. The dibromostyryl ketones were confirmed by two doublets appears at 5.67-5.78 ppm. The substituted 1,3-propanediones were confirmed strong IR spectra at 1622 for the ketonic group. The ¹H NMR spectra of the characteristic spectra for active methylene group were located at 3.85 ppm, the aliphatic region. The structure of targeted compound 6(a-j) were confirmed through characteristic peaks. The IR spectra generally indicate the presence of ν_{NH} 3408 cm⁻¹ and ν_{C=N} 1620 cm⁻¹ show the characteristic frequencies of newly formed functional groups. The NMR spectral data of the newly synthesized compound were confirmed by the δ value of 5.71 with J value of 2.7 Hz. The yield, melting point, physicochemical properties and elemental analyses data are described in Table 1.

Antioxidant activity

The free radicals scavenging activity of the compounds and ascorbic acid was measured in terms of hydrogen donating or radical-scavenging ability using the stable DPPH radical. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical scavenging method was followed to check *in vitro* antioxidant activity of the synthesized compounds.

Compounds 6f, 6g, 6i, and 6j were found to possess good antioxidant properties of 59.07%, 41.33%, 68.3%, and 60.4% scavenging activity, respectively at a concentration of 100 μM compared with 79.73% of standard ascorbic acid. Other compounds in the series were found to possess poor antioxidant potential. It was evident from the *in vitro* antioxidant screening that

the presence of methoxy and nitro substituent on *meta* position of the phenyl ring of the benzodiazepine play crucial role for activity.

The current research revealed the potential of 2-(*p*-hydroxyphenyl)-4-(substitutedphenyl)-1*H*-1,5-benzodiazepine as emerging free radical scavengers. The study helped to establish a structure-activity relationship (SAR) where the substitution on the phenyl moiety of the 1,5-benzodiazepine was found to play profound role and influence over biological activity. The compounds 6f, 6g, 6i, and 6j were found to possess good antioxidant properties of 59.07%, 41.33%, 68.3%, and 60.4% scavenging activity, respectively at a concentration of 100 μ M compared with 79.73% of standard ascorbic acid. The research will open new avenues for the development of antioxidant moieties having perspectives in cancer, inflammation, and several other ailments.

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