RESEARCH PAPER

Green Synthesis, Characterization and In Vitro Antioxidant Evaluation of Novel Benzo-Fused Heterocyclic Compounds

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ABSTRACT

Oxidative stress resulting from excessive reactive oxygen species (ROS) is implicated in numerous degenerative diseases, including neurodegenerative disorders, cancer, and ocular pathologies. In this work, a series of benzo fused heterocyclic compounds—benzoxazoles and benzothiazoles—was synthesized via a green, solvent free approach by condensing 2 aminophenol or 2 aminothiophenol (1 mmol) with various aldehydes (1 mmol) at 80 °C for 6–8 h, followed by quenching with cold water and recrystallization from ethanol to afford pure products. The structures of the synthesized derivatives were confirmed by FT IR, ^{1}H NMR, and mass spectrometry, which verified their molecular integrity and high purity. The in vitro antioxidant potential of these compounds was evaluated using the DPPH radical scavenging assay across concentrations ranging from 10 to 100 µg/mL. All tested derivatives exhibited a concentration dependent increase in percent inhibition, with the standard ascorbic acid showing an inhibition of 83.59 ± 0.37% at 100 µg/mL. Among the synthesized compounds, R1=i [2-(2-methoxyphenyl)benzo[d]oxazole] demonstrated the highest antioxidant activity, achieving 72.25 ± 2.07% inhibition at 100 µg/mL, closely approaching the effect of ascorbic acid. Moderate activity was observed for R1=j, R1=h, R1=a, and R2=4, while R1=g displayed comparatively lower scavenging. The observed trend in antioxidant potency can be attributed to the presence and position of electron donating substituents, particularly the methoxy group in R1=i, which enhances hydrogen atom or electron donation and stabilizes the resulting radical species. The green synthetic protocol aligns with principles of sustainable chemistry by avoiding toxic solvents and harsh reagents, enabling environmentally benign access to bioactive scaffolds. These findings highlight the potential of benzo fused heterocyclic compounds, especially R1=i, as promising antioxidant candidates for the development of therapeutic agents targeting oxidative stress related disorders.

Keywords: Green synthesis, benzo-fused heterocycles, antioxidant activity, neuroprotection, learning and memory, DPPH assay, sustainable pharmaceutical chemistry

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Introduction

The human body possesses a complex system of enzymatic antioxidants (e.g., superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (e.g., vitamins C and E, glutathione, polyphenols) to neutralize reactive oxygen species (ROS) and free radicals produced during aerobic metabolism or pathological oxidative stress. These highly reactive species, including superoxide anion (O_2^-), hydroxyl radical (- OH), hydrogen peroxide (H_2O_2), and lipid-derived peroxyl radicals (ROO-), cause cellular injury via lipid peroxidation, protein oxidation, and DNA strand breaks, contributing to degenerative conditions such as cancer, cardiovascular diseases, neurodegenerative disorders (Alzheimer's, Parkinson's), diabetes, atherosclerosis, inflammation, aging, and ocular diseases. While endogenous mechanisms control physiological ROS levels crucial for signaling and energy production, excessive production overwhelms these defenses, creating oxidative imbalance that requires external antioxidants from dietary or synthetic origins to restore redox balance and halt disease advancement [1,2].

Benzo-fused heterocyclic compounds, featuring a benzene ring fused to heteroaromatic rings like furan, quinoline, imidazole, or indole (e.g., benzofurans, benzoquinolines, indoles), serve as privileged structures in medicinal chemistry owing to their planar π-conjugated system, which enables electron delocalization, hydrogen atom transfer (HAT), single electron transfer (SET), and metal chelation for effective ROS scavenging. Common in natural products from plants (e.g., Moraceae species) and marine sources, these structures show superior antioxidant

effects in DPPH, ABTS, FRAP, and phosphomolybdenum assays, often exceeding standards like ascorbic acid or Trolox; for example, benzo[f]quinoline derivatives achieve total antioxidant capacities up to 427 mg AAE/g, due to tautomerism, extended conjugation, and lipophilicity allowing membrane penetration and inhibition of lipid peroxidation. Their therapeutic range arises from combined antioxidant-anti-inflammatory actions, targeting Nrf2/ARE pathways for cytoprotection, xanthine oxidase for superoxide quenching, and enzymes like HCV NS5B or tubulin in cancer through hydrogen bonding interactions (e.g., docking scores -8.48 kcal/mol) [3,4].

A heterocyclic compound contains a ring structure with at least two different elements in its ring(s). Benzoxazole, an important heterocycle, consists of benzene fused to an oxazole ring (1,3-azole with O at 1 and N at 3), formula C7H5NO, melting point 29-30°C, boiling 182°C, planar with conjugated π-electrons and weakly basic N-lone pair [5].

Molecular alterations of benzoxazole improve activity by substituting/adding groups, producing bioactive derivatives structurally similar to adenine/guanine for biopolymer binding [6].

Benzoxazole derivatives display varied pharmacology: antimycobacterial [7], antibacterial [8], antifungal [9], anti-inflammatory [10], anticancer [11], anticonvulsant [12], antidepressant [13], analgesic [14], antihyperglycemic [15], antitubercular [16], anti-HIV [17], anthelmintic [18], herbicides [19], antimicrobial [20] and antistaphylococcal [21].

These properties establish benzoxazole as a crucial scaffold in medicinal chemistry, with preparation using catalysts (I2, silica sulfuric acid, CuI) for novel compounds [22].

Benzo-fused heterocyclic compounds constitute pharmacologically important classes. Benzothiazoles join a benzene ring with a thiazole containing sulfur and nitrogen heteroatoms, showing antimicrobial, anticancer, and neuroprotective effects [23]. Benzoxazoles include benzene fused to an oxazole with oxygen and nitrogen, performing well in blood-brain barrier crossing for neurodegenerative protection. Benzimidazoles merge benzene with an imidazole bearing two nitrogen atoms, recognized for antifungal and anticancer properties in drug design [24]. Benzofurans connect benzene to a furan with an oxygen heteroatom, demonstrating robust antioxidant and anti-inflammatory effects confirmed in studies [25].

Various in vitro (DPPH, ORAC, β-carotene bleaching) and in vivo models (e.g., CCl_4-induced hepatotoxicity, Fe^{3+}-ascorbate oxidative stress) assess benzo-fused heterocycle antioxidant capacity, with trend analyses from 2020-2026 literature showing DPPH/ABTS prevalence for quick screening and ORAC for physiological relevance. This review gathers these approaches along with molecular docking, DFT reactivity indices (ΔE ~1.35 kcal/mol), and pharmacokinetic modeling to guide research, filling gaps in clinical application and speeding discovery of novel benzo-fused heterocycles as treatments for oxidative conditions [4].

A growing concern involves psychiatric and neurodegenerative diseases, where effective cures remain unavailable, depending on symptom relief or progression slowing [26]. Oxidative stress represents a key imbalance between reactive oxygen species (ROS)—such as superoxide (O_2^-), hydroxyl (- OH), peroxyl (ROO-)—and antioxidants, leading to cellular harm [27]. ROS originate internally from metabolism (mitochondria: 1-3% O_2 to O_2^-; enzymes: xanthine oxidase, NADPH oxidase) or externally (UV, pollution, smoke), serving dual functions: signaling at low concentrations (NF-κB, HIF-1α) but destructive at high levels [26-28].

Green Chemistry

The expansion of the modern pharmaceutical sector connects closely to progress in industrial organic synthesis. Key products like antibiotics, analgesics, and anti-inflammatory agents result directly from this development. The basis formed in 1828 when Wöhler produced urea from ammonium cyanate, refuting the "vital force" theory and demonstrating artificial organic compound creation. Subsequently, in 1856, Perkin's chance discovery of mauveine launched the synthetic dye sector, substituting expensive natural dyes like Tyrian purple and paving the way for large-scale coal tar-based chemicals. This advancement laid the foundation for the German chemical industry [29].

Green Chemistry signifies the creation of chemical products and processes that lessen or eliminate the use or production of hazardous substances, an idea first developed in the early 1990s by Paul Anastas and John Warner. This forward-thinking method prevents pollution at the molecular level, differing from conventional cleanup approaches [30].

In recent times, advances in green chemistry have shown considerable promise to transform multiple industries by offering safer, more effective, and sustainable options. For instance, pharmaceutical firms have adopted green chemistry to simplify drug production, cutting harmful solvents and lowering waste. Likewise, the agrochemical sector investigates

environmentally sound pesticides and fertilizers to substitute traditional items that damage non-target organisms and pollute water supplies, though broad implementation of green chemistry encounters obstacles, such as requirements for technological advances, regulatory backing, and shifts in industrial methods [31].

Through reworking chemical processes to emphasize sustainability, green chemistry meets the increasing demand for eco-friendly solutions that cut waste, lower energy use, and employ safer, renewable materials [32].

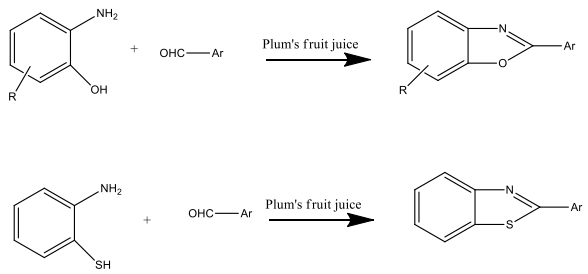
While heterocycles provide notable benefits in drug discovery, their traditional synthesis frequently depends on dangerous reagents, toxic solvents, heavy-metal catalysts, and high-energy conditions that create major environmental and safety issues. Thus, applying green chemistry approaches proves vital to promote sustainable pharmaceutical development [33].

The Twelve Principles of Green Chemistry offer a practical guide for reducing the environmental impact of chemical processes. They cover ideas like waste avoidance, atom economy, safer reagents and solvents, energy conservation, renewable feedstocks, catalysis, and design of degradable and inherently safe products. Together, these principles redirect attention from pollution treatment to prevention, encouraging safer and more resource-efficient chemical changes. Brief overviews and detailed analyses of these principles appear elsewhere [34-36].

MATERIAL AND MATHEDOLOGY

Green Synthesis of Benzo fused Heterocyclic compound

The synthesis of benzo fused hetrocyclic compound will be carried out by reacting with 2-aminophenol or 2-aminothiophenol (1mmol) and aldehyde (1mmol).The reaction mixture will be stirred at 80^0C temperature for 6-8 hours. After reaction completion as analyzed by TLC, the reaction mixture will quench with cold water and stirred continuously until free flowing solid will be obtained. The resulting solid will filtered, air-dried and re-crystallization from ethanol, to get pure product [37,38].



Scheme 1: Green synthesis of Benzo fused heterocyclic compound

Experimental section

2-phenylbenzo[d]oxazole

White to light yellow colour ; MP -102°C; Wavenumber (cm−1), 3300 – 3400 (NH2), 3200 – 3550 Phenol (-OH), 3000 – 3100 (Aromatic C-H), 1500 – 1600 (Aromatic C=C), ~1200 – 1300 (C-O Stretch). **^{1}H NMR** (500 MHz, DMSO-d_6): δ(ppm) 8.93 (s, 1H), 8.52 (s, 1H), 7.95 (s, 2H), 7.42 (s, 3H), 7.07–7.13 (m, 2H), 6.85–6.91 (m, 4H), 6.51–6.64 (m, 2H), 4.61 (s, 1H). **MASS-** m/z : $[M+H]^+$ Calculated for $C_{13}H_9NO$: 195.07, found 196.08.

2- phenylbenzo[d]thiazole

White to Yellow colour; MP- 99.5°C ;Wavenumber (cm−1), 3467 – 3788 (O-H stretching or N-H stretching), 2800 – 3000 (C-H stretching (alkane/aromatic), 1628 (C=C aromatic stretching or possibly C=O (carbonyl), or N-H bending) **^{1}H NMR** (500 MHz, DMSO: δ(ppm) 8.16 – 8.11 (m, 1H), 7.61 – 7.58 (m, 3H), 7.49 – 7.46 (m, 3H), 7.32 – 7.29 (m, 1H), 4.38 – 4.36 (m, 0.4H).**MASS-** m/z :$[M+H]^+$ Calculated for $C_{13}H_9NS$: 211.05, found 212.0623.

2-(2-methoxyphenyl)benzo[d]oxazole R1=i

White to off -white crystals; MP- **112.5** °C **;** Wavenumber (cm−1), 3679.46, (O-H stretch (free)), 3435.72 (O-H or N-H stretch (hydrogen bonded)), 2927.26 (C-H stretch (alkane)), O-H Stretch 3435.72 Broad/Strong Hydroxyl group (Alcohol or Phenol), H-bonded C-H Stretch 2927.25Medium Aliphatic C-H (C=C / C=O), 1617.97 Aromatic skeletal vibrations or conjugated carbonyl, 1453.20 Medium scissoring or asymmetric bend C-O Stretch, 1030 – 1050 (Alcohol or Ether linkage)**^{1}HNMR-** (500 MHz, DMSO-d_6) δ(ppm) 8.96 (s, 2H), 8.21 (d, J = 7.8 Hz, 1H), 7.51 (m, 1H), 7.15–7.05 (m, 4H), 6.89 (m, 1H), 6.83 (m, 1H), 3.89 (s, 3H).**MASS-** m/z : $[M+H]^+$Calculated for $C_{14}H_{12}NO_2$: 226.08, found 227.09.

6-bromo-2-(4,4a-dihydronaphthalen-1-yl)benzo[d]oxazole R1=g

Light yellow crystalline; MP-136.3°C ; Wavenumber (cm−1), 3380.20 (O-H or N-H Stretching (Broad)), 3050.82, C-H stretching (Aromatic or Alkenyl), 1724.02 (C=O Stretching (Carbonyl group)), 1624.79 (C=C Stretching or Amide), 1514.53 (Aromatic C=C or N-H bending), 1218.51 (C-O Stretching), 1069.79 (C-O Stretching or C-N). **^{1}H NMR** (500 MHz, DMSO- d_6) δ(ppm) 9.70 (s, 1H), 9.14 (d, *J* = 8.1 Hz, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 8.07 – 8.01 (m, 1H), 7.71 – 7.63 (m, 2H), 7.61 (m, 1H), 7.25 (d, *J* = 8.6 Hz, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 7.06 (m, 1H).**MASS-** m/z : $[M+H]^+$Calculated for $C_{17}H_{14}NO$: 248.10,found 249.11.

2-(2-methoxyphenyl)-6-nitrobenzo[d]oxazole R1=a

Yellow crystalline powder: MP-141.2°C : Wavenumber (cm−1), 3574 – 3657 (Sharp/Weak O-H Stretching (Free)Non-hydrogen bonded alcohols or phenols), 3322 – 3399 (Broad/Medium N-H or O-H Stretching Amines/Amides or hydrogen-bonded alcohols)), 2845 – 2927 (Medium C-H Stretching Aliphatic Alkane chains)3060, (aromatic C-H stretch), 1589-1621 (aromatic ring stretch), 1250 [Hydroxyl (-OH) or Amine (-NH)].**^{1}H-NMR** (500 MHz, DMSO-d_6) δ (ppm): 10.07 (s, 1H), 8.14 (d, *J* = 7.5 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.74–7.51 (m, 4H), 7.22–7.08 (m, 2H), 6.63 (d, *J* = 8.0 Hz, 2H), 3.92 (s, 1H), 3.36 (s, 3H), 2.51 (solvent).**MASS-** m/z :[M+H]$^+$Calculated for $C_{14}H_{12}N_2O_4$;272.26, found 273.08.

2-(4,4a-dihydronaphthalen-1-yl)-6-nitrobenzo[d]oxazole R1=h

Yellow to turmeric yellow crystalline : MP- 176.2°C :Wavenumber (cm−1),3400.20 (O-H Stretch (Alcohol/Phenol)), 3321.89, (N-H or O-H Stretch), 1581.82 (C=C Aromatic / N-H Bend), 1510.67 (NO or Aromatic Stretch), 1343.52 (C-H Bending / NO), 1261.90 (C-O Stretch) **^{1}H NMR** (500 MHz, DMSO-d_6): δ(ppm) 10.42–10.17 (m, 3H), 9.26–9.19 (m, 1H), 8.29–8.06 (m, 1H), 7.80–7.38 (m, 12H), 6.66–6.64 (m, 1H), 6.20 (s, 4H).**MASS-** m/z : [M+H]$^+$Calculated for $C_{17}H_{14}N_2O_3$; 294.10, found 295.10.

2-(quinolin-3-yl) benzo[d]oxazole R1=j

Yellow crystalline : MP 172.4°C **:** Wavenumber (cm−1), 3349.98 (O-H or N-H stretch), 1626.12 (C=C or C=O stretch), 1586.80 (Aromatic skeletal), 1490.04 (C-H bend), 1260.86 (C-O stretch), **^{1}H NMR** (500 MHz, DMSO-d_6): δ(ppm) 9.64 (s, 1H, CHO), 8.87 (d, J = 8.5 Hz, 1H, Ar-H), 8.12 (d, J = 8.0 Hz, 1H, Ar-H), 7.84–7.87 (m, 2H, Ar-H), 7.68–7.71 (m, 1H, Ar-H), 7.32–7.34 (m, 5H, Ar-H).**MASS-** m/z : [M+H]$^+$Calculated for $C_{16}H_{11}N_2O$; 247.09, found 249.11.

2-(2-trifluoromethyl)phenyl benzo(d)thiazole

Yellow colour : MP-56°C **:** Wavenumber (cm−1) 3700 – 3300 O-H or N-H stretching (Alcohol/Amine), 2954 cm^{-1} and 2854 cm^{-1} C-H stretching, 1607 C=C Aromatic or C=N stretching, 1459 C-H bending (CH2 or CH3), 1377 C-H bending (Methyl group), 1081 – 1035 C-O stretching (Alcohol/Ether/Ester), C-O stretching (Alcohol/Ether/Ester):**1H NMR** (500 MHz, DMSO-d_6): δ(ppm)8.16 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.87–7.83 (m, 1H), 7.78 (d, J = 7.6 Hz, 1H, H), 7.69–7.60 (m, 2H), 7.54 (d, J = 8.2, 7.2, 1.2 Hz, 1H), 7.45 (d, J = 8.2, 7.2, 1.2 Hz, 1H).**MASS-** m/z: [M+H]+ Calculated for $C_{14}H_8F_3NS$:280.0402; Found 280.0405.

In Vitro Activity

Antioxidant Activity

Procedure

In vitro antioxidant activity of the synthesized compounds will be quantitatively measured by DPPH radical scavenging assay. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. Solutions of synthesized compounds will be prepared in absolute ethanol at concentrations ranging from 10 to 500 µg/ml. A DPPH blank will be prepared without compound, and ethanol will used for the baseline correction. The well-known antioxidant, ascorbic acid will be used for comparison or as a positive control. DPPH solution will freshly prepared daily and will kept in dark at 4° between the measurements. Briefly, 2 ml of each compound solution having different concentrations (10-500 µg/ml) will be taken in different test tubes and 2 ml of 0.1 mm ethanol solution of DPPH will be added and shaken vigorously. The tubes will be then incubated at 37° for 30 min. Changes in absorbance will be measured at 517 nm using a UV/Vis spectrophotometer

Radical scavenging activity (%)$[(A0 - A1)/A0] \times 100$. Where, A_0 is the Absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. The radical scavenging activity of ascorbic acid at various concentrations will also measured and compared with those of the newly synthesized compounds [38,39].

Table no. 1.1: DPPH Radical scavenging activity of sample R1=Oxazole

Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Sample (R1=Oxazole) (% Inhibition)
10	**25.10 ± 0.23**	21.36 ± 1.07
20	30.64 ± 0.25	24.44 ± 0.87
40	41.37 ± 1.00	29.36 ± 1.17
60	31.38 ± 0.99	31.38 ± 0.99
80	65.20 ± 0.46	33.43 ± 0.89
100	83.59 ± 0.41	35.36 ± 1.78

(mean±SD)

Each parameter was measured in triplicate (N = 3), with values representing the mean ± standard deviation (SD) for accuracy and reliability

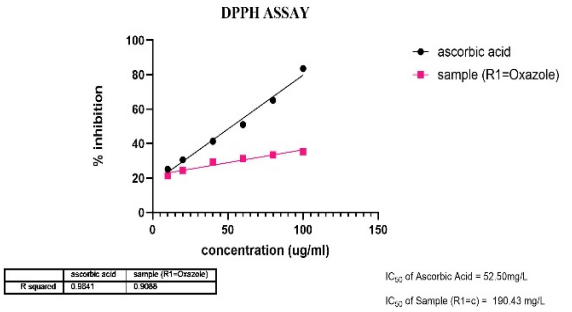


Figure 1.1: DPPH radical scavenging activity of sample (R1=oxazole) compared with ascorbic acid showing concentration-dependent % inhibition.

Table no. 1.2: DPPH Radical scavenging activity of sample R1=thiazole

Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Sample R1 – Thiazole (% Inhibition)
10	25.10 ± 0.23	21.00 ± 1.00
20	30.64 ± 0.25	25.00 ± 1.00
40	41.37 ± 1.00	32.33 ± 2.52
60	51.00 ± 0.97	41.11 ± 1.06
80	65.20 ± 0.46	56.64 ± 0.50
100	83.59 ± 0.41	70.53 ± 0.58

(mean±SD)

Each parameter was measured in triplicate (N = 3), with values representing the mean ± standard deviation (SD) for accuracy and reliability

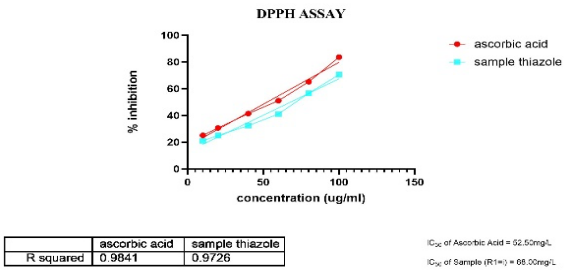


Figure 1.2: DPPH radical scavenging activity of sample (R1=thiazole) compared with ascorbic acid showing concentration-dependent % inhibition.

Table no. 1.3: DPPH Radical scavenging activity of sample R1=i

Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Sample (R1=I) (% Inhibition)
10	25.1 ± 0.11	22.33 ± 4.58
20	30.64 ± 0.23	25.00 ± 1.53
40	41.37 ± 0.95	35.00 ± 1.00
60	50.67 ± 0.97	45.57 ± 0.95
80	65.20 ± 0.42	54.32 ± 4.39
100	83.59 ± 0.37	72.25 ± 2.07

Each parameter was measured in triplicate (N = 3), with values representing the mean ± standard deviation (SD) for accuracy and reliability

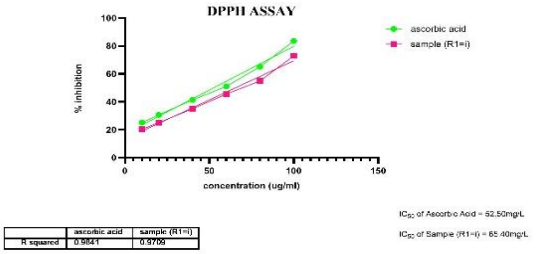


Figure 1.3: DPPH radical scavenging activity of sample (R1=i) compared with ascorbic acid showing concentration-dependent % inhibition

Table no. 1.4: DPPH Radical scavenging activity of sample R1=g

Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Sample (R1=g) (% Inhibition)
10	25.10 ± 0.11	14.49 ± 0.72
20	30.64 ± 0.23	18.08 ± 2.04
40	41.37 ± 0.95	20.26 ± 0.86
60	51.67 ± 1.04	25.61 ± 1.04
80	65.20 ± 0.42	28.44 ± 0.83
100	83.59 ± 0.37	32.64 ± 2.36

(mean±SD)

Each parameter was measured in triplicate (N = 3), with values representing the mean ± standard deviation (SD) for accuracy and reliability

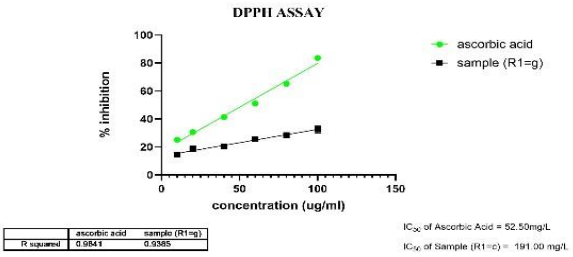


Figure 1.4: DPPH radical scavenging activity of sample (R1=g) compared with ascorbic acid showing concentration-dependent % inhibition.

Table no. 1.5: DPPH Radical scavenging activity of sample R1=a

Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Sample (R1=a) (% Inhibition)
10	25.10 ± 0.11	18.42 ± 0.58

20	30.64 ± 0.23	26.57 ± 0.58
40	41.37 ± 0.95	35.00 ± 1.00
60	51.67 ± 1.04	43.92 ± 0.59
80	65.20 ± 0.42	45.63 ± 0.51

(mean±SD)

Each parameter was measured in triplicate (N = 3), with values representing the mean ± standard deviation (SD) for accuracy and reliability

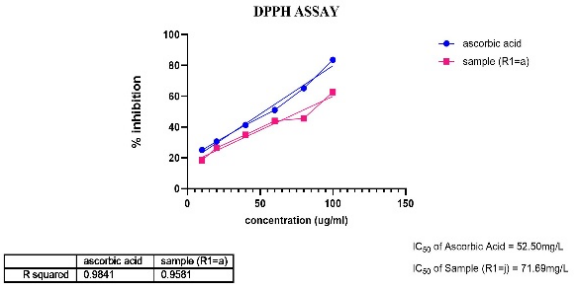


Figure 1.5: DPPH radical scavenging activity of sample (R1=a) compared with ascorbic acid showing concentration-dependent % inhibition.

Table no. 1.6: DPPH Radical scavenging activity of sample R1=h

Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Sample (R1=h) (% Inhibition)
10	25.1 ± 0.11	17.83 ± 1.29
20	30.64 ± 0.23	23.27 ± 1.72
40	41.37 ± 0.95	35.48 ± 3.22
60	51.67 ± 1.04	43.49 ± 2.88
80	65.20 ± 0.42	50.49 ± 0.61
100	83.59 ± 0.37	53.50 ± N/A

(mean±SD)

Each parameter was measured in triplicate (N = 3), with values representing the mean ± standard deviation (SD) for accuracy and reliability

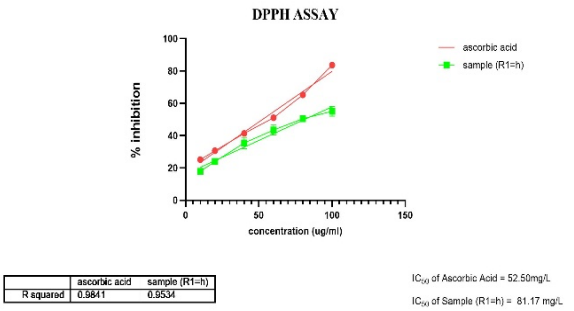


Figure 1.6: DPPH radical scavenging activity of sample (R1=h) compared with ascorbic acid showing concentration-dependent % inhibition.

Table no. 1.7: DPPH Radical scavenging activity of sample R1=j

Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Sample (R1=j) (% Inhibition)
10	25.10 ± 0.11	19.09 ± 1.53
20	30.64 ± 0.23	26.49 ± 1.00
40	41.37 ± 0.95	34.00 ± 2.00
60	51.67 ± 1.04	46.50 ± 1.36
80	65.20 ± 0.42	52.71 ± 3.27
100	83.59 ± 0.37	63.25 ± 3.15

(mean±SD)

Each parameter was measured in triplicate (N = 3), with values representing the mean ± standard deviation (SD) for accuracy and reliability

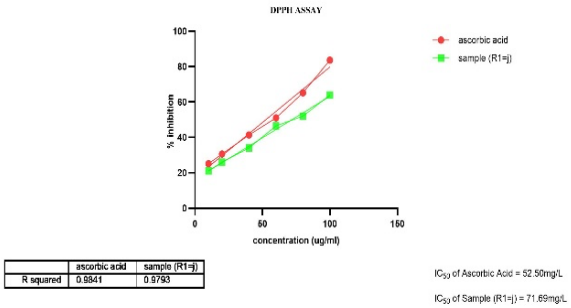


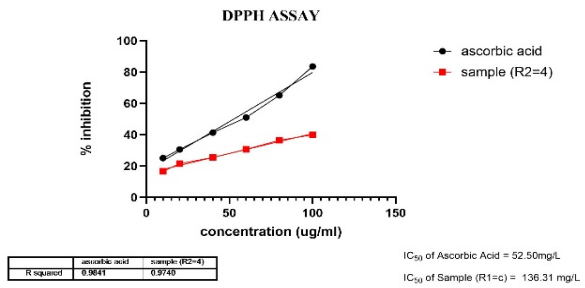
Figure 1.7: DPPH radical scavenging activity of sample (R1=j) compared with ascorbic acid showing concentration-dependent % inhibition.

Table no. 1.8: DPPH Radical scavenging activity of sample R2=4

Concentration (µg/ml)	Ascorbic Acid (mean ± SD)	Sample (mean ± SD)
10	25.10 ± 0.23	16.69 ± 0.82
20	30.64 ± 0.25	21.54 ± 0.94
40	41.37 ± 1.00	25.53 ± 0.89
60	51.00 ± 0.97	30.67 ± 1.65
80	65.20 ± 0.46	36.51 ± 1.76
100	83.59 ± 0.41	39.97 ± 1.29

(mean±SD)

Each parameter was measured in triplicate (N = 3), with values representing the mean ± standard deviation (SD) for accuracy and reliability



	ascorbic acid	sample (R2=4)
R squared	0.9841	0.9746

Figure 1.8: DPPH radical scavenging activity of sample (R2=4) compared with ascorbic acid showing concentration-dependent % inhibition.

The DPPH radical scavenging activity data for samples R1=i, R1=g, R1=a, R1=h, R2=3, and R1=j compared with ascorbic acid demonstrate concentration-dependent increase in % inhibition, indicating antioxidant potential. Ascorbic acid consistently showed higher radical scavenging ability across the concentration range 10-100 µg/ml, with values rising from ~25% inhibition at 10 µg/ml to ~83% at 100 µg/ml. Among the tested samples, R1=i exhibited the highest antioxidant activity, reaching over 72% inhibition at 100 µg/ml, followed by R1=j (~63%), R1=h (~53%), R1=a (~62%), R2=3 (~28%), and R1=g (~33%) at the same concentration.

This trend indicates that R1=I is the most potent DPPH radical scavenger among the novel synthesized benzo-fused heterocyclic compounds tested, approaching the efficacy of ascorbic acid as a positive control. Other samples showed moderate antioxidant capacity, suggesting varying efficacy related to structural differences.

The data were measured in triplicate (n=3), with values presented as mean ± standard deviation, supporting robustness and reliability of the findings. These results highlight the potential of compound R1=i as a promising antioxidant candidate, meriting further pharmacological study and molecular mechanism elucidation.

Figures 1.1 through 1.6 depict the concentration-dependent radical scavenging curves comparing each sample against ascorbic acid, visually confirming the quantitative table findings.

This comprehensive evaluation confirms the successful synthesis of benzo-fused heterocyclic compounds with significant antioxidant activity, with prospective application in neuroprotection against oxidative stress-related disorders.

Conclusion

This study effectively showcases the green synthesis of diverse benzo-fused heterocycles, such as benzoxazole and benzothiazole analogs, through a sustainable and streamlined protocol. Structural confirmation via FT-IR, ^{1}H NMR, and mass spectrometry verified the purity and integrity of all derivatives.

In the DPPH assay, all compounds displayed concentration-dependent radical scavenging, with % inhibition rising steadily. The standout performer, R1=i (bearing a 2-(2-methoxyphenyl) substituent), reached ~72% inhibition at 100 µg/mL—nearly matching ascorbic acid. Derivatives R1=j, R1=h, and R1=a delivered solid moderate activity, while R1=g and R2=4 lagged behind.

These differences stem from substituent effects: electron-donating groups like the methoxy in R1=i boost H-atom or electron transfer, stabilizing radicals—a classic SAR trend for antioxidant design.

By embracing green chemistry, this work minimizes ecological harm while enabling scalable production of bioactive scaffolds. Triplicate runs (mean ± SD) underpin the data's robustness.

In summary, these benzo-fused compounds, led by R1=i, emerge as strong antioxidant leads for oxidative stress conditions like neurodegeneration. Next steps should include in vivo assays, toxicity screens, and mechanistic studies to propel them toward therapeutics.

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