












Synthesis, Characterization, and Evaluation of Biological Activities of Imidazolyl-Isoxazoline Analogue

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Abstract: Synthesis of isoxazoline heterocycle containing benzimidazole moiety with the highest bioactivity using substituted benzaldehyde as starting material is reported in this paper. In the beginning, the precursor benzaldehydes were treated with hydroxylamine hydrochloride to afford respective aldoximes. The resultant compound was subjected to cyclization reaction with allyl chloride in the presence of chloramine-T to afford isoxazoline key intermediate. Finally, benzimidazole was subjected to *S*-alkylation with isoxazoline moiety to afford the title compound with good yield. This method cultivated many advantages like; short reaction time and easy isolation. All the compounds structurally characterized by ¹H NMR, ¹³C NMR, LCMS, IR spectral data, and elemental analysis. Besides, all the synthesized compounds were tested for their antimicrobial and antioxidant activity. The bioactivity was envisioned that the compound **5a** and **5f** exhibited excellent antifungal activity, which may be helpful in developing a lead to inhibit microbes.

Keywords: Isoxazoline; benzimidazole; chloramine-T; allyl chloride; antimicrobial.

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1. Introduction

Isoxazolines [1] are five-membered unsaturated heterocycles containing three carbon atoms, one nitrogen, and oxygen atoms (adjacent) to each other. Heterocycles containing nitrogen and oxygen atom are considered as useful scaffolds for the synthesis of biologically active molecules and liquid crystals [2-4]. Among such classes of compounds, isoxazolines occupy a prime position in bioorganic chemistry for their diverse biological applications. They have been reported to exhibit anticancer [5], antimalarial [6], antimicrobial [7], antioxidant [8], anthelmintic [9], enzyme inhibition [10] and insecticidal activities [11]. Isoxazolines also serves as an essential building block in organic synthesis [12].

The benzo derivative of imidazole: benzimidazole and its derivatives have received considerable attention after it was found to be a part of Vitamin B₁₂ [13]. Benzimidazoles are truly versatile [14], as compounds that have a benzimidazole core are known to have a variety of biological activities such as antiviral [15], anticancer [16], analgesic [17], anthelmintic [18], anti-inflammatory [19], antitumor [20], antibacterial and antifungal activity [21]. Most of the biologically active benzimidazole derivatives are known to be modified at positions 1-, 2- and 5- (or 6-) by various substituents. Modifying the benzimidazole nucleus has thus resulted in anthelmintics [22] (albendazole, mebendazole, thiabendazole) and also many lead compounds in a wide range of therapeutic areas. In particular, mercaptobenzimidazole is used in the synthesis of omeprazole, lansoprazole, and pantoprazole [23], which are protein pump inhibitors and are used as antiulcerous agents in the treatment of stomach and duodenal ulcers [24]. Thus, we have synthesized a series of compounds incorporating two potent heterocycles- isoxazoline and benzimidazole in a single scaffold and to evaluate their biological activity.

2. Materials and Methods

The chemical ingredients, viz., para bromo benzaldehyde, para nitro benzaldehyde, veratraldehyde, anisaldehyde, benzaldehyde, piperonal, allyl chloride were purchased from LOBA Chemie, India. Hydroxylamine hydrochloride was obtained from SRL, India. Sodium acetate, Potassium Carbonate, and Diethyl ether were procured from RANKEM, India. Ethanol was purchased from CHANGSHU YANGYUAN CHEMICAL, China. The proposed structure of the intermediate compounds and that of the final compound were confirmed by the ¹H and ¹³C-NMR spectra obtained using an AGILENT (400 MHz) NMR spectrometer (Deuterated chloroform as solvent procured from SIGMA ALDRICH, USA and tetramethylsilane as internal standard). The following representations denoted the peak types in the spectra: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), and multiplet (m). Infrared spectra (IR) were obtained using a Perkin Elmer spectrophotometer. The ¹H and ¹³C-NMR and LCMS spectra were used for the confirmation of the molecular structure, hydrogen bonding, and the purity of the sample. Antibacterial property and antifungal property of the given compounds was assessed using pure cultures of *Escherichia coli* (MTCC - 443), *Pseudomonas aeruginosa* (MTCC - 2453), *Staphylococcus aureus* (MTCC - 3610), *Bacillus subtilis* (MTCC - 10619), *Enterococcus faecalis* (MTCC - 439), *Aspergillus niger* (MTCC - 3323) and *Candida albicans* (MTCC - 227), procured from Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh, India. Penicillin [25] and Streptomycin were used as standards. The experiment was performed in well plates, and plates were scanned using a microplate reader (Thermo scientific, Multiskan™) at 580-600 nm after the incubation period. Absorbance reading was taken at 517 nm and 541 nm using UV-Vis spectrophotometer (ELICO® SA 165, India) for the assessment of antioxidant activity using DPPH assay and Haemolysis assay, respectively.

3. Results and Discussion

The structures of newly synthesized compounds **3a** and **5a** were confirmed by their analytical and other spectral data. In the ¹H NMR spectra of compound **3a**, three protons (–CH₂ and –CH) of the isoxazoline ring were seen as a doublet of the doublet. Among three protons, –CH is the most deshielded one due to its proximity to oxygen and oxadiazole moiety and it

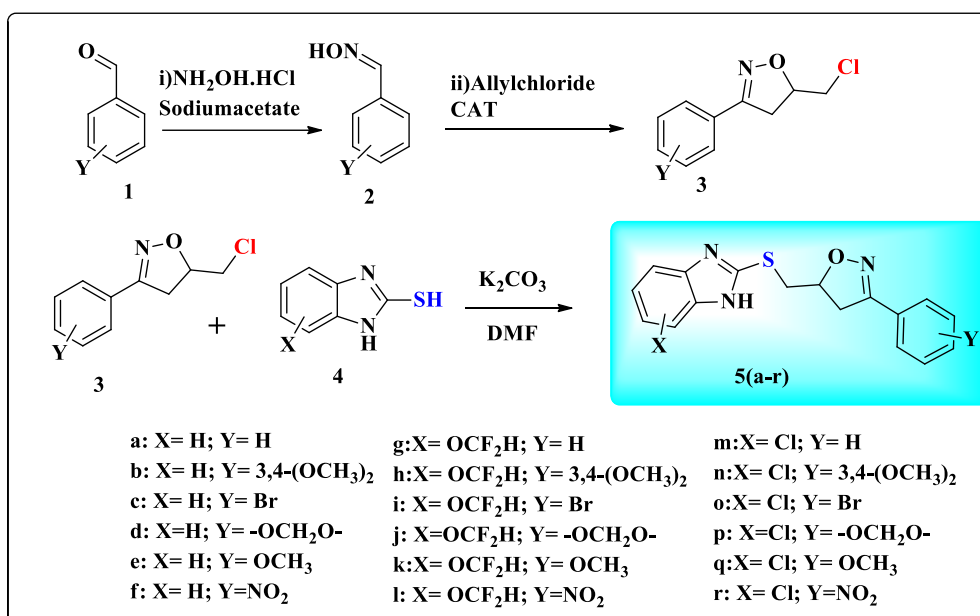
appeared as a doublet of doublets. The methylene protons of the isoxazoline ring ($-\text{CH}_2$), exhibited a typical ABX spin system with $-\text{CH}$ as a doublet of doublets.

The ^1H NMR spectra of the compound **5a** showed peaks at δ 3.10-3.20 (dd, 1H), 3.63-3.66 (dd, 1H), 4.59 - 4.63 (dd, 1H) similarly, the appearance of singlet due to $-\text{CH}_2-$ group of isoxazoline **5a** at δ 5.40 and also disappearance of peaks at δ 12.10 (1H, s) due to $-\text{SH}$ proton confirms the formation of product **5a**.

In ^{13}C NMR spectra, the appearance of peaks at δ 37.8 $-\text{CH}_2$ of and 65.8 $-\text{CH}$ of isoxazoline and also the appearance of a peak at 34.7 of compound **5a** instead of a peak at 45.1 for CH_2Cl of the compound **3a** confirms the formation of the title compound. The mass spectrum of all the compounds showed a molecular ion peak at $[\text{M}+1]$ corresponding to its molecular formula, which confirmed its chemical structure.

3.1. Experimental.

3.1.1. Scheme of synthesis.



Scheme 1. Synthesis of Imidazolyl-isoxazoline derivative.

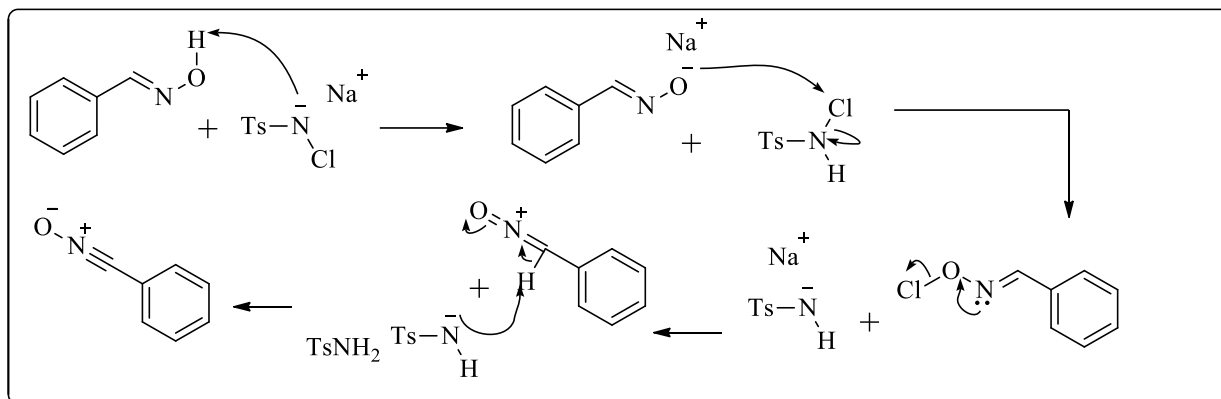
3.1.2. General procedure for the synthesis of aromatic aldoximes.

About 1-1.5g benzaldehyde (1mmol) in 15 ml ethanol was added to a solution of hydroxylamine hydrochloride (1.5mmol) and sodium acetate (1.5mmol) the mixture was heated at 80–90°C for 1h and then it was allowed to cool to room temperature. The precipitate was collected and was taken to the next step.

3.1.3. General procedure for the synthesis of the isoxazoline derivative.

Allyl chloride (1mmol), respective aldoxime (1mmol), and chloramine-T (1.2 mmol) in ethanol (20 mL) were taken in a reaction flask, and it was stirred for 8 hours at room temperature 25°C. The progress of the reaction was monitored by TLC. After completion, Sodium chloride formed was filtered off and washed with ethanol (15 mL). Filtrate and washing were combined, and the solvent was evaporated in a vacuum. The residue was extracted with ether (25 mL \times 3). The extract was washed successively with water (15 mL \times 2),

10% NaOH (15mL ×2), and saturated brine solution (10 mL). The organic layer was dried over anhydrous Na₂SO₄. The crude product was filtered and purified by recrystallization using methanol. The probable mechanism is shown in scheme 2.



Scheme 2. The possible mechanism for the generation of nitrile oxides using chloramine-T.

3.1.4. General procedure for N-alkylation.

Respective isoxazoline derivative (1mmol) and respective benzimidazole derivative (1mmol) and K₂CO₃ (3mmol) and dimethylformamide were stirred for 8 hours at room temperature. The solid product was extracted into the ether layer, and it was dried over anhydrous Na₂SO₄ (**Scheme 1**).

5-[[{(1H-benzo[d]imidazol-2-yl)thio}methyl]-3-phenyl-4,5-dihydroisoxazole (5a):

Prepared from **3a** and **4a**. White solid, Yield: 78%. ¹H NMR (400MHz, DMSO): δ 12.55(s, 1H), 7.65(m, 2H, ArH), 7.46(m, 2H, ArH), 7.10(d, 1H, ArH), 7.09(m, 2H, ArH), 5.10(m, 1H), 3.59(m, 3H), 3.50(m, 2H). ¹³C NMR (100MHz, DMSO): δ 156.2, 147.1, 138.9, 136.0, 131.0, 128.8, 128.2, 123, 115.6, 70.4, 45.0, 39.9. Anal. Calcd for C₁₇H₁₅N₃OS: C - 66.00; H - 4.89; N - 13.58%; Found: C - 65.87; H - 4.76; N - 13.42%. LCMS [M+1]: Calcd for C₁₇H₁₅N₃OS: 310.09, Found: 310.11.

5-[[{(1H-benzo[d]imidazol-2-yl)thio}methyl]-3-(3,4-dimethoxyphenyl)-4,5-

dihydroisoxazole (5b): Prepared from **3b** and **4b**. White solid, Yield: 76%. ¹H NMR (400MHz, DMSO): δ 12.55 (s, 1H), 7.59 (m, 1H, ArH), 7.44 (d, 2H, ArH), 7.40 (m, 1H, ArH), 7.13 (d, 2H, ArH), 6.98 (m, 1H, ArH), 3.9 (m, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.19 - 2.94 (m, 2H), 2.98 - 2.73 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 156.2, 152.1, 149.9, 147.1, 138.9, 127.3, 123.0, 121.3, 114.3, 115.2, 111.9, 70.4, 56.2, 45.0, 39.9. Anal. Calcd for C₁₉H₁₉N₃O₃S: C - 61.77; H - 5.18; N - 11.37%; Found: C - 61.64; H - 5.06; N - 11.16%. LCMS [M+1]: Calcd for C₁₉H₁₉N₃O₃S: 370.44, Found: 370.56.

5-[[{(1H-benzo[d]imidazol-2-yl)thio}methyl]-3-(4-bromophenyl)-4,5-dihydroisoxazole

(5c): Prepared from **3c** and **4c**. White solid, Yield: 75%. ¹H NMR (400MHz, DMSO): δ 12.55 (s, 1H), 7.70 (d, 2H, ArH), 7.64 (d, 2H, ArH), 7.44 (d, 2H, ArH), 7.13 (d, 2H, ArH), 3.9 (m, 2H), 3.19 - 2.98 (m, 2H), 2.94 - 2.73 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 156.2, 147.2, 138.9, 131.7, 129.4, 128.6, 125.4, 123.0, 115.2, 70.4, 45.0, 39.9. Anal. Calcd for C₁₇H₁₄BrN₃OS: C - 52.59; H - 3.63; N - 10.82%; Found: C - 52.15; H - 3.14; N - 10.38%. LCMS [M+1]: Calcd for C₁₇H₁₄BrN₃OS: 389.28, Found: 389.36.

5-[[{(1H-benzo[d]imidazol-2-yl)thio}methyl]-3-(benzo[d][1,3]dioxol-5-yl)-4,5-

dihydroisoxazole (5d): Prepared from **3d** and **4d**. White solid, Yield: 72%. ¹H NMR (400MHz, DMSO): δ 12.55 (s, 1H), 7.59 (m, 1H, ArH), 7.44 (d, 2H, ArH), 7.40 (m, 1H, ArH), 7.13 (d, 2H, ArH), 6.98 (m, 1H, ArH), 6.06 (s, 2H), 3.9 (m, 2H), 3.19 - 2.94 (m, 2H), 2.98 - 2.73 (m,

2H). ¹³C NMR (100MHz, DMSO): δ 156.2, 151.1, 148.9, 147.2, 138.9, 127.3, 123.0, 121.0, 115.3, 114.2, 111.9, 101.2, 70.4, 45.0, 39.9. Anal. Calcd for C₁₈H₁₅N₃O₃S: C - 61.18; H - 4.28; N - 11.89%; Found: C - 61.42; H - 4.21; N - 11.46%. LCMS [M+1]: Calcd for C₁₈H₁₅N₃O₃S: 354.39, Found: 354.41.

5-*[(1H-benzo[d]imidazol-2-yl)thio]methyl*-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (5e): Prepared from **3e** and **4e**. White solid, Yield: 76%. ¹H NMR (400MHz, DMSO): δ 12.57 (s, 1H), 7.57 (d, 2H, ArH), 7.48 (d, 1H, ArH), 7.32 (d, 1H, ArH), 7.01 (d, 2H, ArH), 6.97 (d, 2H, ArH), 5.02 (s, 1H), 3.58 (s, 3H), 3.54 (m, 3H), 3.50 (m, 1H). ¹³C NMR (100MHz, DMSO): δ 162.9, 156.2, 147.1, 138.9, 128.7, 123, 122, 115.4, 114.2, 70.4, 55.8, 45, 39.9. Anal. Calcd for C₁₈H₁₇N₃O₂S: C - 63.70; H - 5.05; N - 12.38%; Found: C - 63.62; H - 5.01; N - 12.14%. LCMS [M+1]: Calcd for C₁₈H₁₇N₃O₂S: 340.10, Found: 340.24.

5-*[(1H-benzo[d]imidazol-2-yl)thio]methyl*-3-(4-nitrophenyl)-4,5-dihydroisoxazole (5f): Prepared from **3f** and **4f**. Yellow solid, Yield: 72%. ¹H NMR (400MHz, DMSO): δ 12.54 (s, 1H), 8.34 (d, 2H, ArH), 8.03 (d, 2H, ArH), 7.44 (d, 2H, ArH), 7.13 (d, 2H, ArH), 3.9 (m, 2H), 3.19 - 2.98 (m, 2H), 2.94 - 2.78 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 156.2, 150.2, 147.1, 138.9, 136.5, 127.7, 123.0, 115.2, 70.4, 45.0, 39.9. Anal. Calcd for C₁₇H₁₄N₄O₃S: C - 57.62; H - 3.98; N - 15.81%; Found: C - 57.43; H - 3.09; N - 15.22%. LCMS [M+1]: Calcd for C₁₇H₁₄N₄O₃S: 355.38, Found: 355.56.

5-*[(6-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio]methyl*-3-phenyl-4,5-dihydroisoxazole (5g): Prepared from **3g** and **4g**. White solid, Yield: 70%. ¹H NMR (400MHz, DMSO): δ 12.73 (s, 1H), 7.57 (s, 1H, ArH), 7.32 (s, 1H), 7.14 (d, 1H, ArH), 6.96 (s, 4H), 5.02 (m, 1H), 3.77 (s, 3H), 3.53 (d, 2H), 3.23 (s, 1H). ¹³C NMR (100MHz, DMSO): δ 167.3, 156.2, 156.1, 147.2, 139.9, 136.0, 131.2, 130.0, 128.2, 116.2, 111.5, 100.8, 70.4, 45.0, 39.9. Anal. Calcd for C₁₈H₁₅F₂N₃O₂S: C - 57.59; H - 4.03; N - 11.19%; Found: C - 57.51; H - 4.01; N - 11.02%. LCMS [M+1]: Calcd for C₁₈H₁₅F₂N₃O₂S: 376.39, Found: 376.45.

5-*[(6-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio]methyl*-3-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazole (5h): Prepared from **3h** and **4h**. White solid, Yield: 71%. ¹H NMR (400MHz, DMSO): δ 12.57 (s, 1H), 7.49 (m, 2H), 7.40 (s, 1H), 7.35 (m, 1H), 7.15 (s, 1H), 6.92 (m, 2H), 5.02 (m, 1H), 3.80 (s, 6H), 3.21 (dd, 1H), 3.11 (m, 2H), 2.97 (dd, 1H). ¹³C NMR (100MHz, DMSO): δ 167.3, 156.1, 156.2, 152.1, 149.9, 147.1, 139.9, 131.2, 127.3, 121.0, 116.2, 114.3, 111.9, 111.5, 100.8, 70.4, 56.3, 45.0, 39.9. Anal. Calcd for C₂₀H₁₉F₂N₃O₄S: C - 55.17; H - 4.40; N - 9.65%; Found: C - 55.16; H - 4.02; N - 9.44%. LCMS [M+1]: Calcd for C₂₀H₁₉F₂N₃O₄S: 436.44, Found: 463.57.

3-(4-bromophenyl)-5-*[(6-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio]methyl*-4,5-dihydroisoxazole (5i): Prepared from **3i** and **4i**. White solid, Yield: 74%. ¹H NMR (400MHz, DMSO): δ 12.55 (s, 1H), 7.70 (d, 2H, ArH), 7.64 (d, 2H, ArH), 7.56 (m, 1H, ArH), 7.36 (s, 2H), 7.12 (m, 1H, ArH), 6.93 (m, 1H, ArH), 3.90 (m, 2H), 3.19 - 2.98 (m, 2H), 2.94 - 2.73 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 167.3, 156.2, 156.1, 147.1, 139.9, 131.7, 131.2, 128.6, 127.6, 125.4, 116.2, 111.5, 100.8, 70.4, 45.0, 39.9. Anal. Calcd for C₁₈H₁₄BrF₂N₃O₂S: C - 47.59; H - 3.11; N - 9.25%; Found: C - 47.32; H - 3.24; N - 9.36%. LCMS [M+1]: Calcd for C₁₈H₁₄BrF₂N₃O₂S: 455.29, Found: 455.34.

3-(benzo[d][1,3]dioxol-5-yl)-5-*[(6-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio]methyl*-4,5-dihydroisoxazole (5j): Prepared from **3j** and **4j**. White solid, Yield: 70%. ¹H NMR (400MHz, DMSO): δ 12.56 (s, 1H), 7.59 (m, 1H, ArH), 7.56 (m, 1H, ArH), 7.40 (m, 1H, ArH), 7.35 (m, 1H, ArH), 6.98 (m, 1H, ArH), 6.93 (m, 1H, ArH), 6.06 (s, 2H), 3.91 (m, 2H), 3.19 - 2.94 (m, 2H), 2.98 - 2.73 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 167.3, 156.2,

156.1, 151.1, 148.9, 147.2, 139.9, 131.2, 127.3, 121.0, 116.3, 114.3, 111.9, 101.3, 100.8, 70.4, 45, 39.9. Anal. Calcd for C₁₉H₁₅F₂N₃O₄S: C - 54.41; H - 3.60; N - 10.02%; Found: C - 54.24; H - 3.42; N - 10.42%. LCMS [M+1]: Calcd for C₁₉H₁₅F₂N₃O₄S: 420.40, Found: 420.57.

5-[(6-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio]methyl]-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (5k): Prepared from **3k** and **4k**. White solid, Yield: 75%. ¹H NMR (400MHz, DMSO): δ 12.57 (s, 1H), 7.90 (d, 2H), 7.49 (m, 3H), 7.40 (s, 1H), 7.35 (m, 1H), 7.10 (d, 1H), 5.02 (m, 1H), 3.83 (s, 3H), 3.20 (dd, 1H), 3.10 (m, 2H), 2.95 (dd, 1H). ¹³C NMR (100MHz, DMSO): δ 167.3, 162.9, 156.2, 156.1, 147.1, 139.9, 131.2, 128.7, 122, 116.2, 114.4, 111.5, 100.8, 70.4, 55.7, 45, 39.9. Anal. Calcd for C₁₉H₁₇F₂N₃O₃S: C - 56.29; H - 4.23; N - 10.36%; Found: C - 56.65; H - 4.23; N - 10.65%. LCMS [M+1]: Calcd for C₁₉H₁₇F₂N₃O₃S: 406.42, Found: 406.48.

5-[(6-(difluoromethoxy)-1H-benzo[d]imidazol-2-yl)thio]methyl]-3-(4-nitrophenyl)-4,5-dihydroisoxazole (5l): Prepared from **3l** and **4l**. Yellow solid, Yield: 72%. ¹H NMR (400MHz, DMSO) δ: 12.56 (s, 1H), 8.34 (d, 2H, ArH), 8.07 (d, 2H, ArH), 7.56 (m, 1H, ArH), 7.36 (s, 2H), 7.12 (m, 1H, ArH), 6.93 (m, 1H, ArH), 3.19 - 2.94 (m, 2H), 2.98 - 2.73 (m, 2H). ¹³C NMR (100MHz, DMSO) δ: 167.3, 156.1, 156.2, 150.2, 147.3, 139.9, 136.5, 131.2, 127.7, 116.2, 111.5, 100.8, 70.4, 45.0, 39.9. Anal. Calcd for C₁₈H₁₄F₂N₄O₄S: C - 51.43; H - 3.36; N - 13.33%; Found: C - 51.43; H - 3.11; N - 13.12%. LCMS [M+1]: 421.39, Found: 420.41.

5-[(6-chloro-1H-benzo[d]imidazol-2-yl)thio]methyl]-3-phenyl-4,5-dihydroisoxazole (5m): Prepared from **3m** and **4m**. White solid, Yield: 71%. ¹H NMR (400MHz, DMSO) δ: 12.52 (s, 1H), 8.40 (s, 1H), 7.90 (d, 2H), 7.53 (m, 4H), 7.49 (d, 1H), 5.05 (m, 1H), 3.17 (dd, 1H), 3.12 (m, 2H), 2.90 (dd, 1H). ¹³C NMR (100MHz, DMSO) δ: 156.2, 147.1, 140.3, 137, 136, 131, 129.2, 128.2, 124.1, 116.6, 115.8, 70.4, 45, 39.9. Anal. Calcd for C₁₇H₁₄ClN₃OS: C - 59.38; H - 4.10; N - 12.22%; Found: C - 59.11; H - 4.01; N - 12.32%. LCMS [M+1]: Calcd for C₁₇H₁₄ClN₃OS: 344.06, Found: 344.01.

5-[(6-chloro-1H-benzo[d]imidazol-2-yl)thio]methyl]-3-(3,4-dimethoxyphenyl)-4,5-dihydroisoxazole (5n): Prepared from **3n** and **4n**. White solid, Yield: 76%. ¹H NMR (400MHz, DMSO): δ 12.56 (s, 1H), 8.36 (s, 1H, ArH), 7.59 (m, 1H, ArH), 7.51 (m, 1H, ArH), 7.14 (m, 1H, ArH), 6.98 (m, 1H, ArH), 3.92 (m, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.19 - 2.94 (m, 2H), 2.98 - 2.73 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 167.3, 156.1, 156.2, 152.1, 149.9, 147.1, 139.9, 131.2, 127.3, 121, 116.2, 114.3, 111.9, 111.5, 100.8, 70.4, 56.3, 45.0, 39.9. Anal. Calcd for C₁₉H₁₈ClN₃O₃S: C, 56.50; H, 4.49; N, 10.40%; Found: C: 56.33, H: 4.43 N: 10.61%. LCMS [M+1]: Calcd for C₁₇H₁₄ClN₃OS: 404.08, Found: 404.15.

3-(4-bromophenyl)-5-[(6-chloro-1H-benzo[d]imidazol-2-yl)thio]methyl]-4,5-dihydroisoxazole (5o): Prepared from **3o** and **4o**. White solid, Yield: 74%. ¹H NMR (400MHz, DMSO): δ 12.56 (s, 1H), 8.36 (m, 1H, ArH), 7.70 (d, 2H, ArH), 7.64 (d, 2H, ArH), 7.51 (m, 1H, ArH), 7.14 (m, 1H, ArH), 3.90 (m, 2H), 3.19 - 2.98 (m, 2H), 2.94 - 2.73 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 156.2, 147.1, 140.3, 137.0, 131.7, 129.4, 129.2, 128.6, 124.1, 115.8, 70.5, 45.0, 39.9. Anal. Calcd for C₁₇H₁₃BrClN₃OS: C - 48.30; H - 3.10; N - 9.94%; Found: C - 48.21; H - 3.12; N - 9.45%. LCMS [M+1]: Calcd for C₁₇H₁₃BrClN₃OS: 424.96, Found: 424.98.

3-(benzo[d][1,3]dioxol-5-yl)-5-[(6-chloro-1H-benzo[d]imidazol-2-yl)thio]methyl]-4,5-dihydroisoxazole (5p): Prepared from **3p** and **4p**. White solid, Yield: 72%. ¹H NMR (400MHz, DMSO): δ 12.56 (s, 1H), 8.36 (d, 1H, ArH), 7.59 (m, 1H, ArH), 7.52 (m, 1H, ArH), 7.40 (m, 1H, ArH), 7.14 (m, 1H, ArH), 6.98 (m, 1H, ArH), 6.06 (s, 2H), 3.19 - 2.94 (m, 2H), 2.98 - 2.73 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 156.2, 151.1, 148.9, 147.1, 140.3, 137.0, 129.2, 127.3, 124.1, 121.1, 116.6, 115.8, 114.4, 111.9, 101.2, 70.4, 45, 39.9. Anal. Calcd for

C₁₈H₁₄ClN₃O₃S: C - 55.74; H - 3.64; N - 10.83%; Found: C - 55.44; H - 3.46; N - 10.23%. LCMS [M+1]: Calcd for C₁₈H₁₄ClN₃O₃S: 388.84, Found: 388.91.

5-[[{(6-chloro-1H-benzo[d]imidazol-2-yl)thio}methyl]-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (5q): Prepared from **3q** and **4q**. White solid, Yield: 76%. ¹H NMR (400MHz, DMSO): δ 12.56 (s, 1H), 7.57 (d, 1H, ArH), 7.10 (m, 3H, ArH), 6.97 (d, 2H, ArH), 5.02 (m, 1H), 3.75 (s, 1H, ArH), 3.54 (m, 3H), 3.50 (m, 1H). ¹³C NMR (100MHz, DMSO): δ 162.5, 156.2, 147.1, 140.3, 137.0, 129.2, 128.7, 124.1, 122.1, 116.6, 115.8, 114.4, 70.4, 55.6, 45, 39.9. Anal. Calcd for C₁₈H₁₆ClN₃O₂S: C - 57.83; H - 4.31; N - 11.24%; Found: C - 57.21; H - 4.27; N - 11.07%. LCMS [M+1]: Calcd for C₁₈H₁₆ClN₃O₂S: 374.07, Found: 374.11.

5-[[{(6-chloro-1H-benzo[d]imidazol-2-yl)thio}methyl]-3-(4-nitrophenyl)-4,5-dihydroisoxazole (5r): Prepared from **3r** and **4r**. Yellow solid, Yield: 71%. ¹H NMR (400MHz, DMSO): δ 12.56 (s, 1H), 8.36 (m, 1H ArH), 8.34 (d, 2H, ArH), 8.04 (d, 2H, ArH), 7.52 (m, 1H, ArH), 7.12 (m, 1H, ArH), 3.91 (m, 2H), 3.19 - 2.98 (m, 2H), 2.94 - 2.78 (m, 2H). ¹³C NMR (100MHz, DMSO): δ 156.2, 150.2, 147.1, 140.3, 137.0, 136.5, 129.2, 127.7, 124.1, 116.7, 115.7, 70.4, 45, 39.9. Anal. Calcd for C₁₇H₁₃ClN₄O₃S: C - 52.51; H - 3.37; N - 14.41%; Found: C - 52.43; H - 3.16; N - 8.14%. LCMS [M+1]: Calcd for C₁₇H₁₃ClN₄O₃S: 389.83, Found: 389.92.

3.2. Biological Assay.

3.2.1 Assessment of Antioxidant Activity Using DPPH Assay.

In vitro, antioxidant activities of all the synthesized compounds were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, which is a rapid and convenient technique for screening the antioxidant activities of the antioxidants. Standard was taken as 100 % inhibition, and our compounds were compared with it. A higher percent value indicated greater antioxidant activity. Percent of inhibition values higher than 80% usually implied effective activities in antioxidant properties. The results were shown in **Graph 1**. Most of the synthesized compounds exhibited potent antioxidant activities. Compounds **5a**, **5b**, **5c**, **5d**, **5g**, **5k**, **5n**, and **5o** showed excellent radical scavenging activities with percent inhibition above 70%. Among all compounds **5c**, **5g** and **5o** showed excellent antioxidant activities with striking antioxidant values of higher than 80% inhibition, demonstrating greater antioxidant activities of these compounds in the assay. Based on the above observation, compounds having no substitution on benzimidazole moiety were found to be the most potent antioxidants than the compounds with electron-withdrawing Cl and OCF₂H substituents whereas substitution of electron releasing groups at phenyl ring of isoxazoline showed least antioxidant activity and the activity enhanced with substitution of electron-withdrawing groups. From the above observation, we can easily notice that the electron releasing group on benzimidazole and electron-withdrawing group on the phenyl group of isoxazoline were found to be good antioxidants.

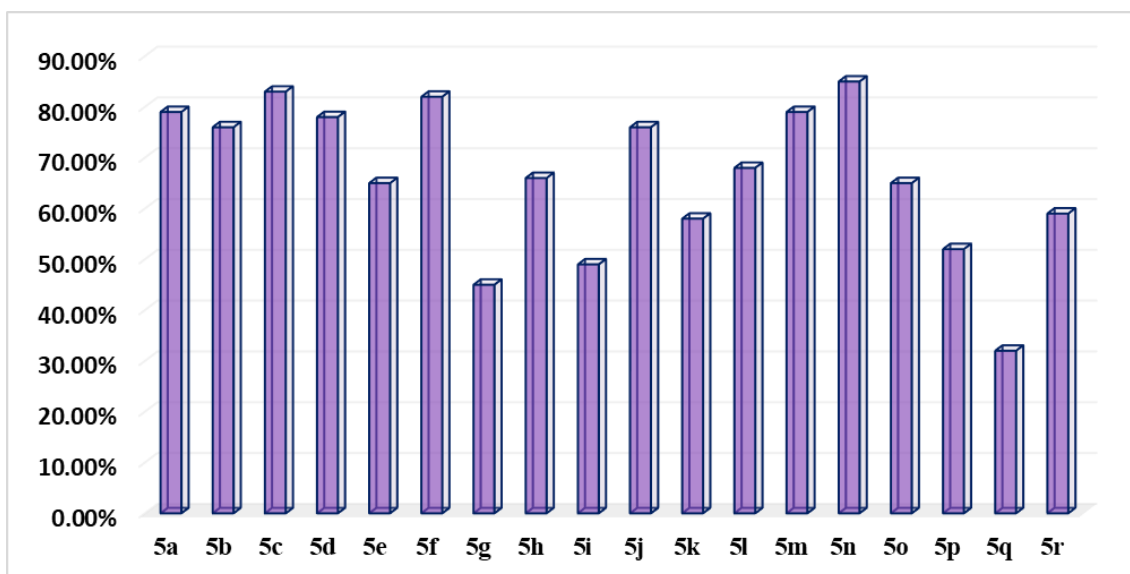
3.2.2 Assessment of Antimicrobial Activity.

All the synthesized compounds were evaluated for their antimicrobial activity using both Gram-negative (*E.coli*, *P.aeruginosa*, *S.typhimurium*), Gram-positive (*B.subtilis*, *E.faecalis*) bacteria, and Fungi (*A.niger*, *C.albicans*). A considerable number of compounds have been recognized exhibiting excellent to moderate inhibitory activity compared to standard percentage antimicrobial activity was calculated (%) (**Table 1**). Compounds without electron-

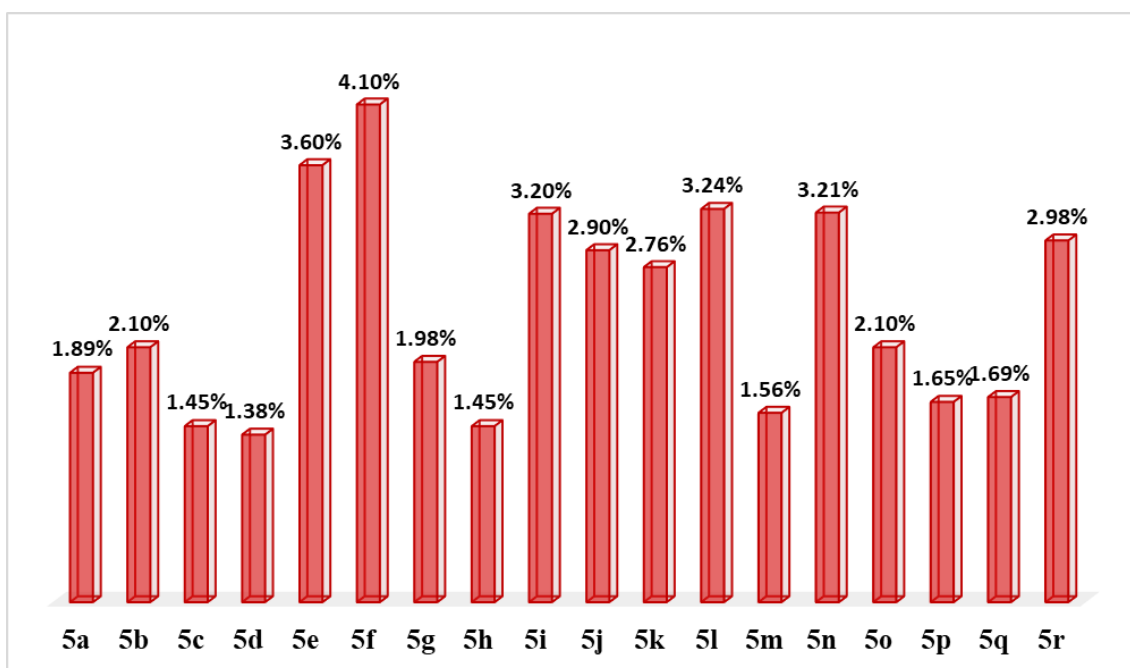
withdrawing substituents on the benzimidazole ring exhibited better antimicrobial activity than the compounds with substitutions. Compounds with electron-withdrawing groups on the phenyl ring of isoxazoline **5a**, **5c**, **5f**, **5g**, **5l**, **5m**, and **5o** showed excellent activity with lower MIC against bacterial strains. Other compounds **5a**, **5c**, **5f**, **5g**, **5h**, **5i**, **5m**, and **5o** possessed good antifungal properties with lower MIC values. From the above discussion, **5a** and **5f** were found to exhibit excellent antimicrobial activity.

3.2.3 Haemolysis Assay.

All the synthesized compounds were evaluated for their Haemolysis activity using Fresh chicken blood. Compounds **5a**, **5c**, **5d**, **5h**, **5m**, **5p**, and **5q** have shown lower hemolysis on blood (**Graph 2**).



Graph 1. Antioxidant activity comparison of synthesized compounds (5a-5r).



Graph 2. Hemolysis assay comparison of synthesized compounds (5a-5r).

Table 1. Percentage antimicrobial activity (%).

Entry	Bacteria					Fungi	
	Gram-negative			Gram-positive		<i>A.niger</i>	<i>C.albicans</i>
	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>S.typhimurium</i>	<i>B.subtilis</i>	<i>E.faecalis</i>		
5a	64	69	76	65	75	78	67
5b	76	53	46	46	64	73	76
5c	54	47	34	47	56	76	34
5d	82	76	72	77	74	56	66
5e	67	83	56	75	34	65	76
5f	49	52	76	65	77	35	48
5g	78	43	65	83	82	67	82
5h	43	68	73	75	56	74	56
5i	66	75	46	85	78	65	76
5j	72	58	54	67	54	56	56
5k	45	67	65	74	78	54	57
5l	82	86	79	45	34	68	65
5m	78	75	75	56	77	82	56
5n	73	65	65	33	67	67	71
5o	52	57	67	48	45	54	46
5p	55	85	46	71	65	45	73
5q	65	67	46	76	36	65	65
5r	80	56	64	43	81	56	55
Std	100	100	100	100	100	100	100

Std: Penicillin and Streptomycin

4. Conclusions

It is evident from the results that the compounds bearing electron-withdrawing groups Br and NO₂ in phenyl ring of isoxazoline moiety and without substitution on benzimidazole moiety are better antioxidant agents. In conclusion, we have designed and synthesized three series of benzimidazole derived 2-isoxazolines derivatives as potential antioxidants, antibacterial agents with different groups in the phenyl ring. Of all the compounds synthesized, compounds **5a**, **5c**, and **5f** exhibited stronger radical scavenging activity and antimicrobial activity with lower hemolysis.

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Conflicts of Interest

The authors declare no conflict of interest.

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