Research Article

Synthesis and Biological Evaluation of Novel 5,7-Dichloro-1,3-benzoxazole Derivatives

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A new class of 5,7-dichloro-1,3-benzoxazole derivatives **4–11** were synthesized by fusing 5,7-dichloro-2-hydrazino-1,3-benzoxazole **3** nucleus with aliphatic acids, active methylene compounds, and with selected esters to form heterocyclic ring systems like 1,2,4-triazoles, pyrazoles, and triazine moieties. The compound **3** on diazotization reaction affords the tetrazole compound. The synthesized compounds were characterized by ¹H NMR, IR, Mass, and ¹³C NMR spectral data and screened for cytotoxic, antimicrobial, antioxidant, and antilipase activities. The compounds **4**, **5**, and **8** have shown significant antimicrobial activities, whereas compounds **6** and **8** have been emerged as leading cytotoxic agents. The compounds **9**, **10**, and **11** were found to be strong enzyme inhibitors.

1. Introduction

Wide-spread interest in the chemistry of benzoxazole derivatives in large number of products has attracted the researchers because of their potential applications in medicinal field. Benzoxazole derivative constitutes an important class of heterocyclic compounds and shows various types of biological properties such as antiviral, antineoplastic, anti-HIV-1, antitubercular, anthelmintic, antimicrobial, and antifungal activities [1, 2]. In the last few years, 2-substituted benzoxazole derivatives have been studied extensively for their biological importance.

The thiadiazole-containing 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles were seldom reported with biological activity. The chemistry of triazoles and their derivatives has always been highlighted in study of lead compound discovery study of their variety of biological activities including antifungal, antibacterial, antitubercular, anticancer, and analgesic activities [3–10]. In support for our study, pyrazoles are also an interesting group of compounds, many of which

possess broad spectrum of pharmacological properties. The synthetic analogues of pyrazoles have been known to exhibit industrial, agricultural, and biological applications [11–18]. In the present work, our group is focused on synthesis of dichlorobenzoxazole derivatives to form various fused heterocyclic ring systems like 1,2,4-triazoles, pyrazoles, tetrazole and triazine moieties with benzoxazole nucleus and screened for some selected biological activities.

2. Experimental Section

2.1. Chemistry. Melting points were recorded on electrothermal melting point apparatus and are uncorrected. ¹H NMR and ¹³C NMR spectra were recorded on Bruker 400 MHz spectrometer in Indian Institute of Science, Bangalore, Karnataka, India. The chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal standard. LC-MS were obtained using C 18 column on Shimadzu, LCMS 2010A, Japan. The Fourier Transform Infrared (FT-IR) spectra of the compounds were taken in KBr pellet (100 mg)

TABLE 1: Physical data of compounds 4–11.

Compound	Molecular formula	Molecular weight	M.P. (°C)	Yield (%)
4	C ₈ H ₃ Cl ₂ N ₃ O	228.04	190–192	93
5	C ₉ H ₅ Cl ₂ N ₃ O	242.06	218–219	95
6	C ₇ H ₂ Cl ₂ N ₄ O	229.02	68–69	87
7	C ₁₁ H ₇ Cl ₂ N ₃ O ₂	284.09	135–138	89
8	C ₁₀ H ₆ Cl ₂ N ₄ O ₂	285.08	162–165	79
9	C ₁₂ H ₉ Cl ₂ N ₃ O	282.10	130–132	90
10	C ₈ H ₃ Cl ₂ N ₃ O ₂	244.03	185–187	85
11	C ₉ H ₃ Cl ₂ N ₃ O ₃	272.04	160–161	84

using Shimadzu (FT-IR) spectrophotometer. The elemental analysis was carried out using VarioMICROV1.7.0 (Elemental Analysersysteme GmbH) instrument. The column chromatography was performed using silica gel (230–400 mesh). Silica gel GF254 plates from Merck were used for TLC and spots located either by UV or dipping in potassium permanganate solution. The chemicals were purchased from Sigma-Aldrich Co. and from SD Fine Chemicals. The solvents for column chromatography were of reagent grade and were purchased from commercial source. The yields are of purified compounds and are not optimized. The yield, melting point, molecular formula, and molecular weight of the compounds were recorded in Table 1.

2.1.1. Preparation of 5,7-Dichloro-1,3-benzoxazole-2-thiol (1). Methanol (50 mL) and potassium hydroxide (1.1eq) were stirred for 10 min and followed by slow addition of carbon disulphide (1.1eq) at room temperature. To the reaction mass, 2-amino-4,6-dichlorophenol (1eq) was added with stirring. The reaction mass was refluxed for 6 hr on water bath. The completion of the reaction was monitored by TLC. The reaction mass was poured on to ice cold water and acidified with glacial acetic acid (pH 6). The obtained solid was filtered, dried, and recrystallized using ethanol.

Yield (95%), M.P. 198–199°C.

2.1.2. Preparation of 5,7-Dichloro-2-(ethylthio)-1,3-benzoxazole (2). To a mixture of sodium hydroxide (10 mmol) and compound 1 (10 mmol) in di-methyl sulphoxide (10 mL), ethyl iodide (10 mmol) was added dropwise. The reaction mass was stirred about 1 hr, and the completion of the reaction was confirmed by TLC. The reaction mass was poured on to ice cold water, filtered the obtained solid, dried, and recrystallized using hexane.

Brown color solid, yield (90%), M.P. 38–39°C; IR (KBr) cm⁻¹: 2974 (CH alkyl), 1608 (C=N), 1491 (C=C), 756 (C–Cl); ¹H NMR (CHCl₃) ppm: δ 1.4 (t, 3H, CH₃), δ 3.26 (q, 2H, CH₂), δ 7.1 (s, 1H, Ar), δ 7.4 (s, 1H, Ar H); ¹³C NMR (CHCl₃): δ 13.22 (CH₃), 14.29 (CH₂), 108.2, 116.5, 122.4, 128.3, 129.62, 140.7, 153.2; MS: (M⁺) *m/z* 248, *m/z*⁺² 250, *m/z*⁺⁴ 252; Anal.Calcd for C₉H₇Cl₂NOS (248.1): C 43.56, H 2.84, N 5.64; found: C 43.55, H 2.82, N 5.62.

2.1.3. Preparation of 5,7-Dichloro-2-hydrazino-1,3-benzoxazole (3). The compound 3 (10 mmol) was taken in a round-bottomed flask and treated with hydrazine hydrate (15 mmol) in ethanol (15 mL) and refluxed for 3 hr. The reaction mixture was cooled and filtered. The obtained solid was recrystallized from ethanol.

Brown color, pluffy solid, yield (70%), M.P. 220–222°C; IR (KBr) cm⁻¹: 3349 (NH₂), 3283 (NH), 1617 (C=N), 1453 (C=C), 771 (C–Cl); ¹H NMR (DMSO-*d*₆) ppm: δ 4.6 (s, 2H, NH₂ D₂O exchangeable), δ 7.18 (s, 1H, Ar H), δ 7.29 (s, 1H, Ar H), δ 9.3 (s, 1H, NH D₂O exchangeable); ¹³C NMR (DMSO-*d*₆): δ 108.9, 116.4, 123.4, 130.09, 130.66, 139.2, 153.8; MS: (M⁺) *m/z* 218, *m/z*⁺² 220, *m/z*⁺⁴ 222; Anal.Calcd for C₇H₅Cl₂N₃O (218.04): C 38.56, H 2.31, N 19.27; found: C 38.53, H 2.30, N 19.25.

2.1.4. Preparation of 6,8-Dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole (4). The mixture of compound 3 (10 mmol), formic acid (10 mL), and catalytic amount of concentrated hydrochloric acid was heated under reflux for 5 hr. The reaction mixture was allowed to cool to room temperature and poured onto water (50 mL). The obtained solid was filtered, washed with ethanol, dried, and recrystallized using methanol.

Half white solid; IR (KBr) cm⁻¹: 1627 (C=N), 1459 (C=C), 748 (C–Cl); ¹H NMR (DMSO-*d*₆) ppm: δ 7.17 (s, 1H, Ar H), δ 7.35 (s, 1H, Ar H), δ 12.18 (s, 1H, =CH); ¹³C NMR (DMSO-*d*₆): δ 111.2, 115.5, 117.3, 122.9, 130.2, 132.23, 141.9, 155.9; MS: (M⁺) *m/z* 228, *m/z*⁺² 230, *m/z*⁺⁴ 232; Anal.Calcd for C₈H₃Cl₂N₃O (228.04): C 42.14, H 1.33, N 18.43; found: C 42.11, H 1.30, N 18.42.

2.1.5. Preparation of 6,8-Dichloro-3-methyl[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole (5). The compound 3 (10 mmol) was dissolved in glacial acetic acid (20 mL) and stirred under reflux for 10 hr. The reaction mixture was allowed to cool to room temperature and poured onto water (50 mL). The solid thus formed was collected by filtration. The crude solid was washed with ethanol (20 mL), dried, and recrystallized from methanol.

Buff color solid; IR (KBr) cm⁻¹: 2990 (CH₃), 1630 (C=N), 1465 (C=C), 773 (C–Cl); ¹H NMR (DMSO-*d*₆) ppm: δ 1.9 (s, 1H, CH₃), δ 7.3 (s, 1H, Ar H), δ 7.4 (s, 1H, Ar H); ¹³C NMR (DMSO-*d*₆): δ 29.79 (CH₃), 116.15, 117.4, 124.82, 130.9, 132.5, 143.04, 145.21, 158.71; MS: (M⁺) *m/z* 242, *m/z*⁺² 244, *m/z*⁺⁴ 246; Anal.Calcd for C₉H₅Cl₂N₃O (242.06): C 44.66, H 2.08, N 17.39; found: C 44.62, H 2.06, N 17.41.

2.1.6. Preparation of 5,7-Dichlorotetrazolo[5,1-*b*][1,3]benzoxazole (6). A solution of sodium nitrite (1.04 g, 15 mmol) in 10 mL of water was added dropwise to an ice cold solution of compound 3 (10 mmol) in acetic acid (10 mL). The reaction mixture was kept for stirring about an hour on ice bath at –5°C. The reaction mixture was allowed to stand for overnight at room temperature. Then, it was poured onto water (100 mL). The solid precipitated was filtered off and crystallized from methanol.

Light yellow color solid; IR (KBr) cm^{-1} : 1640 (C=N), 1450 (C=C), 740 (C-Cl); ^1H NMR (CHCl_3) ppm: δ 7.2 (s, 1H, Ar H), δ 7.39 (s, 1H, Ar H); ^{13}C NMR (CHCl_3): δ 115.32, 116.9, 125.12, 130.2, 141.41, 145.4, 156.34; MS: (M^+) m/z 229, m/z^{+2} 231, m/z^{+4} 233; Anal. Calcd for $\text{C}_7\text{H}_2\text{Cl}_2\text{N}_4\text{O}$ (229.02): C 36.71, H 0.88, N, 24.46; found: C 36.69, H 0.83, N 24.42.

2.1.7. Preparation of 2-(5,7-Dichloro-1,3-benzoxazol-2-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (7). The compound **3** (10 mmol) and ethyl acetoacetate (0.7 g, 10 mmol) mixture was stirred under reflux in absolute ethanol (30 mL) for 8 hr. The reaction mixture was allowed to cool to room temperature, poured onto cold water (50 mL). The precipitate was filtered off, dried, and crystallized from methanol.

Light brown color solid; IR (KBr) cm^{-1} : 2998 (alkyl), 1720 (C=O), 1630 (C=N), 1453 (C=C), 726 (C-Cl); ^1H NMR (DMSO- d_6) ppm: δ 3.3 (s, 2H, CH_2), δ 3.37 (s, 3H, CH_3), δ 7.28 (s, 1H, Ar H), δ 7.4 (s, 1H, Ar H); ^{13}C NMR (DMSO- d_6): δ 13.5 (CH_3), 28 (CH_2), 110.25, 115.33, 120.34, 124.4, 130.5, 142.2, 144.12, 156.43, 162.24; MS: (M^+) m/z 284, m/z^{+2} 286, m/z^{+4} 288; Anal. calcd for $\text{C}_{11}\text{H}_7\text{Cl}_2\text{N}_3\text{O}_2$ (284.09): C 46.50, H 2.48, N 14.79; found: C 46.49, H 2.46, N 14.78.

2.1.8. Preparation of 5-Amino-2-(5,7-dichloro-1,3-benzoxazol-2-yl)-2,4-dihydro-3H-pyrazol-3-one (8). To a freshly prepared warmed ethanolic sodium ethoxide solution, compound **3** (10 mmol) and ethyl cyanoacetate (1.3 g, 10 mmol) mixture was added. The mixture was stirred under reflux for 8 hr. The reaction mixture was allowed to cool to room temperature, then poured onto cold water and neutralized with acetic acid. The solid product was filtered off, washed with water, ethanol, dried, and crystallized from methanol.

Light brown color solid; IR (KBr) cm^{-1} : 3295 ($-\text{NH}_2$), 1730 (C=O), 1637 (C=N), 1444 (C=C), 778 (C-Cl); ^1H NMR (DMSO- d_6) ppm: δ 3.4 (s, 2H, CH_2), δ 6.37 (s, 2H, $-\text{NH}_2$ D_2O exchangeable), δ 7.2 (s, 1H, Ar H), δ 7.3 (s, 1H, Ar H); ^{13}C NMR (DMSO- d_6): δ 28.2 (CH_2), 111.41, 114.71, 121.01, 124.5, 129.6, 142.9, 144.76, 157.15, 161.93; MS: (M^+) m/z 286, m/z^{+2} 288, m/z^{+4} 290; Anal. Calcd for $\text{C}_{10}\text{H}_6\text{Cl}_2\text{N}_4\text{O}_2$ (285.08): C 42.13, H 2.12, N 19.65; found: C 42.10, H 2.10, N 19.64.

2.1.9. Preparation of 5,7-Dichloro-2-(3,5-dimethyl-1H-pyrazol-1-yl)-1,3-benzoxazole (9). The compound **3** (10 mmol) and acetyl acetone (10 mmol) mixture in absolute ethanol (30 mL) was stirred under reflux for 5 hr. The reaction mixture was allowed to cool at room temperature and poured onto water. The precipitate was filtered off, dried, and crystallized from methanol.

Light brown color solid; IR (KBr) cm^{-1} : 2998 (alkyl), 1637 (C=N), 1447 (C=C), 750 (C-Cl); ^1H NMR (DMSO- d_6) ppm: δ 3.0 (s, 3H, CH_3), δ 3.04 (s, 3H, CH_3), δ 6.3 (s, 1H, =CH), δ 7.63 (s, 1H, Ar H), δ 7.83 (s, 1H, Ar H); ^{13}C NMR (DMSO- d_6): δ 13.7 (CH_3), 13.92 (CH_3), 111.1, 116.32, 118.17, 124.8, 130.8, 142.9, 143.83, 144.4, 154.38, 155.24; MS: (M^+) m/z 282,

m/z^{+2} 284, m/z^{+4} 286; Anal. Calcd for $\text{C}_{12}\text{H}_9\text{Cl}_2\text{N}_3\text{O}$ (282.1): C 51.09, H 3.22, N 14.89; found: C 51.08, H 3.21, N 14.88.

2.1.10. Preparation of 6,8-Dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3(2H)-one (10). The compound **3** (10 mmol) and Ethyl chloroformate (10 mmol) mixture in ethanol was refluxed for 3 hr. The reaction mass was cooled to room temperature and filtered the solid. The obtained solid was recrystallized using methanol.

Half white color solid; IR (KBr) cm^{-1} : 3200 (NH), 1725 (C=O), 1622 (C=N), 1448 (C=C), 779 (C-Cl); ^1H NMR (DMSO- d_6) ppm: δ 7.2 (s, 1H, Ar H), δ 7.29 (s, 1H, Ar H), δ 8.18 (s, 1H, =NH); ^{13}C NMR (DMSO- d_6): δ 113.81, 119.31, 123.29, 130.21, 142.2, 144.54, 155.41, 163.55; MS: (M^+) m/z 244, m/z^{+2} 246, m/z^{+4} 248; Anal. Calcd for $\text{C}_8\text{H}_3\text{Cl}_2\text{N}_3\text{O}_2$ (244.03): C 39.37, H 1.24, N 17.22; found: C 39.35, H 1.23, N 17.20.

2.1.11. Preparation of 7,9-Dichloro-2H-[1,2,4]triazine[3,4-b][1,3]benzoxazole-3,4-dione (11). The compound **3** (10 mmol) and diethyl oxalate (10 mmol) were dissolved in ethanol (20 mL) and refluxed for 3 hr. The reaction mass was cooled to room temperature and filtered the solid. The obtained solid was recrystallized using methanol.

Half white color solid; IR (KBr) cm^{-1} : 3223 (NH), 1730 (C=O), 1620 (C=N), 1439 (C=C), 771 (C-Cl); ^1H NMR (DMSO- d_6) ppm: δ 7.3 (s, 1H, Ar H), δ 7.32 (s, 1H, Ar H), δ 8.5 (s, 1H, =NH); ^{13}C NMR (DMSO- d_6): δ 115.11, 119.23, 123.43, 130.55, 142.09, 145.64, 155.92, 162.91, 163.55; MS: (M^+) m/z 272, m/z^{+2} 274, m/z^{+4} 276; Anal. Calcd for $\text{C}_9\text{H}_3\text{Cl}_2\text{N}_3\text{O}_3$ (272.04): C 39.73, H 1.11, N 15.45; found: C 39.71, H 1.08, N 15.42.

2.2. Biological Activities

2.2.1. Antibacterial Activity. The antibacterial efficacy of the compounds was tested against Gram-positive bacteria, namely, *Staphylococcus aureus*, *Bacillus cereus*, and against Gram-negative bacteria, namely, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* by agar well diffusion method [19]. The 24 hr old Muller-Hinton broth cultures of test bacteria were swabbed on sterile Muller-Hinton agar plates using sterile cotton swab followed by punching wells of 6 mm with the help of sterile cork borer. The standard drug (Chloramphenicol, 1 mg/mL of sterile distilled water), compounds **4–11** (20 mg/mL of 10% DMSO), and control (10% DMSO) were added to, respectively, labeled wells. The plates were allowed to stand for 30 minutes and incubated at 37°C for 24 hr in upright position, and the zone of inhibition was recorded.

2.2.2. Minimum Inhibitory Concentration (MIC). MIC was determined by micro-dilution method using serially diluted compounds (0 to 20000 $\mu\text{g/mL}$) in nutrient broth tubes. The standardized 0.1 mL of inoculum (10^7 CFU/mL) of test bacteria was added to each tube and incubated at 37°C for 24 hr. Two control tubes were maintained for each organism. The

lowest concentration (highest dilution) of the compounds that produced no visible growth when compared with the control tubes was regarded as MIC [20].

2.2.3. Antioxidant Activity. DPPH Assay. The radical scavenging ability of synthesized compounds and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on DPPH free radical. Different concentrations (25, 50, 100, 200, and 400 $\mu\text{g/mL}$) of compounds and standard were prepared in methanol. In clean and labeled test tubes, 2 mL of DPPH solution (0.002% in methanol) was mixed with 2 mL of different concentrations of compounds and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-visible spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity was calculated using the formula: Scavenging activity (%) = $A - B/A \times 100$, where A is the absorbance of DPPH and B is the absorbance of DPPH in standard combination [21].

Ferric Reducing Assay. Different concentrations (25, 50, 100, 200 and 400 $\mu\text{g/mL}$) of compounds and standard (tannic acid) in 1 mL of methanol were mixed in separate tubes with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The tubes were placed in water bath for 20 minutes at 50°C, cooled rapidly, and mixed with 2.5 mL of 10% trichloroacetic acid and 0.5 mL of 0.1% ferric chloride. The amount of iron (II)-ferricyanide complex formed was determined by measuring the formation of Perl's Prussian blue at 700 nm after 10 minutes. The increase in absorbance of the reaction mixture indicated the increased reducing power [22].

2.2.4. Pancreatic Lipase Inhibitory Activity.

Extraction of Lipase from Chicken (*Gallus domesticus*) Pancreas. Pancreas of freshly slaughtered chicken were collected, washed, and placed in ice cold sucrose solution (0.01 M). The pancreas was homogenized in 0.01 M sucrose, centrifuged. The supernatant liquid was separated and subjected to ammonium sulphate precipitation (50% saturation). The pellet obtained after centrifugation was dissolved in sucrose solution and again saturated with 50% ammonium sulphate and centrifuged. The pellet obtained was dissolved in phosphate buffer and used as enzyme source [23].

Determination of Chicken Pancreatic Lipase Activity. The activity of lipase was determined by incubating an emulsion containing 8 mL of olive oil, 0.4 mL of phosphate buffer, and 1 mL of chicken pancreatic lipase for 1 hr in rotary shaker, followed by stopping the reaction by the addition of 1.5 mL of a solution containing acetone and 95% ethanol (1 : 1). The liberated fatty acids were determined by titrating the solution against 0.02 M NaOH (standardized by 0.01 M oxalic acid) using phenolphthalein as an indicator [24, 25].

Lipase Inhibitory Activity. Lipase inhibitory activity of different concentrations of synthesized compounds (2.5, 5, 10, and 20 mg/mL) was tested by mixing 100 μL of each compound, 8 mL of oil emulsion, and 1 mL of chicken pancreatic lipase followed by incubation for 60 minutes. The reaction was stopped by adding 1.5 mL of a solution

containing acetone and 95% ethanol (1 : 1). The liberated fatty acids were determined by titrating the solution against 0.02 M NaOH using phenolphthalein as an indicator [26]. The orlistat was used as standard lipase inhibitor, and the percentage inhibition of lipase activity was calculated using the formula:

Lipase inhibition = $A - B/A \times 100$, where A is lipase activity, and B is activity of lipase when incubated with the compounds.

2.2.5. Cytotoxic Activity. The brine shrimp lethality test was conducted to determine cytotoxic activity of compounds [27]. Brine shrimp *Artemianauplii* eggs (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water, which was prepared with 10 gm of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500 mL of distilled water. After 36–48 hr, the phototropic shrimps were collected by pipette for bioassay. Twenty-five shrimps were transferred to each vial containing different concentrations of compounds. The artificial sea water was added to make up to 5 mL and incubated at 25°C, and survived shrimps were counted microscopically in the stem of the pipette against a lightened background after 24 hr.

3. Results and Discussion

3.1. Chemistry. The compound 5,7-dichloro-2-(ethylthio)-1,3-benzoxazole **2** was treated with hydrazine hydrate to afford 5,7-dichloro-2-hydrazino-1,3-benzoxazole **3**. The compound **3** was reported in another route [28], but it is possible to synthesize the intermediate by direct method (Scheme 1). The compound **3** was characterized by ^1H NMR, which exhibited two singlets at δ 4.6, δ 9.3 for $-\text{NH}_2$ and $-\text{NH}$ protons, respectively (D_2O exchangeable). The compound **3** on treatment with different reagents gives compounds **4–11** (Scheme 2).

The compound **3** on reacting with aliphatic acids like formic acid and acetic acid afforded 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole derivative **4** and **5**, respectively. In ^1H NMR, the presence of $-\text{CH}_3$ at δ 1.9 confirmed the formation of compound, **5** whereas it did not appear in compound **4**. The compound **4** shows a singlet at δ 12.18 for triazole proton. The compound **3** reacted with nitrous acid in presence of acetic acid yielded 5,7-dichlorotetrazolo[5,1-*b*][1,3]benzoxazole **6**, the formation of tetrazole was confirmed by the absence of $-\text{NH}$ and $-\text{NH}_2$ protons and the presence of only two aromatic protons at δ 7.2 and 7.39. The compound 2-(5,7-dichloro-1,3-benzoxazol-2-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one **7** was formed by the reaction between compound **3** and ethyl acetoacetate.

The ethyl cyanoacetate treated with compound **3** yields 5-amino-2-(5,7-dichloro-1,3-benzoxazol-2-yl)-2,4-dihydro-3H-pyrazol-3-one **8**. On the other hand, 5,7-dichloro-2-hydrazino-1,3-benzoxazole **3** was condensed with acetyl acetone to form 5,7-dichloro-2-(3,5-dimethyl-1H-pyrazol-1-yl)-1,3-benzoxazole **9** in excellent yield. Two singlets

SCHEME 1: Synthetic route for compound 3.

SCHEME 2: Synthesis of compounds 4–11. i: HCOOH, ii: CH₃COOH, iii: NaNO₂/AcOH, iv: CH₃COCH₂COOC₂H₅, v: CNCH₂COOC₂H₅, vi: CH₃COCH₂COCH₃, vii: ClCOOC₂H₅, viii: (COOC₂H₅)₂.

for six protons at δ 3.0–3.04 confirmed the formation of compound 9. The compound 3 was cyclized with ethyl chloroformate and with diethyl oxalate furnished 6,8-dichloro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazol-3(2*H*)-one 10 and 7,9-dichloro-2*H*-[1,2,4]triazine[3,4-*b*][1,3]benzoxazole-3,4-dione 11, respectively.

3.2. Biological Evaluation. The results of antibacterial activity of compounds 4–11 were shown in Table 2. In the antibacterial study, the compounds have shown inhibition of test bacteria. Among synthesized compounds, marked inhibition of test bacteria was observed to compounds 4, 5, and 8, while least activity was observed to compounds 7 and 9.

Minimum inhibitory concentration of synthesized compounds against test bacteria ranged from 250 $\mu\text{g/mL}$ to 7500 $\mu\text{g/mL}$. The compounds 4, 5, and 8 caused high inhibition at low concentration and are reported in Table 3.

Figure 1 explains antioxidant activity at different concentrations of compounds 4–11 in methanol and ascorbic acid in terms of free radical scavenging ability which was evaluated using DPPH free radical assay. The compounds exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPH, and the activity was found to be dose dependent. The compound 5 was shown to be more potent than ascorbic acid, and results were tabulated in Table 4.

The result of reducing power at different concentrations of compounds 4–11 and tannic acid is represented in Table 5. In this study, the absorbance was found to be increased with the dose of compounds and standard, which is suggestive reducing power (Figure 2). The compounds 4, 6, 7, and 11 showed remarkable reducing power.

Inhibitory activity on chicken pancreatic lipase at different concentrations of compounds in DMSO was determined using olive oil as the substrate. It was found that, the activity

TABLE 2: Antibacterial activity of compounds **4–11**.

Compounds	Zone of inhibition of test bacteria (in cm)				
	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
4	2.8	1.9	2.3	2.6	2.1
5	2.4	2.5	2.2	2.6	2.2
6	2.4	2.0	1.3	1.6	1.3
7	1.3	1.4	1.4	1.8	1.8
8	3.0	2.1	2.2	2.4	2.6
9	1.0	0.8	0.8	1.1	0.8
10	1.8	2.0	1.5	1.9	1.8
11	1.9	1.6	1.7	1.8	1.8
DMSO	0.0	0.0	0.0	0.0	0.0
Standard	3.1	3.0	2.8	2.7	2.8

Standard-Chloramphenicol.

TABLE 3: MIC of compounds **4–11**.

Compounds	MIC in $\mu\text{g/mL}$				
	<i>B. cereus</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
4	250	750	500	500	750
5	500	500	750	500	750
6	500	1000	2000	1500	2000
7	2000	2000	2000	1500	1500
8	250	750	750	500	500
9	5000	7500	7500	1000	7500
10	750	750	1000	750	750
11	750	1000	750	750	750
DMSO	0.0	0.0	0.0	0.0	0.0
Standard	250	250	250	250	250

Standard-Chloramphenicol.

TABLE 4: DPPH radical scavenging activity of compounds **4–11**.

Compounds	DPPH radical scavenging activity (%) of different concentrations ($\mu\text{g/mL}$) of compounds 4–11				
	400	200	100	50	25
4	85.71	73.01	68.25	60.32	53.96
5	97.56	95.12	93.90	92.68	91.46
6	73.01	57.14	55.55	53.96	47.62
7	95.24	93.65	93.65	92.06	90.47
8	96.82	95.04	93.05	87.30	85.71
9	90.47	85.71	73.01	60.32	53.96
10	92.68	87.80	71.95	67.07	64.63
11	93.65	90.47	88.89	76.19	71.42
Standard	97.33	95.12	93.14	90.33	86.69

Standard-acetic acid.

TABLE 5: Ferric reducing activity of compounds **4–11**.

Compounds	Ferric reducing activity of different concentrations ($\mu\text{g/mL}$) of compounds 4–11 (%)				
	25	50	100	200	400
4	0.43	0.53	0.55	0.59	0.66
5	0.45	0.48	0.51	0.55	0.61
6	0.49	0.51	0.55	0.61	0.68
7	0.51	0.54	0.65	0.68	0.73
8	0.41	0.44	0.49	0.51	0.54
9	0.41	0.47	0.52	0.55	0.59
10	0.46	0.50	0.53	0.56	0.62
11	0.53	0.58	0.61	0.66	0.71
Tannic acid	0.90	0.76	0.73	0.69	0.61

of lipase was affected when incubated with the compounds. The inhibitory activity was found to be dose dependent that is, higher inhibition of enzyme was observed on increasing the concentrations of compounds (Figure 3). Lipase inhibition was more in case of compound **11**. The results are tabulated in Table 6.

The degree of lethality was found to be directly proportional to the concentration of the compounds. The highest mortality was observed at 1000 $\mu\text{g/mL}$ concentration of compounds. The most potent activity was observed in case of compounds **6** and **8**, whereas the mortality was 100% at the lowest concentration of compounds (Figure 4). LC_{50}

TABLE 6: Pancreatic lipase inhibitory activity of compounds 4–11.

Compounds	Inhibition of lipase activity (%)				
	20 mg/mL	10 mg/mL	5 mg/mL	2.5 mg/mL	0 mg/mL
4	35.19	20.07	10.00	06.55	0.0
5	39.84	30.54	20.46	09.44	0.0
6	37.90	22.79	21.55	10.95	0.0
7	50.69	35.96	23.30	12.01	0.0
8	57.28	42.94	29.05	14.34	0.0
9	59.80	38.68	27.82	15.42	0.0
10	59.80	41.39	25.50	16.45	0.0
11	72.86	54.94	40.67	19.77	0.0
Standard	96.61	94.30	93.12	89.21	0.0

Standard-Orlistat.

FIGURE 1: DPPH radical scavenging activity of compounds 4–11.

of compound 5, 10, and 11 was found to be 896.05, 22.85, and 31.99 µg/mL, respectively, and the LC_{50} values were determined by linear regression using Origin 6.0 software.

The results are mentioned in Table 7.

4. Conclusion

This study reports the successful synthesis of the title compounds via different routes in good yield. The target molecules were characterized and confirmed by 1H NMR, IR, Mass, and ^{13}C NMR spectral analysis and screened for selected biological activities. The compounds 4, 5, and 8 showed marked antibacterial activity, and compounds 10 and 11 exhibited potent pancreas lipase inhibitors, whereas compound 5 acted as scavengers and reducing agents. The compounds 6 and 8 showed potent cytotoxic activity. So, the derivatives of 5,7-dichloro-2-hydrazino-1,3-benzoxazole 3 were found to be biologically potent-fused heterocycles.

FIGURE 2: Ferric reducing activity of compounds 4–11.

FIGURE 3: Pancreatic lipase inhibitory activity of compounds 4–11.

FIGURE 4: Cytotoxic activity of compounds 4–11.

TABLE 7: Cytotoxic activity of compounds 4–11.

Compounds	% Mortality of Shrimps at different concentrations ($\mu\text{g/mL}$)		
	10	100	1000
4	0.0	0.0	0.0
5	0.0	20.0	50.0
6	100.0	100.0	100.0
7	0.0	0.0	0.0
8	100.0	100.0	100.0
9	0.0	0.0	0.0
10	27.0	87.0	100.0
11	20.0	80.0	100.0

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