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Article

# Synthesis, Characterization, Density Functional Theory, Molecular Docking Studies, and Biological Activities of 6,8-dichloro-3-phenyl[1,2,4]triazolo[3,4-b][1,3]benzoxazole Derivatives

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**ABSTRACT**

Triazole and benzoxazole nucleus are the most adorable moieties in the field of medicinal chemistry. In our prior research, we employed synthesis of target molecules bearing both active nucleuses of triazole and benzoxazole derivatives. The compounds 6,8-dichloro-3-phenyl[1,2,4]triazolo[3,4-b][1,3]benzoxazole derivatives **(4-11)** were synthesized by reacting 5,7-Dichloro-2-hydrazino-1,3-benzoxazole (3) with various compounds containing carboxylic groups through conventional method. The target molecules have been characterized by infrared, $^{1}$H NMR, $^{13}$C NMR, and mass spectral studies. The synthesized molecules subjected for antibacterial, insecticidal, and antioxidant activities to know the efficacy of molecules. In addition to this computational density functional theory using B3LYP/LANL2DZ method in the ground state was performed. The calculations were done to confirm the geometry of the molecules and also the highest occupied molecular orbital-lowest unoccupied molecular orbital excitation energy levels also calculated. Further, in addition to this, molecular docking studies were performed with protein receptor PDB: 3MNG exhibits the highest binding interactions with all the compounds.

**Key words:** Benzoxazole, Density functional theory, Highest occupied molecular orbital, Insecticidal activity, Lowest unoccupied molecular orbital, Molecular docking, and Triazole.

## 1. INTRODUCTION

Heterocyclic compounds have been important and major classes of organic compounds occur widely in nature and in a variety of non-natural compounds various compounds such as alkaloids antibiotics essential amino acids hemoglobin chlorophyll hormones a large number of synthetic drugs and dyes contain heterocyclic ring system as basic moiety heterocycles such as triazoles, benzoxazoles, and pyridine pyrimidine thiamine purine indole are known to play an important role in biological process in the form of vitamins nucleic acid proteins and enzymes. The benzoxazoles are a large chemical family used as antimicrobial agents against a wide spectrum of microorganisms. The high therapeutic activities of the related drugs have encouraged medicinal chemists to synthesize a large number of novel chemotherapeutic agents. The incorporation of the benzoxazole nucleus is an important synthetic strategy in drug discovery. Most of the natural product containing benzoxazole moiety are endowed with useful medicinal properties. Benzoxazole can be considered as structure isosteres [1] of the naturally occurring purine and pyrimidine bases, which allow them to interact easily with polymers of a living system [2].

Their importance is due to their versatile application in the field of drugs and pharmaceuticals. Zoxazolamine, which is benzoxazole analog, is mainly used as skeletal muscle relaxant [3]. Moreover, some benzoxazole derivatives have been demonstrated to be potent antimicrobial [4], anti-HIV [5], analgesic [6], anti-inflammatory [7], anti-cancer agents [8], and topoisomerase-I inhibitory activity [9]. 1,2,4-Triazole derivatives are the important class of heterocyclic compounds containing nitrogen atom in their ring skeleton. The chemistry of triazoles and their derivatives was found to be the highlight of study in lead compound discovery and biological screening and study of their various biological activities including antifungal, antibacterial, antitubercular, anticancer, and analgesic [10]. Hence, they have been studied for over a century and the research is still going on. Triazoles occupied a unique position in biological activities.

In view of the above observation, we have synthesized some novel dichlorobenzoxazole derivatives to form various fused heterocyclic ring systems such as 1,2,4-triazoles moiety and screening for possible biological and pharmacological activities also subjected for density functional theory (DFT) studies and molecular docking studies for the respective synthesized compounds.

## 2. RESULTS AND DISCUSSION

### *2.1. Chemistry*

The compound **3** is the intermediate compound for the synthesis of compounds **6a-e.** The compound **3** was obtained by the reaction of 5,7-dichloro-2-(ethylthio)-1,3-benzoxazole **2** with hydrazine

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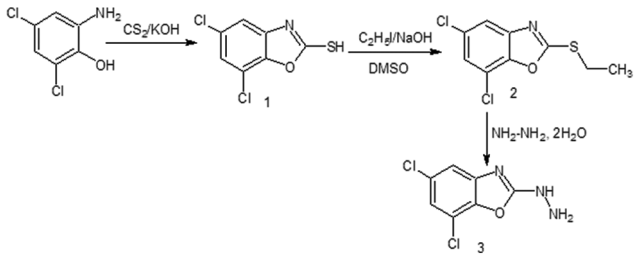
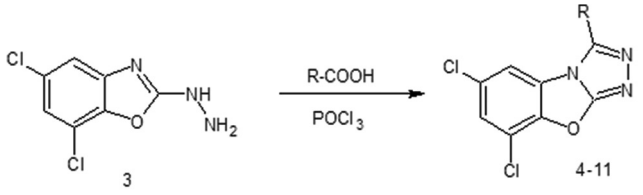
hydrate [Scheme 1]. The compounds 6,8-dichloro-3-phenyl[1,2,4] triazolo[3,4-b][1,3]benzoxazole derivatives **(4-11)** are presented in Table 1 were synthesized using 5,7-dichloro-2-hydrazino-1,3-benzoxazole **3** and compounds with carboxylic acid groups [Scheme 2]. The structures of newly synthesized compounds were confirmed by infrared (IR), $^{1}$H NMR, $^{13}$C NMR liquid chromatography-mass spectrometry (LCMS), and elemental analysis. Broad singlet at δ 4.6 for two protons of –$NH_2$ and another broad singlet at δ 9.3 for –NH proton (D2O exchangeable) in $^{1}$H NMR evidences the formation of compound **3**. The IR spectrum of compound **4** showed disappearance of peaks for 2-hydrazino groups and a new peak was observed for $NH_2$ group at 3321 $cm^{-1}$. The $^{1}$H NMR spectrum of compound **4** shows multiplet between δ 7.4 and 8 for the 7 aromatic protons also shows absence of NH and $NH_2$ protons signal. It was further confirmed by its mass spectrum, which exhibited a molecular ion peak at m/z 304 equivalent to its molecular weight.




**Scheme 1:** Synthetic route for compounds **1-3**




**Scheme 2:** Synthetic route for compounds **4-11**

### *2.2. Biological Evaluation*

#### *2.2.1. Antimicrobial activity*

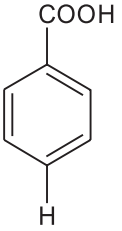
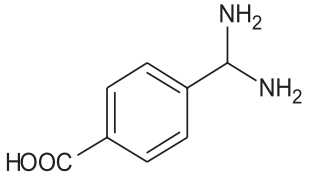
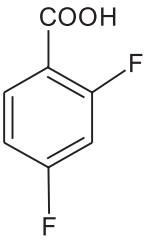
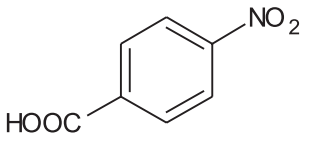
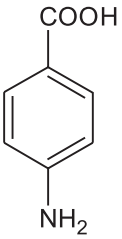
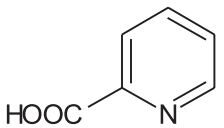
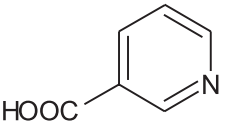
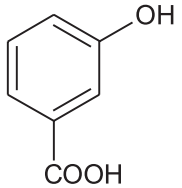
The antimicrobial activity was tested against bacterial strains by agar well diffusion method [11]. The compounds **4** and **5** showed promising antibacterial activity and the compound **8** exhibits potent antibacterial activity. The results are tabulated in Table 2.

#### *2.2.2. Antioxidant activity*

The antioxidant activity at different concentrations, namely, 25, 50, 100, 200, and 400 µg/mL of the compounds **4-11** and ascorbic acid was tested on the basis of the radical scavenging effect of the stable DPPH free radical assay [12,13]. The compound **11** showed better antioxidant activity followed by the compound **5** and **7.** The obtained results are recorded in Table 3.

#### *2.2.3. Insecticidal activity*

The compounds **4-11** were subjected to insecticidal activity to know the mortality of the compounds against *Aedes aegypti* mosquito

| Compound | R | Compound | R |
|---|---|---|---|
| **4** |  | **8** |  |
| **5** |  | **9** |  |
| **6** |  | **10** |  |
| **7** |  | **11** |  |

larvae [14]. The compound **11** exhibited higher mortality as compared to other synthesized compounds. The results aere recorded in Table 4.

### *2.2.4. Computational studies*

Molecular geometries of the singlet ground state of the derivatives **6**, **9,** and **11** were fully optimized in the gas phase at the DFT/B3LYP 6-31G (d, p) [15] and DFT/B3LYP LANL2DZ basic sets 11. The optimized geometry of the studied molecules, highest occupied molecular orbital (HOMO), and lowest unoccupied molecular orbital (LUMO) frontier molecular orbitals (FMOs) was visualized with supporting software chemcraft with chem3D and Gaussian view [16,17].

The optimized geometry of the title derivatives, bond lengths, bond angles, and dihedral angles corresponding to the optimized geometry of the title compound has been obtained using the DFT/B3LYP method. The energies of FMOs are important properties in several chemical and pharmacological processes. The HOMO measures the electron-donating ability to cede an electron, and LUMO as an electron acceptor represents the ability to receive an electron. Thus, the higher the energy HOMO (EHOMO) is, the greater the electron-donating capacity will be, and the lower the energy LUMO (ELUMO) is, the smaller the resistance to accept electrons will be 12, 13. In addition, the energy gap reflects the biological activity of molecules. The calculated values of HOMO and LUMO energies and HOMO–LUMO band gap of the compounds **6**, **9,** and **11** are schematized in Figures 1-3.

The energy gap of the FMOs suggests that the structure is stable. In addition, FMOs provide information regarding a molecule's chemical reactivity and kinetic stability. Furthermore, the FMOs help in determining a molecule's most reactive site. The calculated energy value of HOMO and LUMO orbitals are 1.549eV, 1.587eV, and 1.984eV, respectively. The FMO's energy gap (ΔEHOMO-LUMO) of the mentioned compounds was found to be 1.549eV. The lower value of the HOMO and LUMO energy gap showed that the studied molecule has high chemical reactivity, biological activity, and polarizability. The relative reactivity sites in a species for both electrophilic and nucleophilic attack can be calculated using the molecular electrostatic potential and the results were tabulated in Table 5.

**Table 1:** Physical data of compounds **4-11**

| Compound | Molecular Formula | Molecular Weight | M.P. (°C) | Yield (%) |
|---|---|---|---|---|
| **4** | $C_{14}H_7Cl_2N_3O$ | 304 | 235 | 75 |
| **5** | $C_{14}H_5Cl_2F_2N_3O$ | 340 | 244 | 79 |
| **6** | $C_{14}H_5Cl_2N_4O$ | 319 | 231 | 81 |
| **7** | $C_{13}H_6Cl_2N_4O$ | 305 | 281 | 71 |
| **8** | $C_{15}H_{11}Cl_2N_5O$ | 348 | 238 | 85 |
| **9** | $C_{14}H_6Cl_2N_4O_3$ | 349 | 211 | 88 |
| **10** | $C_{13}H_6Cl_2N_4O$ | 305 | 286 | 70 |
| **11** | $C_{14}H_7Cl_2N_3O_2$ | 320 | 221 | 65 |

### *2.2.5. Molecular docking studies*

Molecular docking work was performed with the Hex molecular modeling package version 8.0 [18]. Protein docking studies of the synthesized compounds **6, 9,** and **11**. For macromolecular docking studies, chemical structures of the synthesized **6, 9,** and **11** were drawn using Chem. Draw Ultra 3D optimization was performed using Chem. Draw 3D Ultra software and stored as pdb files. Hex docking was carried out by setting suitable parameters, outlined in Table 6 and this docking score can be interpreted as the interaction energy. A more negative E-total energy value implies that a strong interaction exists between drug and receptor which leads to the inhibition of receptor activity and the results are presented in Figures 4-6 [19]. Molecular docking work was performed with the Hex molecular modeling package version 8.0.

## 3. EXPERIMENTAL SECTION

### *3.1. Chemistry*

Melting points were recorded on electrothermal melting point apparatus and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded on Bruker 400 MHz spectrometer. Chemical shifts were shown in δ values (ppm) with tetramethylsilane as an internal standard. LC-MS were obtained using Atlantis DC18 (50×4.6) mm column on Shimadzu, LCMS 2010A, Japan. The 0.1% HCOOH and methanol were used as mobile phase at the flow rate of 1 mL/min. The Fourier transform infrared (FTIR) spectra of compounds were taken in KBr pellets (100 mg) using Shimadzu FT-IR spectrophotometer. Column chromatography was performed using silica gel (60–120 mesh). Silica gel GF254 plates from Merck were used for thin layer chromatography (TLC) and the spots were observed by ultraviolet (UV) light. The chemicals were purchased from Sigma Aldrich Co. and solvents for column chromatography were of reagent grade and purchased from commercial source.

#### *3.1.1. Preparation of 5,7-Dichloro-1, 3-benzoxazole-2-thiol* ***(1)***

To a solution of methanol (50 mL), KOH (1.1eq) was added and stirred for 10 min followed by slow addition of $CS_2$ at room temperature. To

**Table 2:** Antibacterial activity of Compounds **4-11**

| Compound | Zone of inhibition of test bacteria (in cm) | | | | |
|---|---|---|---|---|---|
| | *Bacillus cereus* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Klebsiella pneumoniae* | *Escherichia coli* |
| **4** | 2.9 | 2.0 | 2.4 | 2.7 | 2.2 |
| **5** | 2.5 | 2.6 | 2.3 | 2.7 | 2.3 |
| **6** | 2.5 | 2.1 | 1.4 | 1.7 | 1.4 |
| **7** | 1.4 | 1.5 | 1.5 | 1.9 | 1.9 |
| **8** | 2.0 | 1.7 | 1.8 | 1.9 | 1.9 |
| **9** | 1.1 | 0.9 | 0.9 | 1.2 | 0.9 |
| **10** | 1.9 | 2.1 | 1.6 | 2.0 | 1.9 |
| **11** | 3.1 | 2.2 | 2.3 | 2.5 | 2.7 |
| **DMSO** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Standard** | 3.2 | 3.1 | 2.9 | 2.8 | 2.9 |

**Table 3:** DPPH radical scavenging activity of compounds **4-11**

| Compounds | Scavenging activity of different concentrations (µg/mL) in % | | | | |
|---|---|---|---|---|---|
| | 25 | 50 | 100 | 200 | 400 |
| **4** | 63.96 | 65.32 | 78.25 | 83.01 | 87.71 |
| **5** | 83.71 | 85.30 | 91.05 | 92.04 | 94.82 |
| **6** | 57.62 | 63.96 | 65.55 | 67.14 | 77.01 |
| **7** | 88.47 | 90.06 | 91.65 | 93.65 | 96.24 |
| **8** | 72.42 | 76.19 | 83.89 | 87.47 | 91.65 |
| **9** | 63.96 | 67.32 | 71.01 | 80.71 | 88.47 |
| **10** | 66.63 | 69.07 | 73.95 | 78.80 | 80.68 |
| **11** | 90.46 | 91.68 | 92.90 | 94.12 | 96.56 |
| **Ascorbic acid** | 88.69 | 90.33 | 93.14 | 95.12 | 97.33 |

**Table 4:** Insecticidal activity of compounds **4-11**

| Compound | % Mortality of larvae, concentration (µg/mL) | | | |
|---|---|---|---|---|
| | 24 h | | 48 h | |
| | 10 | 20 | 10 | 20 |
| **4** | 35 | 45 | 60 | 75 |
| **5** | 25 | 50 | 65 | 70 |
| **6** | 40 | 50 | 60 | 78 |
| **7** | 25 | 45 | 45 | 60 |
| **8** | 35 | 50 | 70 | 75 |
| **9** | 25 | 45 | 60 | 75 |
| **10** | 35 | 40 | 45 | 55 |
| **11** | 55 | 65 | 80 | 85 |
| **Melathione** | 100 | 100 | 100 | 100 |

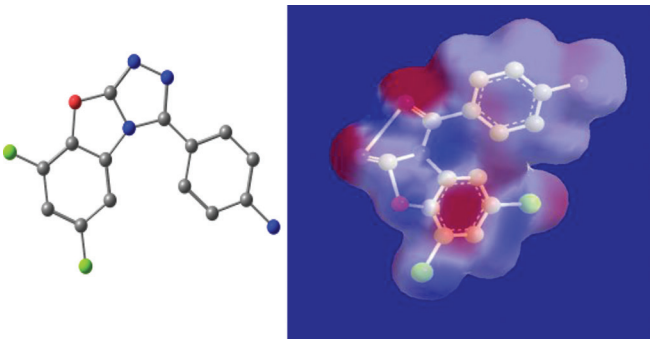
**Table 5:** LUMO and HOMO energy values of compounds **6, 9,** and **11**

| Compounds | $-E_{LUMO}$ (eV) | $-E_{HOMO}$ (eV) | $E_{Gap}$ (eV) |
|---|---|---|---|
| **6** | −7.512 eV | −9.061 eV | 1.549 eV |
| **9** | −7.525 eV | (−9.112 eV | 1.587 eV |
| **11** | −7.601 eV | −9.585 eV | 1.984 eV |

**Table 6:** Molecular docking scores of Compounds **6, 9**, and **11**

| Docking studies of compounds with *Escherichia coli* MurB enzyme (PDB code: 2MBR) and showed a minimum binding energy and good affinity toward the active pocket comparable with the standard drug Ciprofloxacin | |
|---|---|
| **Compounds** | **Docking Score** |
| **6** | −165.4 Kcal/mol |
| **9** | −172.8 Kcal/mol |
| **11** | −180.1 Kcal/mol |
| **Ciprofloxacin** | −202.3 Kcal/mol |

the reaction mass, 2-amino-4,6-dichlorophenol was added on stirring. The reaction mass was refluxed for 6 h on water bath. The completion



Optimized geometry and molecular electrostatic potential surface.

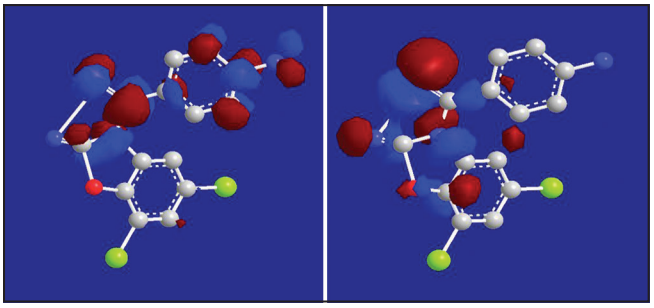
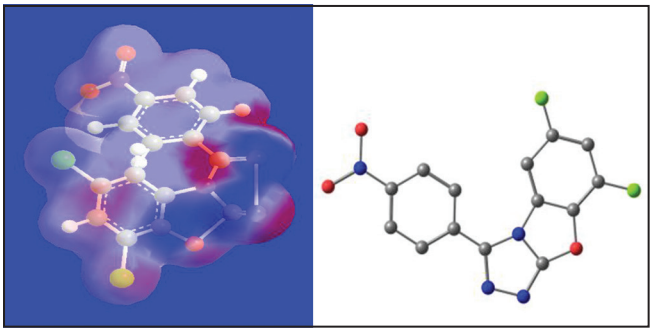


**Figure 1:** HOMO and LUMO orbital's for compound **6.**



Optimized geometry and molecular electrostatic potential surface.

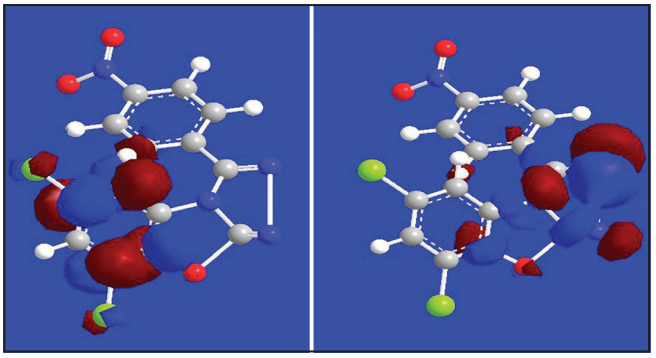
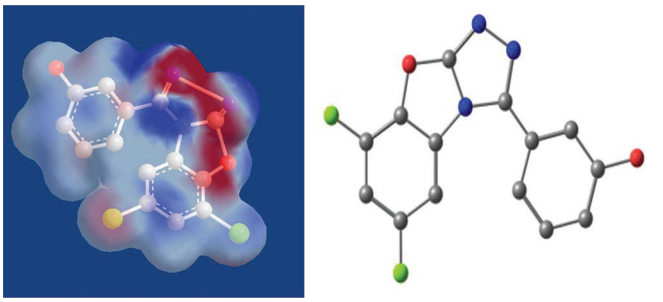


**Figure 2:** HOMO and LUMO orbital's for compound **9.**

of the reaction was confirmed by TLC and the reaction mass was poured onto ice-cold water, followed by acidification with glacial acetic acid (pH 6). The obtained solid was filtered, dried, and recrystallized using ethanol. Yield (95%), M.P. 198°C.

*3.1.2. Preparation of 5,7-Dichloro-2-(ethylthio)-1, 3-benzoxazole* ***(2)***

To a mixture of sodium hydroxide (10 mol) and compound **1** (10 mol) in DMSO (10 mL), ethyl iodide (10 mol) was added dropwise. The



Optimized geometry and molecular electrostatic potential surface

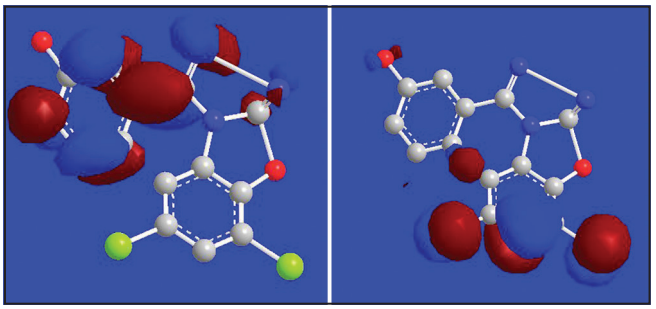


**Figure 3:** HOMO and LUMO orbital's for compound **11.**

reaction mass was stirred about 1 h and the completion of the reaction was confirmed by TLC. The reaction mass was poured onto ice-cold water. The obtained solid was filtered, dried, and recrystallized using hexane.

Yield (90%), M.P. 38°C, IR (KBr) $cm^{-1}$: 2974 (CH alkyl), 1608 (C=N), 1491 (C=C), 756 (C-Cl); $^1$H NMR ($CHCl_3$) ppm: δ 1.4 (t, 3H, $CH_3$), δ 3.26 (q, 2H, $CH_2$), δ 7.1 (s,1H, Ar H) δ 7.4 (s,1H, Ar H), $^{13}$C NMR ($CHCl_3$) ppm: δ 124.85–144.4 (7C, $sp^2$ carbon atoms), δ 14.29 ($CH_2$), δ 13.22 ($CH_3$), Analyses: $C_9H_7Cl_2NOS$ (248.1). *M/z* 248, $M^{+2}$ 250, $M^{+4}$ 252, Required: C (43.56%) H (2.84%) N (5.64%) Found: C (43.54) H (2.82), N (5.63).

*3.1.3. Preparation of 5,7-Dichloro-2-hydrazino-1,3-benzoxazole (3)*

The compound **2** (10 mol) and hydrazine hydrate (15 mol) in ethanol (15 mL) was taken in a round-bottomed flask and refluxed for 3 h. The completion of the reaction was confirmed by TLC and the reaction mixture was cooled and filtered. The obtained solid was recrystallized from ethanol.

Yield (70%), M.P. 220°C, IR (KBr) $cm^{-1}$: 3349 ($NH_2$), 3283 (NH), 1617 (C=N), 1453 (C=C), 771 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 4.6 (s, 2H, -$NH_2$, $D_2O$ exchangeable), δ 9.3 (s,1H, -NH, $D_2O$ exchangeable), δ 7.18 (s,1H, Ar H) δ 7.29 (s,1H, Ar H); $^{13}$C NMR (DMSO-$d_6$): 122.9–

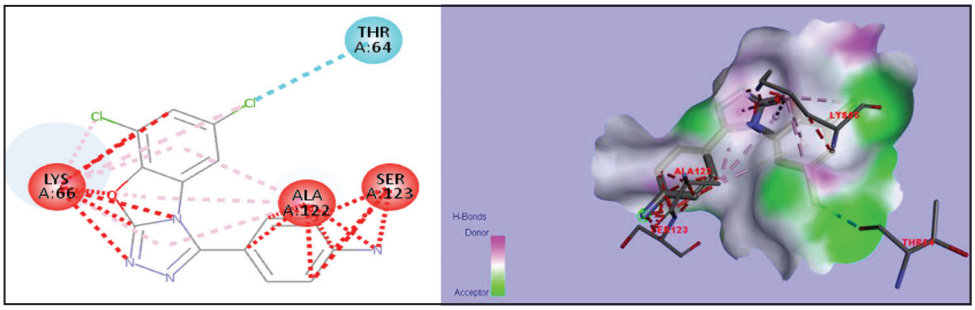



**Figure 4:** 2D and 3D structure of compound **6** with receptor *Escherichia coli* MurB enzyme (PDB code: 2MBR).

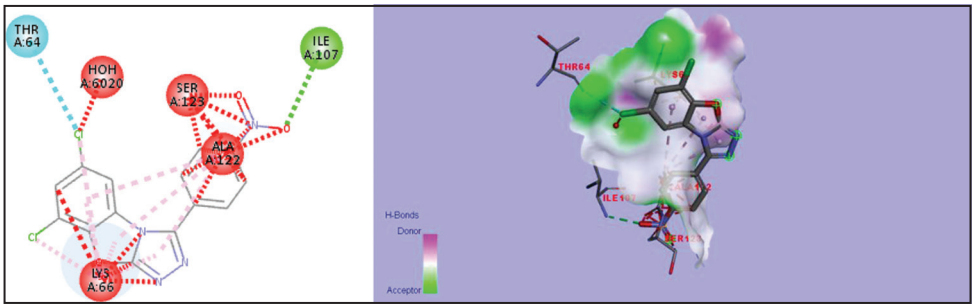



**Figure 5:** 2D and 3D structure of compound **9** with receptor *Escherichia coli* MurB enzyme (PDB code: 2MBR).

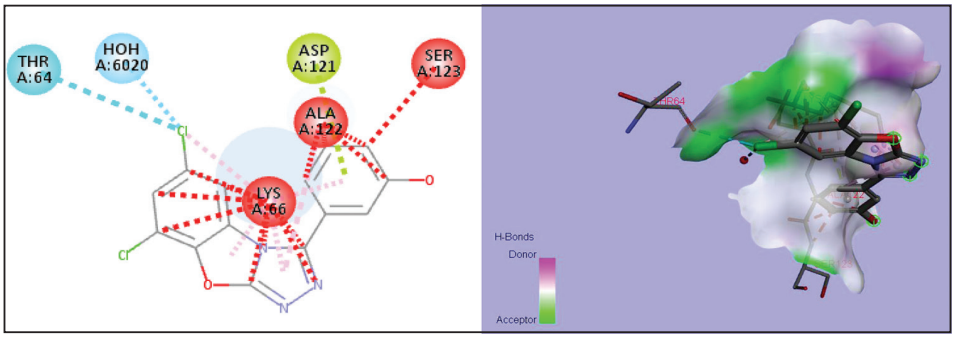



**Figure 6:** 2D and 3D structure of compound **11** with receptor *Escherichia coli* MurB enzyme (PDB code: 2MBR).

143.0 (7C, $sp^2$ carbon atoms), Analyses: $C_7H_5Cl_2N_3O$ (218.04). *M/z* 218, $M^{+2}$ 220, $M^{+4}$ 222, Required: C (38.56%) H (2.31%) N (19.27%) Found: C (38.53%) H (2.30%) N (19.25%).

### *3.1.4. Preparation of 6,8-dichloro-3-phenyl[1,2,4]triazolo[3,4-b][1,3]benzoxazole **4***

Compound **3** (0.01 mol) was taken in a round bottom flask and treated with benzoic acid (0.01 mol) in 20 mL of $POCl_3$ and refluxed for 6 hours. The reaction mixture was cooled and neutralized with sodium carbonate also filtered the obtained solid. The obtained solid was recrystallized from ethanol.

The compounds **5-11** were prepared using the same procedure.

Yield (75%), M.P. 235°C, IR (KBr) $cm^{-1:}$ 1619 (C=N), 1459 (C=C), 769 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 7.18 (s,1H, Ar H) δ 7.29 (s,1H, Ar H) δ 7.41 (m,5H, Ar H); $^{13}$C NMR (DMSO-$d_6$): 122.9–143.0 (14C, $sp^2$ carbon atoms), Analyses: $C_{14}H_7Cl_2N_3O$ (304.1). *M/z* 304, $M^{+2}$ 306, $M^{+4}$ 308, Required: C (55.29%) H (2.32%) N (13.82%) Found: C (55.33%) H (2.34%) N (13.95%).

### *3.1.5. 6,8-dichloro-3-(2,4-difluorophenyl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole **5***

Yield (79%), M.P. 244°C, IR (KBr) $cm^{-1:}$ 1611 (C=N), 1451 (C=C), 761 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 7.10 (s,1H, Ar H) δ 7.21 (s,1H, Ar H) δ 7.24 (s,1H, Ar H) δ 7.46 (d,1H, Ar H) δ 7.49 (d,1H, Ar H); $^{13}$C NMR (DMSO-$d_6$): 119.9–144.0 (14C, $sp^2$ carbon atoms), Analyses: $C_{14}H_5Cl_2F_2N_3O$ (340.1). *M/z* 340, $M^{+2}$ 344, $M^{+4}$ 346, Required: C (49.44%) H (2.34%) N (12.35%) Found: C (49.48%) H (2.38%) N (12.91%).

### *3.1.6. 4-(6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)aniline 6*

Yield (81%), M.P. 231°C, IR (KBr) $cm^{-1}$:3422 (NH2), 1622 (C=N), 1457 (C=C), 769 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 7.17 (s,1H, Ar H) δ 7.28 (s,1H, Ar H) δ 7.61 (m,4H, Ar H) δ 4.4 (s, 2H, -$NH_2$, $D_2O$ exchangeable); $^{13}$C NMR (DMSO-$d_6$): 115.9–145.0 (14C, $sp^2$ carbon atoms), Analyses: $C_{14}H_8Cl_2N_4O$ (319.1). *M/z* 319, $M^{+2}$ 321, $M^{+4}$ 323, Required: C (52.69%) H (2.53%) N (17.56%) Found: C (52.72%) H (2.55%) N (17.91%).

### *3.1.7. 6,8-dichloro-3-pyridin-3-yl[1,2,4]triazolo[3,4-b][1,3]benzoxazole 7*

Yield (71%), M.P. 281°C, IR (KBr) $cm^{-1:}$ 1623 (C=N), 1450 (C=C), 762 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 7.19 (s,1H, Ar H) δ 7.30 (s,1H, Ar H) δ 7.47 (m,4H, Ar H); $^{13}$C NMR (DMSO-$d_6$): 120.1–147.0 (14C, $sp^2$ carbon atoms), Analyses: $C_{13}H_6Cl_2N_4O$ (305.1). *M/z* 305, $M^{+2}$ 307, $M^{+4}$ 309, Required: C (51.17%) H (1.98%) N (18.36%) Found: C (51.31%) H (1.24%) N (18.41%).

### *3.1.8. 1-[4-(6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)phenyl]methanediamine **8***

Yield (85%), M.P. 238°C, IR (KBr) $cm^{-1}$:3521 (NH2), 1611 (C=N), 1441 (C=C), 758 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 2.63 (s, 1H, CH) δ 7.27 (s,1H, Ar H) δ 7.38 (s,1H, Ar H) δ 7.61 (d,2H, Ar H) δ 7.64 (d,2H, Ar H) δ 3.1 (s, 4H, -$NH_2$, $D_2O$ exchangeable); $^{13}$C NMR (DMSO-$d_6$): 22 (1C sp3 carbon) 119.9–147.0 (14C, $sp^2$ carbon atoms), Analyses: $C_{15}H_{11}Cl_2N_5O$ (348.18). *M/z* 348, $M^{+2}$ 350, $M^{+4}$ 352, Required: C (51.74%) H (3.18%) N (20.11%) Found: C (51.79%) H (3.22%) N (20.91%).

### *3.1.9. 6,8-dichloro-3-(4-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole **9***

Yield (88%), M.P. 211°C, IR (KBr) $cm^{-1}$:1635 (C=N), 1461 (C=C), 766 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 7.23 (s,1H, Ar H) δ 7.36 (s,1H, Ar H) δ 7.66 (d,2H, Ar H) δ 7.84 (d,2H, Ar H); $^{13}$C NMR (DMSO-$d_6$): 118.1–144.2 (14C, $sp^2$ carbon atoms), Analyses: $C_{14}H_6Cl_2N_4O_3$ (349.1). *M/z* 349, $M^{+2}$ 351, $M^{+4}$ 353, Required: C (48.16%) H (1.73%) N (16.5%) Found: C (48.21%) H (1.75%) N (16.71%).

### *3.1.10. 6,8-dichloro-3-pyridin-2-yl[1,2,4]triazolo[3,4-b][1,3]benzoxazole **10***

Yield (70%), M.P. 286°C, IR (KBr) $cm^{-1:}$ 1621 (C=N), 1451 (C=C), 761 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 7.13 (s,1H, Ar H) δ 7.33 (s,1H, Ar H) δ 7.43 (m,4H, Ar H); $^{13}$C NMR (DMSO-$d_6$): 190.1–146.0 (14C, $sp^2$ carbon atoms), Analyses: $C_{13}H_6Cl_2N_4O$ (305.1). *M/z* 305, $M^{+2}$ 307, $M^{+4}$ 309, Required: C (51.17%) H (1.98%) N (18.36%) Found: C (51.31%) H (1.24%) N (18.41%).

### *3.1.11. 3-(6,8-dichloro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)phenol **11***

Yield (65%), M.P. 221°C, IR (KBr) $cm^{-1:}$ 3630 (OH), 1611 (C=N), 1443 (C=C), 759 (C-Cl), $^1$H NMR (DMSO- $d_6$)ppm: δ 5.13 (s,1H, OH) δ 7.28 (s,1H, Ar H) δ 7.39 (s,1H, Ar H) δ 7.69 (d, 2H, Ar H) δ 7.89 (d,2H, Ar H); $^{13}$C NMR (DMSO-$d_6$): 121.1–144.1 (14C, $sp^2$ carbon atoms), Analyses: $C_{14}H_7Cl_2N_3O$ (320.1). *M/z* 320, $M^{+2}$ 322, $M^{+4}$ 324, Required: C (52.53%) H (2.20%) N (13.13%) Found: C (52.63%) H (2.24%) N (13.23%).

## *3.2. Biological Activities*

### *3.2.1. Antibacterial activity*

The antibacterial efficacy of compounds was tested against two Gram-positive bacteria, namely, *Staphylococcus aureus* and *Bacillus cereus,* and three Gram-negative bacteria, namely, *Pseudomonas aeruginosa*, *Klebsiella pneumonia,* and *Escherichia coli* by agar well diffusion method [20]. Twenty-four hold 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 in 10% DMSO), and control (10% DMSO) were added to, respectively, labeled wells. The plates are allowed to stand for 30 min and were incubated at 37°C for 24 h in upright position and the zone of inhibition was recorded.

### *3.2.2. Antioxidant activity*

3.2.2.1. DPPH assay

The radical scavenging ability of synthesized compounds and the ascorbic acid (standard) was tested on the basis of the radical scavenging effect on DPPH free radical. Different concentrations of compounds **4-11** and standard, namely, 25, 50, 100, 200, and 400 µg/mL 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 the dark for 30 min 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 × 100, where A is the absorbance of DPPH and B is the absorbance of DPPH and in standard combination [21].

### *3.2.3. Insecticidal activity*

Insecticidal activity of compounds **4-11** was tested against second and third-instar larvae of *A. aegypti* mosquito. The concentration of the compounds (0.5 mg/mL) was prepared in 10% DMSO and added to sterile labeled beakers containing 25 mL of water. Twenty larvae were placed in each of the beakers containing compounds **4-11**. A control was kept in 10% DMSO. The insecticidal effect was determined by counting the number of dead larvae after 24 h. The dead larvae were identified when they failed to move after probing with a needle in siphon or cervical region. Each test was repeated thrice; the percentage of larval mortality was determined.

## 4. CONCLUSION

The new series of benzoxazole–triazole derivatives with ease and procedural simplicity are the key aspects of the synthesis. It was observed that the synthesized target molecules have been confirmed by spectral studies. The result of the antibacterial screening revealed that, among the title compounds, compounds **11**, **4,** and **5** showed higher inhibition while the other compounds displayed moderate to low inhibition. In the case of anti-oxidant activity, the compound **11** exhibited effective scavenging activity while compounds **5** and 7 have shown moderate activity. Insecticidal activity revealed that the compound **11** has exhibited very good mortality among the compounds. Optimized geometries, HOMO-LUMO energy gaps of derivatives **6, 9,** and **11** showed the chemical stability at the B3LYP/LANL2DZ level of theory. The docking score of the targeted receptor supporting the antibacterial activity of the compounds as the compound with more binding energy shows prominent zone of inhibition.

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