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# SYNTHESIS, CHARACTERIZATION AND EVALUATION OF *IN VITRO* CYTOTOXIC, ANTIBACTERIAL, ANTIOXIDANT ACTIVITY AND MOLECULAR DOCKING STUDIES OF NITRO SUBSTITUTED BENZOXAZOLE LINKED THIAZOLIDINE DERIVATIVES

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## ABSTRACT

The 3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-2-phenyl-1,3-thiazolidin-4-one derivatives 6(a-j) have been synthesized by reacting compounds 2-[2-benzylidenehydrazinyl]-6-nitro-1,3-benzoxazole derivatives 5(a-j) with thioacetic acid in dioxane using $ZnCl_2$ as catalyst. The chemical structures of the compounds were elucidated by IR, $^1$H NMR, $^{13}$C NMR and Mass spectral studies. The organisms such as *Escherichia coli* (ATTC-8739), *Staphylococcus aureus* (ATTC-6538), *Vibrio cholera* (ATTC-9027), *Bacillus subtilis* (ATTC-6633), *Staphylococcus epidermidis* (ATTC-12228) and *Salmonella typhimurium* (ATTC-23564) have been used to study the antimicrobial activity of synthesized compounds. MIC was done with effective result, the selected synthesized derivatives showed potent cytotoxic activity against Peripheral Blood Mononuclear cancer Cells. The antioxidant activity has been carried out to know the scavenging effect of targeted molecules and the results were supported by the *in silico* molecular docking studies.

**KEYWORDS:** Benzoxazole, Schiff base, thioacetic acid, thiozolidinine, Peripheral Blood Mononuclear Cells.

## INTRODUCTION

Benzoxazoles are active and medicinally significant compounds.[1] Benzoxazoles are found as fused azoles and an important class of heterocyclic scaffold in biologically active skeleton. During recent years there have been some interesting developments in the pharmacology of benzoxazole derivatives.[2] The substituted benzoxazoles have been shown to exhibit antitumor,[3] antioxidant, antihelmintic,[4] cyclooxygenase inhibitory,[5] antifungal,[6] antitubercular,[7] 5HT 3 receptor antagonists,[8] anti-inflammatory, analgesic & cyclin dependent kinase inhibitory,[9] 5-lipoxygenase inhibitory,[10] melatonin receptor agonist,[11] anticancer,[12] antibacterial[13] and anti-HIV-1[14] activities. As a consequence of this feature, benzoxazole derivatives which have multiple biological activities have been known for a long time.

In recent years, thiazolidinones have been the most extensively investigated classes of organic compounds. The presence of a thiazolidine ring in penicillin and related derivatives was the first recognition of its occurrence in nature. Thiazolidine-4-one represents a prevalent scaffold in drug discovery. Literature survey showed that thiazolidinone ring system exhibit a variety of biological activities such as antimicrobial[15], antifungal,[16] antiviral,[17] antituberculostatic,[18] anti-HIV,[19] cardioprotective,[20] anticancer,[21] anticonvulsant,[22] anti-inflammatory[23] and analgesic propertie.[24]

So on course literature survey, our work has been presented here to synthesize benzoxazole nucleus with thiazolidine moiety also to know the combined pharmacological effect of benzoxazole with thiazolidine moiety.

## 2. EXPERIMENTAL SECTIONS

### *(i)* 2-[(2*E*)-2-(4-chlorobenzylidene) hydrazinyl]-6-nitro-1, 3-benzoxazole 5(a)

To the compound **4** (0.01mol) in ethanol (30ml) added substituted aldehydes **5 (a)** (0.01mol) with few drops of glacial acetic acid. The reaction mixture was refluxed for 5-6 hr and cooled. The solid separated was filtered, washed with water, dried and recrystallized from ethanol to get compound **5(a).**

The compounds **5(b-j)** were prepared by following similar method of procedure by using **(b-j)** aromatic aldehydes.

***(ii)* 3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-2-phenyl-1,3-thiazolidin-4-one 6 (a)**

The compound **6(a)** (0.01mol) was dissolved in 1,4-dioxin (20ml) with sulfanylacetic acid (0.01mol) in the presence of pinch of zinc chloride and refluxed for 8 hr. Then reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol to get compound **6(a).**

The compounds **6(b-j)** can be prepared by following similar method

Yield: 0.2710 (78 %), m.p. 206-208$^0$ C; IR (KBr $\nu_{max}$ cm$^{-1}$): 1670 (C=O), 3287 (NH); $^1$H NMR (DMSO-d$_6$, 400MHz) δ : 6.83-7.82 (m, 8H, Ar-H), 9.58 (s, 1H, NH), 3.45 (s, 1H, $CH_2$), 5.92 (s, 1H, N-CH). $^{13}$C NMR (DMSO-d$_6$,100 MHz) δ: 168.45, 156.52, 155.23, 152.14, 150.41, 149.36, 148.35, 146.19, 143.65, 141.31, 137.08, 135.65, 131.19, 77.39, 28.46. MS (CI) *m/z*(%): M$^{+1}$, 357. Anul.: Calcd. for $C_{16}H_{12}N_4O_4S$: C (53.93), H (3.39), N (15.72). Found: C (53.84), H (3.36), N (15.67).

**(iii)2-(4-chlorophenyl)-3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-1,3-thiazolidin-4-one 6 (b)**

Yield: 0.3143 (86 %), m.p. 176-178$^0$ C; IR (KBr $\nu_{max}$ cm$^{-1}$): 1672 (C=O), 3291 (NH); $^1$H NMR (DMSO-d$_6$, 400MHz) δ : 6.87-7.85 (m, 7H, Ar-H), 9.63 (s, 1H, NH), 3.54 (s, 1H, $CH_2$), 5.98 (s, 1H, N-CH). $^{13}$C NMR (DMSO-d$_6$, 100 MHz) δ: 169.26, 154.25, 153.32, 151.41, 149.19, 148.38, 147.99, 147.06, 144.82, 140.16, 138.95, 134.65, 130.50, 77.39, 28.46. MS (CI) *m/z*(%): M$^{+1}$, 391, M$^{+2}$, 393. Anul.: Calcd. for $C_{16}H_{11}ClN_4O_4S$: C (49.17), H (2.84), N (14.35). Found: C (49.14), H (2.89), N (14.40).

**(iv) 3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-2-(4-nitrophenyl)-1,3-thiazolidin-4-one 6 (c)**

Yield: 0.3064 (82 %), m.p. 210-212$^0$ C; IR (KBr $\nu_{max}$ cm$^{-1}$): 1675 (C=O), 3285 (NH);

$^1$H NMR (DMSO-d$_6$, 400MHz) δ : 6.92-7.72 (m, 7H, Ar-H), 9.72 (s, 1H, NH), 3.48 (s, 1H, $CH_2$), 5.99 (s, 1H, N-CH). $^{13}$C NMR (DMSO-d$_6$,100 MHz) δ: 168.36, 155.81, 153.64, 152.70, 150.08, 149.33, 147.79, 146.58, 145.69, 140.15, 139.91, 134.39, 132.18, 78.76, 27.95. MS (CI) *m/z*(%): M$^{+1}$, 401. Anul.: Calcd. for $C_{16}H_{11}N_5O_6S$: C (47.88), H (2.76), N (17.45). Found: C (47.79), H (2.72), N (17.41).

**(v)2-(4-methoxyphenyl)-3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-1,3-thiazolidin-4-one6(d)**

Yield: 0.3810 (83 %), m.p. 163-165$^0$ C; IR (KBr $\nu_{max}$ cm$^{-1}$): 1678 (C=O), 3287 (NH); $^1$H NMR (DMSO-d$_6$, 400MHz) δ : 6.89-7.92 (m, 7H, Ar-H), 9.68 (s, 1H, NH), 3.56 (s, 1H, $CH_2$), 5.97 (s, 1H, N-CH). $^{13}$C NMR (DMSO-d$_6$, 100 MHz) δ: 167.18, 156.12, 155.64, 154.55, 152.92, 150.18, 149.76 148.72, 147.68, 144.74, 140.09, 138.67, 136.38, 77.19, 28.62.

MS (CI) *m/z*(%):$M^{+1}$, 386. Anul.: Calcd. for $C_{17}H_{14}N_4O_5S$: C (52.84), H (3.65), N (14.50). Found: C (52.79), H (3.62), N (14.48).

**(vi)2-(4-methylphenyl)-3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-1,3-thiazolidin-4-one 6 (e)**

Yield: 0.3267 (76 %), m.p. 159-162$^0$ C; IR (KBr $\nu_{max}$ $cm^{-1}$): 1677 (C=O), 3286 (NH); $^1$H NMR (DMSO-$d_6$, 400MHz) δ : 6.79-7.98 (m, 7H, Ar-H), 9.54 (s, 1H, NH), 3.52 (s, 1H, $CH_2$), 5.82 (s, 1H, N-CH), 1.8 (s, 3H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 167.52, 154.68, 153.16, 151.61, 150.73, 149.25, 148.18 146.28, 145.58, 143.92, 140.19, 137.35, 135.64, 78.24, 29.61, 15.78. MS (CI) *m/z*(%):$M^{+1}$, 370. Anul.: Calcd. for $C_{17}H_{14}N_4O_4S$: C (55.34), H (3.81), N (15.513). Found: C (55.31), H (3.78), N (15.72).

**(vii)2-(4-hydroxyphenyl)-3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-1,3-thiazolidin-4-one 6 (f)**

Yield: 0.2860 (82 %), m.p. 179-182$^0$ C; IR (KBr $\nu_{max}$ $cm^{-1}$): 1685 (C=O), 3289 (NH); $^1$H NMR (DMSO-$d_6$, 400MHz) δ : 6.82-7.89 (m, 7H, Ar-H), 9.49 (s, 1H, NH), 3.59 (s, 1H, $CH_2$), 5.92 (s, 1H, N-CH), 5.8 (s, H, OH). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 168.31, 156.18, 154.49, 152.73 151.91, 150.38, 149.51 147.36, 146.68, 144.15, 143.38, 138.16, 134.73, 79.46, 28.29. MS (CI) *m/z*(%): $M^{+1}$, 372. Anul.: Calcd. for $C_{16}H_{12}N_4O_5S$: C (51.61), H (3.25), N (15.05). Found: C (51.58), H (3.22), N (15.02).

**(viii)2-(2-chloroquinolin-3-yl)-3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-1,3-thiazolidin-4-one 6 (g)**

Yield: 0.3650 (86 %), m.p. 198-200$^0$ C; IR (KBr $\nu_{max}$ $cm^{-1}$): 1679 (C=O), 3283 (NH); $^1$H NMR (DMSO-$d_6$, 400MHz) δ : 6.78-7.97 (m, 8H, Ar-H), 9.52 (s, 1H, NH), 3.61 (s, 1H, $CH_2$), 5.98 (s, 1H, N-CH). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 168.15, 155.38, 154.65, 153.42, 152.12, 150.06, 150.16 148.48, 147.85, 145.56, 144.71, 143.52, 140.24, 138.73, 132.68, 71.82, 28.87. MS (CI) *m/z*(%):$M^{+1}$, 441, $M^{+2}$, 443. Anul.: Calcd. for $C_{19}H_{12}ClN_5O_4S$: C (51.65), H (2.74), N (15.85). Found: C (51.61), H (2.69), N (15.80).

**(ix)2-(2-chloro-7-nitroquinolin-3-yl)-3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-1,3 thiazolidin-4-one6 (h)**

Yield: 0.2501 (76 %), m.p. 280-282$^0$ C; IR (KBr $\nu_{max}$ $cm^{-1}$): 1675 (C=O), 3291 (NH); $^1$H NMR (DMSO-$d_6$, 400MHz) δ : 6.72-7.95 (m, 7H, Ar-H), 9.57 (s, 1H, NH), 3.49 (s, 1H, $CH_2$), 5.82 (s, 1H, N-CH). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 169.15, 156.09, 155.18, 154.31, 153.60, 150.14, 149.29, 147.98, 146.11, 145.56, 144.72, 141.3, 138.02, 133.49,

131.85, 129.15, 78.88, 29.63. MS (CI) *m/z*(%):$M^{+1}$, 486, $M^{+2}$, 488. Anul.: Calcd. for $C_{19}H_{11}ClN_6O_6S$: C (46.87), H (2.28), N (17.26). Found: C (46.84), H (2.23), N (17.22).

**(x)2-(2-chloro-7-methoxyquinolin-3-yl)-3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-1,3-thiazolidin-4-one 6 (i)**

Yield: 0.3291 (74 %), m.p. 212-214$^0$ C; IR (KBr $\nu_{max}$ $cm^{-1}$): 1671 (C=O), 3295 (NH); $^1$H NMR (DMSO-$d_6$, 400MHz) δ : 6.79-7.98 (m,7H, Ar-H), 9.62 (s, 1H, NH), 3.52 (s, 1H, $CH_2$), 5.89 (s, 1H, N-CH), 4.65 (s, 3H, O-$CH_3$). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 167.89, 156.18, 154.35, 153.98, 152.17, 150.05, 149.40, 148.19, 147.23, 145.71, 143.56, 140.62, 138.33, 137.08, 135.65, 131.19, 77.39, 28.46. MS (CI) *m/z*(%):$M^{+1}$, 471, $M^{+2}$, 473. Anul.: Calcd. for $C_{16}H_{12}N_4O_4S$: C (53.93), H (3.39), N (15.72). Found: C (53.84), H (3.36), N (15.67).

**(xi)2-(2,6-dichloroquinolin-3-yl)-3-[(6-nitro-1,3-benzoxazol-2-yl)amino]-1,3-thiazolidin-4-one 6 (j)**

Yield: 0.3360 (85 %), m.p. 218-220$^0$ C; IR (KBr $\nu_{max}$ $cm^{-1}$): 1684 (C=O), 3296 (NH); $^1$H NMR (DMSO-$d_6$, 400MHz) δ : 6.85-7.82 (m, 7H, Ar-H), 9.62 (s, 1H, NH), 3.58 (s, 1H, $CH_2$), 5.86 (s, 1H, N-CH). $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 168.37, 155.40, 154.39, 153.10, 152.12, 150.75, 149.51 148.48, 145.66, 143.90, 141.94, 136.08, 133.64, 130,50, 125.60, 123.64, 75.81, 30.12. MS (CI) *m/z*(%):$M^{+1}$, 476, $M^{+2}$, 478, $M^{+3}$, 480. Anul.: Calcd. for $C_{19}H_{11}Cl_2N_2O_4S$: C (47.91), H (2.33), N (14.70). Found: C (47.87), H (2.29), N (14.66).

## 3. RESULT AND DISCUSSION

### *Chemistry*

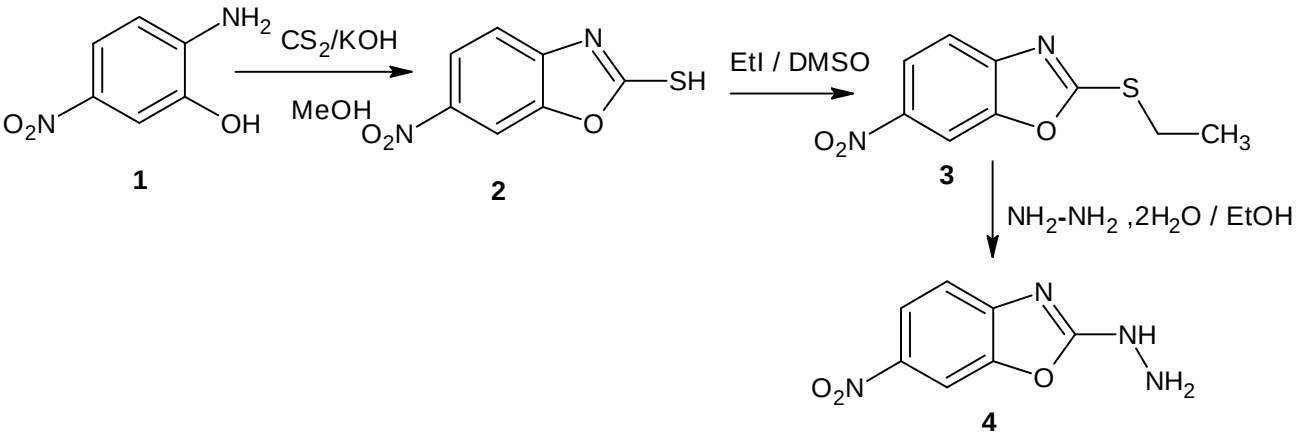
The 2-amino-5-nitrophenol **1** was treated with carbon disulphide and potassium hydroxide in presence of methanol as solvent to give a compound 6-nitro-1,3-benzoxazole-2-thiol **2**. To the compound **2,** iodoethane and sodium hydroxide was added and DMSO was (solvent) to get 2-(ethylsulfanyl)-6-nitro-1,3-benzoxazole **3**. Then the compound **3** was reacted with hydrazine hydride in ethanol to get the intermediate compound 2-hydrazinyl-6-nitro-1,3-benzoxazole **4**[25] **(Scheme1)**. The compound **4** was characterized by $^1$H NMR, which exhibited two singlet's at $\delta$ 4.6 and $\delta$ 9.3 for $-NH_2$ and –NH protons, respectively ($D_2O$ exchangeable). The compound **4** is an intermediate compound to the synthesis of targeted molecule **6(a–j)**.

The derivatives **5(a–j)** were synthesized using 5-chloro-2-hydrazinyl-1,3-benzoxazole **4** on reacts with aromatic aldehyde **(a-j).** The newly synthesized molecules exhibited 1614 $cm^{-1}$ for

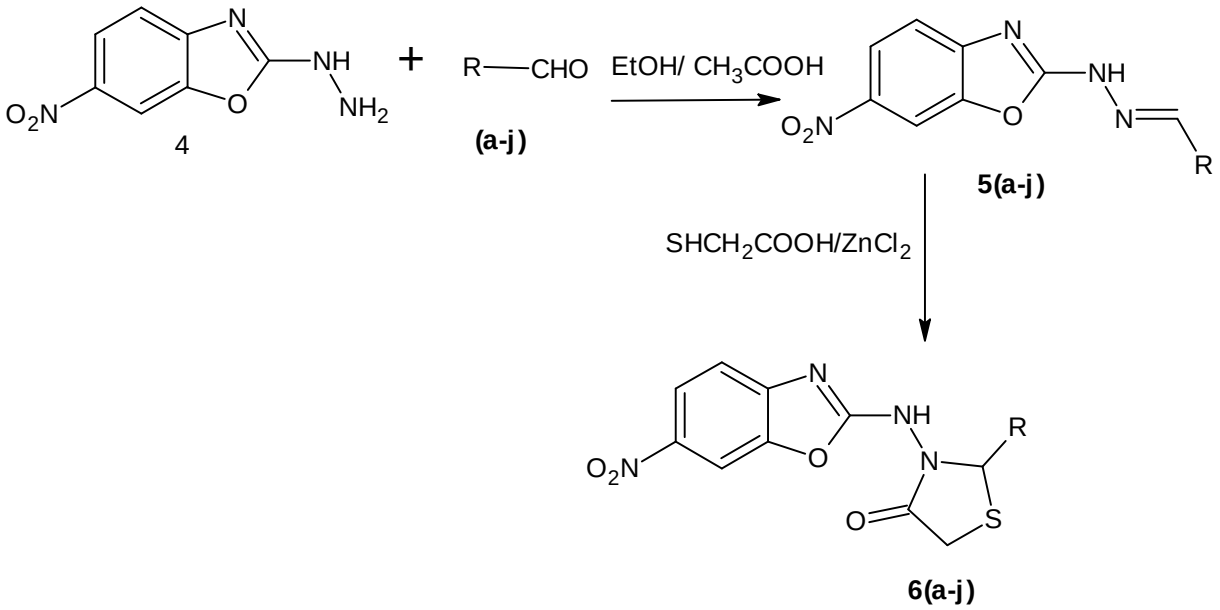
N=CH IR observation and $^{1}$H NMR showed $\delta$ 12.00 (s, N=CH) confirms the disappearance of $NH_2$ proton.

The Schiff's base **5(a–j)** were treated with thioacetic acid and pinch of zinc chloride in presence of dioxane as solvent to forms thioazolidinone derivatives **6(a–j) (Scheme 2)**. The target molecules were confirmed by IR, $^{1}$H NMR, $^{13}$C NMR, Mass, and elemental analysis. IR spectrum showed strong absorption frequency at 1700 $cm^{-1}$ for (C=O), 3287 $cm^{-1}$ for (NH) groups and $^{1}$H NMR value exhibited at $\delta$ 6.83-7.65 (m, 7H, Ar-H), 9.43 (s, 1H, -NH), 3.32 (s, 1H, -$CH_2$), 5.56 (s, 1H, -N-CH). The disappearance N=CH in Schiff's base and forms thioazolidinone ring as cyclization product supports by spectral data's. The Mass spectra's are concurrence with Molecular weights of the target compounds.

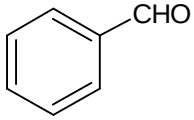
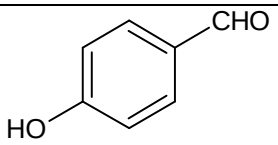
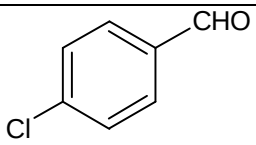
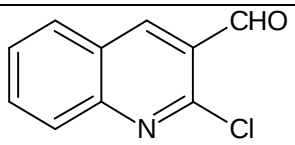
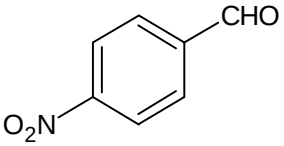
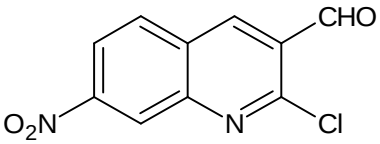
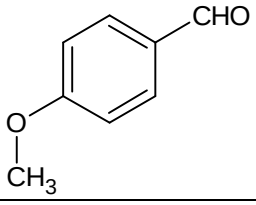
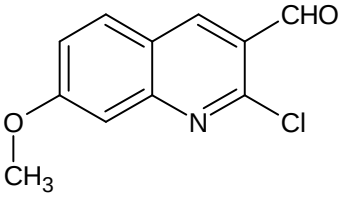
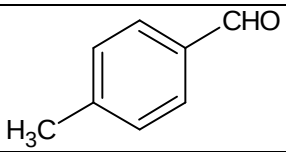
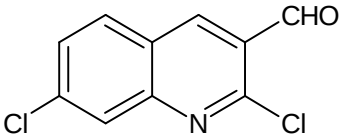
The constructed molecules **6(a-j)** have been screened for antibacterial, minimum inhibition concentration, antioxidant activity and molecular docking studies. In antibacterial study, few compounds have showed potent zone of inhibition **(Table-1 and Figure-1)**. Among the synthesized compounds, marked zone of inhibition of bacteria was observed in compounds **6c, 6f, 6g, 6h, 6i** and **6j,** in comparison of standard drug Chloramphenicol. Compounds **6(a-j)** were evaluated for their minimum inhibitory concentration (**Table-2**) to determine their distinct zone of inhibition of bacteria at different concentration. Compounds **6c, 6f, 6g, 6h**, **6i** and **6j** were found marked zone of inhibition in four different concentrations (25µg/mL, 50µg/mL, 75µg/mL and 100 µg/mL) against gram positive and gram negative bacteria. It was found that quinoline substituent derivatives showed marked zone of inhibition with observation of effective microbial activity results. The selected compound **6c, 6h, 6i** and **6j** exhibited more than 70% of cytotoxicity against Peripheral Blood Mononuclear cancer Cells (**Table-3)** and antioxidant activity was done with effective free radical scavenge (**Table-4** and **Figure-2**). The synthesized compounds interact with receptor **PDB ID: 3FLY** amino acids, which displayed the higher binding energy (**Table-5** and **Figure-3**) for the derivatives **6a**, **6b, 6c, 6d, 6e** and **6f** as compared to other derivatives.




**Scheme-1: Synthetic route for the preparation of compound.**




**Scheme-2: Synthetic route for the preparation of compound 6(a-j)**

| Comp | R | Comp | R |
|---|---|---|---|
| **a** |  | **f** |  |
| **b** |  | **g** |  |
| **c** |  | **h** |  |
| **d** |  | **i** |  |
| **e** |  | **j** |  |

## 4. Biological activity of the synthesized compounds 6(a-j)

### 4.1 Antibacterial Activity

The newly synthesized benzoxazole linked thiazolidinone derivatives were tested for antibacterial activity against bacterial strains, *Escherichia coli* (ATTC-8739), *Staphylococcus aureus* (ATTC-6538), *Vibrio cholera* (ATTC-9027), *Bacillus subtilis* (ATTC-6633), *Staphylococcus epidermidis* (ATTC-12228) and *Salmonella typhimurium* (ATTC-23564) by agar well diffusion method [26]. The 24 hr old Mueller-Hinton broth culture of test bacteria were swabbed on sterile Mueller-Hint on 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 **6(a-j)** (250 µg/ml of 10% DMSO), and control (10% DMSO) were added to the respectively labeled wells. The plates were allowed to stand for 30 minutes and were incubated at $37^0$ C for 24 hr in upright position and the zone of inhibition was recorded and tabulated in **Table-1** and graphically represented in **figure-1**.

**Table 1: Antibacterial activity of compounds 6(a-j).**

| Compounds | Zone of inhibition in mm | | | | | |
|---|---|---|---|---|---|---|
| | Gram positive bacteria | | | Gram negative bacteria | | |
| | S. aureus | B. subtilis | S. epidermis | V. cholerae | S. typhi | E. coli |
| 6a | 22. 01±0.02 | 23.03±0.01 | 21.11±0.04 | 24.01±0.83 | 21.02±0,21 | 20.02±0.07 |
| 6b | 21.00±1.01 | 21.04±0.31 | 20.07±0.25 | 24.01±0.65 | 24.09±0.63 | 19.90±0.32 |
| 6c | **25.39±0.77** | 23.01±0.0 | 24.99±0.90 | 23.02±0.75 | **25.00±0.98** | 21.03±0.33 |
| 6d | 21.00±0.98 | 20.00±0.98 | 22.00±0.54 | 23.07±0.64 | 22.99±0.22 | **23.05±0.88** |
| 6e | 19.00±0.85 | 18.98±0.69 | 21.47±0.49 | 19.06±0.55 | 22.68±0.9 | 18.54±0.23 |
| 6f | 24.00±0.48 | 21.09±1.20 | 23.29±0.22 | 24.02±0.77 | 23.19±9.90 | 20.28±09 |
| 6g | 20.99±0.66 | 22.08±0.43 | **25.06±0.0** | 24.09±0.76 | 24.08±0.22 | 19.00±0.78 |
| 6h | 24.57±0.45 | 24.32±0.85 | 23.02±0.66 | **25.02±0.00** | **26.00±0.99** | 22.02±0.12 |
| 6i | 24.77±0.26 | 23.92±0.37 | 22.09±0.75 | **26.99±0.24** | **25.98±0.56** | 20.98±0.29 |
| 6j | 22.67±9.09 | **26.23±0.39** | 25.09±2.98 | 24.88±0.27 | **25.09±0.39** | 21.09±1.29 |
| DMSO | 00 | 00 | 00 | 00 | 00 | 00 |
| Std | **28.55±98** | **29.29±0.00** | **28. 09±0.33** | **28.00±0.88** | **30.38±0.44** | **26.09±0.36** |

**Standard:** Chloramphenicol **Solvent:** DMSO

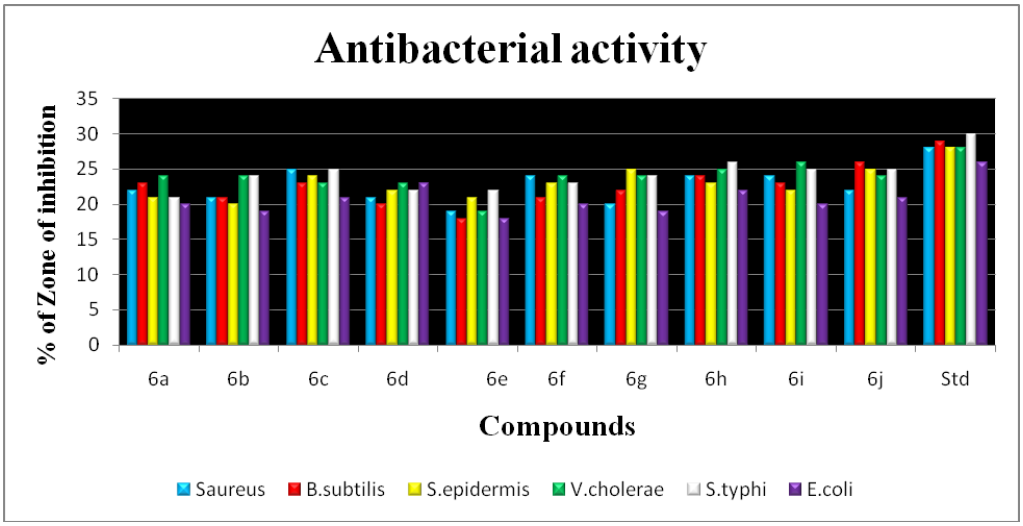



**Figure-1: Antibacterial activity of compounds 6(a-j).**

### 4.2 Minimum Inhibitory Concentration (MIC).

The MIC of all the synthesized compounds 6(b, c, f, g, h, i &j) were determined by micro dilution method [27]. The respective clinical strains were spread separately on the medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The synthesized compounds at different concentrations (25, 50, 75 and 100 $\mu$g/ml), were loaded into corresponding wells and Controls (10% DMSO) were added to the respectively labeled wells. The drugs Chloramphenicol were used as standard for the comparison of antibacterial activities, respectively. The results were recorded in mm and presented in **Table-2.**

**Table 2: Minimum inhibition concentration of compounds 6(b, c, f, g, h, i &j).**

| | Gram positive bacteria | | | | | | | | | | | | Gram negative bacteria | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S. Aureus | | | | B. Cereus | | | | S. Epidermis | | | | V. cholerae | | | | S. Typhi | | | | E. Coli | | | |
| Comp | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 |
| **6b** | 19 | 20 | 22 | 24 | **21** | **24** | **23** | **22** | 16 | 18 | 18 | 20 | 15 | 19 | 18 | 19 | **19** | **21** | **23** | **25** | 18 | 19 | 19 | 22 |
| **6c** | **20** | **22** | **23** | **25** | 19 | 20 | 20 | 22 | 17 | 19 | 19 | 21 | 18 | 19 | 20 | 21 | 18 | 19 | 20 | 21 | 20 | 21 | 22 | 23 |
| **6f** | **21** | **22** | **25** | **23** | 16 | 17 | 17 | 19 | **20** | **22** | **23** | **25** | 16 | 18 | 19 | 21 | 16 | 19 | 19 | 20 | **20** | **22** | **23** | **25** |
| **6g** | 16 | 17 | 17 | 19 | 19 | 21 | 19 | 20 | 16 | 19 | 19 | 20 | 18 | 19 | 19 | 20 | **18** | **19** | **25** | **23** | **19** | **20** | **22** | **25** |
| **6h** | 19 | 21 | 22 | 24 | 17 | 18 | 19 | 20 | 15 | 17 | 18 | 19 | **21** | **22** | **24** | **25** | **20** | **22** | **26** | **22** | 16 | 18 | 20 | 24 |
| **6i** | 14 | 16 | 16 | 18 | 17 | 18 | 19 | 20 | 18 | 19 | 20 | 21 | **21** | **22** | **23** | **25** | **19** | **21** | **23** | **25** | 16 | 17 | 17 | 19 |
| **6j** | 18 | 18 | 20 | 21 | 17 | 19 | 21 | 22 | **19** | **21** | **23** | **25** | 18 | 20 | 22 | 23 | 17 | 19 | 21 | 22 | **21** | **23** | **23** | **25** |
| **DMSO** | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| **STD** | 29 | | | | 30 | | | | 29 | | | | 28 | | | | 29 | | | | 28 | | | |

**Standard:** Chloramphenicol **Solvent:** DMSO

### 4.3 Cytotoxic activity

**Preparation of Peripheral Blood Mononuclear Cells (PBMCs) or Buffy Coat**

Blood samples from healthy volunteers were collected by venipuncture and transferred into 2 ml heparin coated vacutainers. It was diluted to 1:1 ratio with PBS (Phosphate buffer solution, pH 7.0) layered onto 4 mL Ficol without getting mixed up. It was further separated by centrifuging at 1,000 rpm for 30 min at room temperature. During the centrifugation the PBMCs move from plasma and suspend as the density gradient. Plasma was removed down to 1 cm above buffy coat and discarded the white layer lying on top of the red cells. The buffy coat layer was washed twice with PBS. Roswell Park Memorial Institute (Gibco, Life Technologies) medium was prepared by mixing 10 ml of Fetal bovine serum (Invitrogen) and 200µl antimycotic [Antibiotic antimycotic solution with Streptomycin (10mg/20ml), 10,000 U Penicillin, Amphotericin B and 0.9% normal saline]. This mixture (4ml) was dispensed into falcon tubes, 30µL ofPhytohemagglutin (Invitrogen) and 200µl of PBMCs were incubated at the atmosphere of 95% air and 5% $CO_2$ at 37°C for 4 hr.[27]

About **10µg/ml**, **50µg/ml** and **100 µg/ml** of the compounds of 1ml were added to the respectively labeled PBMCs tubes and incubated for 72 hr at the earlier mentioned conditions. After 72 hr, cell viability was determined by the trypan-blue dye exclusion method.[28]

Trypan blue exclusion test cells were clarified by centrifuging at 1000 rpm for 30 min at room temperature. The supernatant liquid was discarded and to the solution 10µL of PBMCs, 10µL of tryphan blue was added and incubated for 10min at room temperature. About 10µL of incubated sample was loaded on previously cleaned Haemocytometer and counted the number of live cells, total cells and dead cells at four corners under Trinucular microscope,

Nikon Eclipse E200. The percentage of cell viability and non-viability was tabulated in Table-3.

**Table 3: Cytotoxic activity of newly synthesized derivatives against PBMCs.**

| Sample | Total cells | Live cells | Dead cells | % of Cells viability | %of cells non-viability |
|---|---|---|---|---|---|
| 6c-10µg/ml | 78 | 29 | 49 | 37.1 | 62.8 |
| 6c-50µg/ml | 82 | 38 | 44 | 46.3 | 53.7 |
| **6c-100µg/ml** | **186** | **46** | **140** | **24.73** | **75.26** |
| **6h-10µg/ml** | **162** | **42** | **120** | **25.92** | **74** |
| 6h-50µg/ml | 188 | 84 | 104 | 44.68 | 55.32 |
| 6h-100µg/ml | 88 | 45 | 43 | 51.13 | 48.86 |
| 6i-10µg/ml | 171 | 63 | 108 | 36.8 | 63.2 |
| **6i-50µg/ml** | **163** | **48** | **115** | **29.4** | **70.6** |
| 6i-100µg/ml | 96 | 57 | 39 | 59.38 | 40.62 |
| 6j-10µg/ml | 154 | 61 | 93 | 39.6 | 60.4 |
| 6j-50µg/ml | 119 | 48 | 71 | 40.33 | 59.66 |
| **6j-100µg/ml** | **136** | **40** | **96** | **29.4** | **70.6** |
| Control | 118 | 17 | 101 | 14.4 | 85.5 |

### 4.4 Antioxidant Activity

***DPPH Assay***

The free radical scavenging ability of synthesized compounds **6(a-j)** and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on a DPPH free radical. Different concentrations (5, 10, 15, 20 and 25 $\mu$g/ml) of compounds and standard were prepared in methanol. In clean and labeled test tubes, 3 ml of DPPH solution (0.002% in methanol) was mixed with 00, 05, 10, 15, 20 and 25 $\mu$g/ml of different concentrations of compounds and standard separately, makeup the solution up to 4 ml by adding methanol. 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 results were tabulated in **Table-4** and graphically represented in **figure-2**. 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.[30]

**Table 4: antioxidant activity of the compound 6(a-j).**

| Compounds | Scavenging activity of different concentration (µg/ml) in % | | | | |
|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 |
| 6a | 28.36 | 32.59 | 33.61 | 38.93 | 44.67 |
| 6b | 50.81 | 53.00 | 54.40 | 58.78 | 62.38 |
| 6c | **73.96** | **78.68** | **82.39** | **87.94** | **88.54** |
| 6d | 50.92 | 60.18 | 64.73 | 67.12 | 70.15 |
| 6e | **75.08** | **78.38** | **81.69** | **82.63** | **85.37** |
| 6f | 53.67 | 58.30 | 61.71 | 64.38 | 69.09 |
| 6g | **78.02** | **84.29** | **87.64** | **89.38** | **92.15** |
| 6h | **80.56** | **80.69** | **82.60** | **83.63** | **84.78** |
| 6i | **78.68** | **82.32** | **85.56** | **86.37** | **91.67** |
| 6j | **81.19** | **83.38** | **84.95** | **85.61** | **88.60** |
| Ascorbic acid | 86.71 | 88.57 | 90.04 | 93.62 | 96.33 |

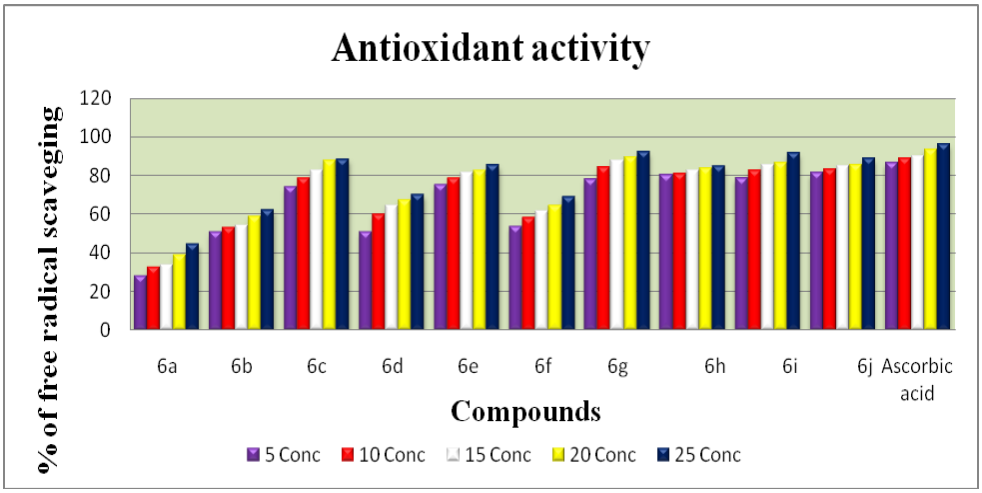



**Figure-2: antioxidant activity of the compound 6(a-j).**

### 4.5 Molecular docking studies

Molecular docking study was performed with the Hex molecular modeling package version 8.2.[31] Docking study of the synthesized compounds **7(a-j)** were evaluated against the receptor (*Pdb: 3MGN*). In the present study, an effort was made to evaluate their antioxidant behavior, we have selected receptor (*Pdb: 3MGN*) to obtained docking scores (binding interaction energy). The results were tabulated in **Table-5** and interaction modes are shown in **figure-3**. The synthesized molecules **6(a-j)** binds with various amino acids in the receptor (*Pdb: 3MGN*) in the active pocket sites and given a molecular interaction energy (E-total value) in range of -243.37 to -271.12 (Kcal/mol). The benzoxazole linked qunoline compounds such as **6e, 6g, 6h, 6i** and **6j** showed higher binding energy as compared with the compounds **6a, 6b, 6c, 6d,** and **6f**. The estimated binding affinity of molecules **6(a-j)** with the complex hydrogen network and other interactions with amino acids were PHE128, LEU97,

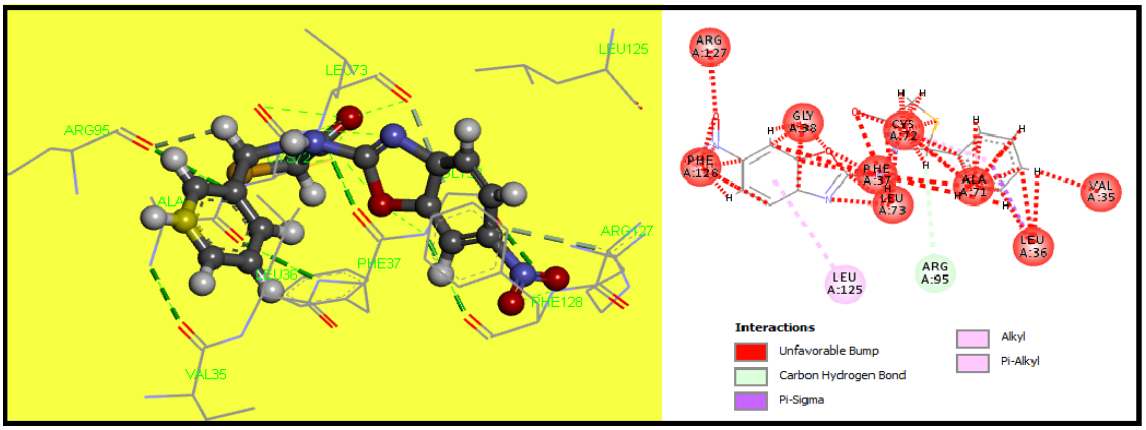
VAL39, LEU110, LEU125, GLY38, ARG127, HIS51, PHE37, GLY38, ARG127, HIS51, LEU36, VAL39, LEU127, LEU96, CYS72, LEU97, GLY38, ARG127, PHE37, MET130, LEU36, PHE29, ALA71, LEU97, VAL39, PRO40, LEU125, ARG127 and LEU36, which were presented in active sites receptor respectively. It explained the role of hydrogen bond formation and other interactions for effective enzyme binding.

**Table 5: Docking result of synthesized compounds in the binding site of receptor.**

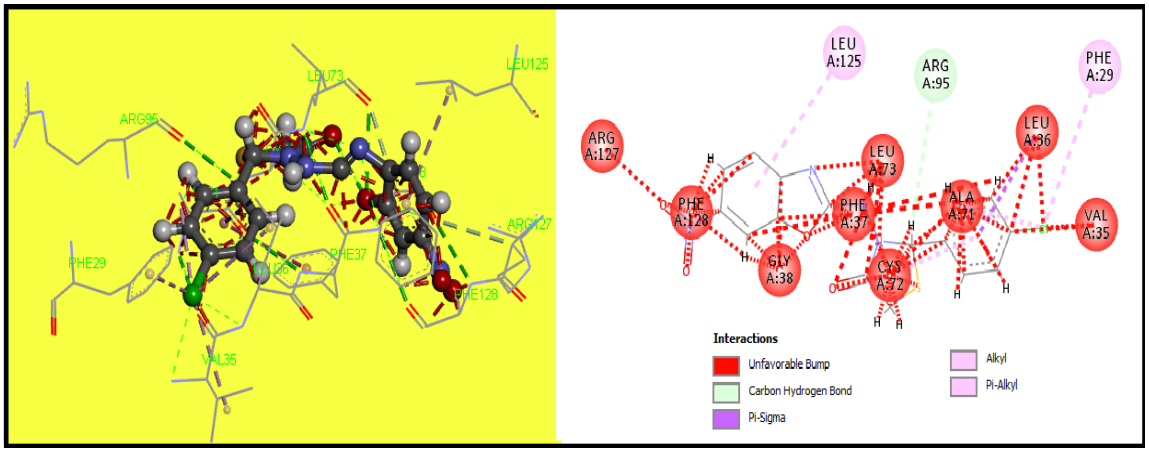
| Entry | Receptor PDB code | ΔG (Kcal/mol) |
|---|---|---|
| **6a** | 3MGN | *-252.19* |
| **6b** | 3MGN | *-243.37* |
| **6c** | 3MGN | *-245.12* |
| **6d** | 3MGN | *-254.31* |
| **6e** | 3MGN | ***-259.13*** |
| **6f** | 3MGN | *-244.10* |
| **6g** | 3MGN | ***-266.41*** |
| **6h** | 3MGN | ***-266.41*** |
| **6i** | 3MGN | ***-271.12*** |
| **6j** | 3MGN | ***-266.35*** |

a. A close-up three dimensional view of the docked pose of compounds structure were shown in the surface model and the ligand have been shown in the ball and stick model (colors by atom).

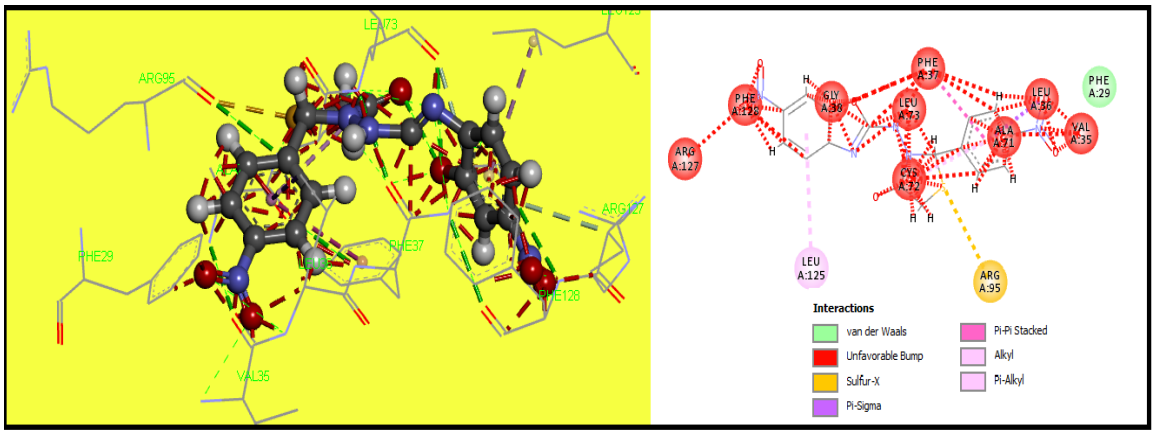
b. Two dimensional interactions of synthesized compounds with receptor *PDB: 3MGN*.



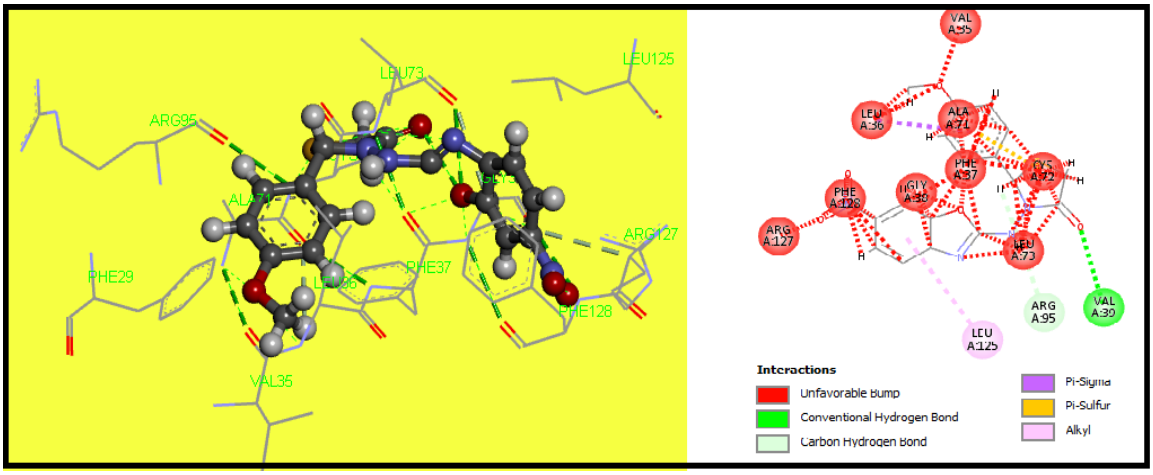

**Structure 6a: 3D & 2D view**



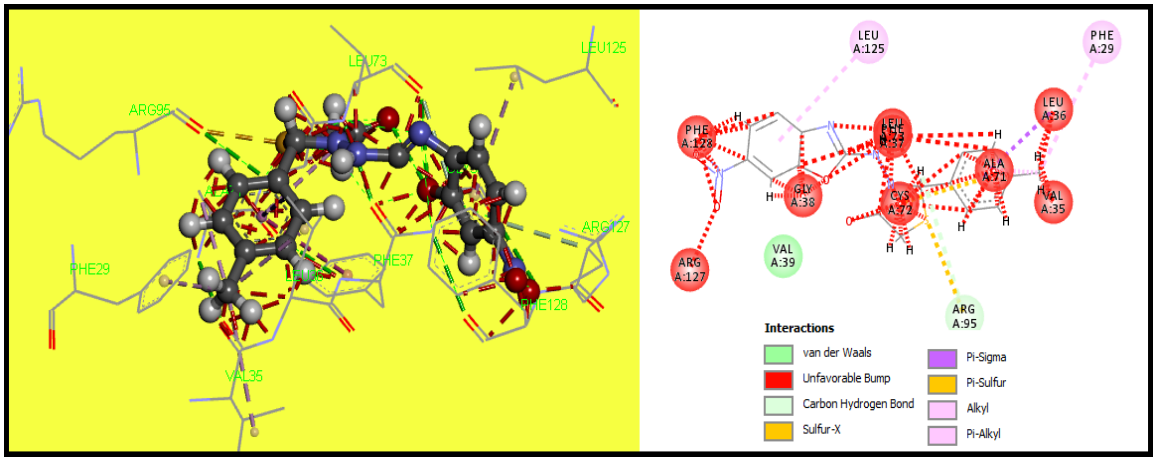

**Structure 6b: 3D & 2D view**



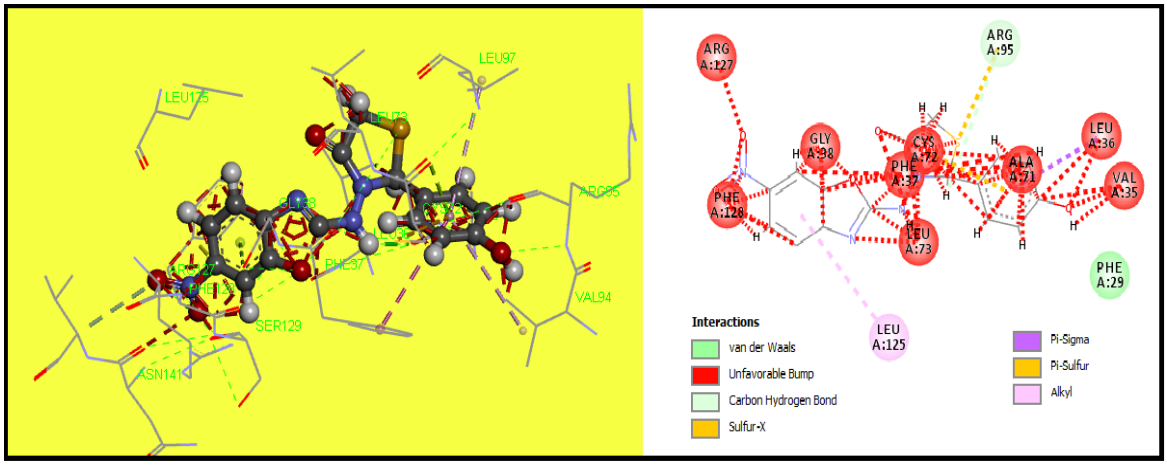

**Structure 6c: 3D & 2D view**



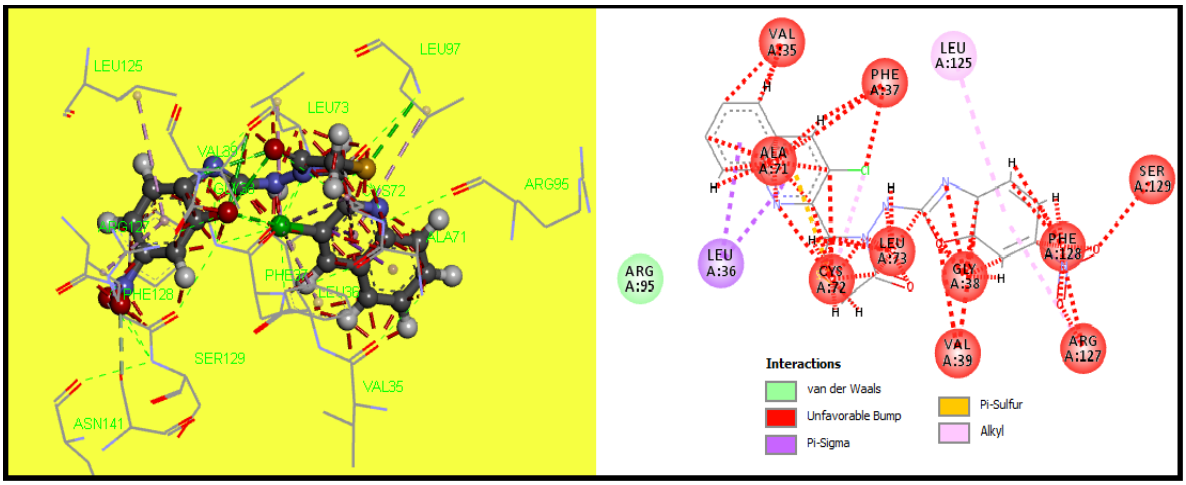

**Structure 6d: 3D & 2D view**



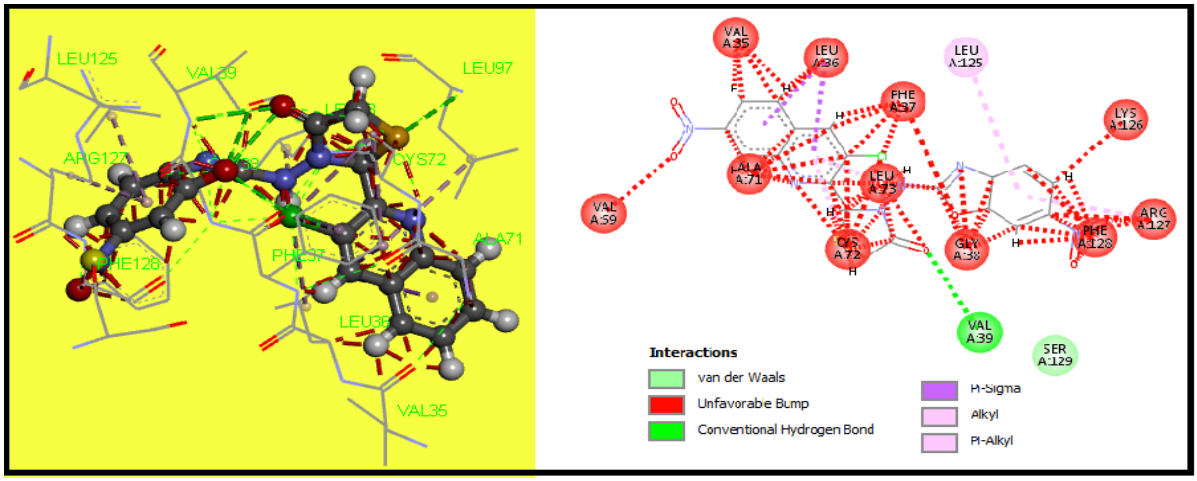

**Structure 6e: 3D & 2D view**



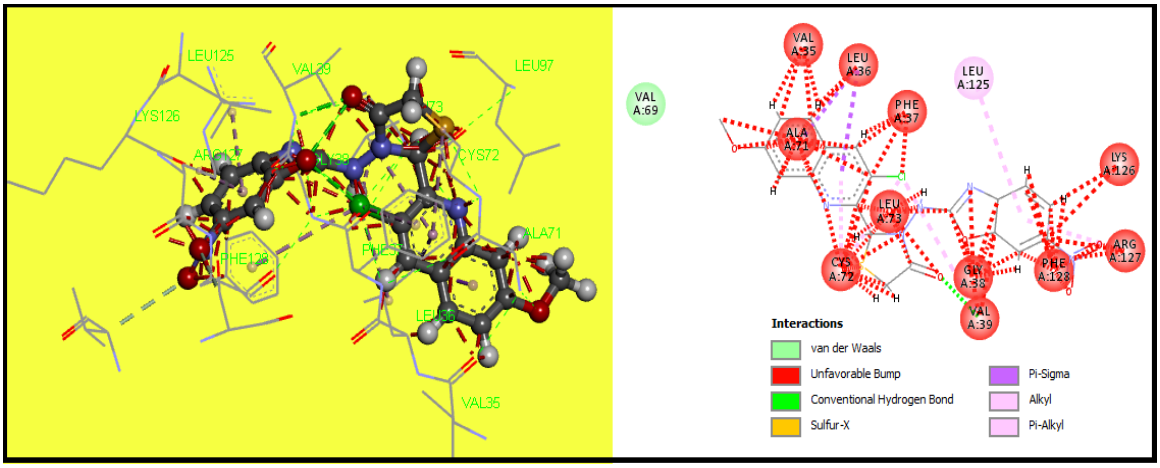

**Structure 6f: 3D & 2D view**



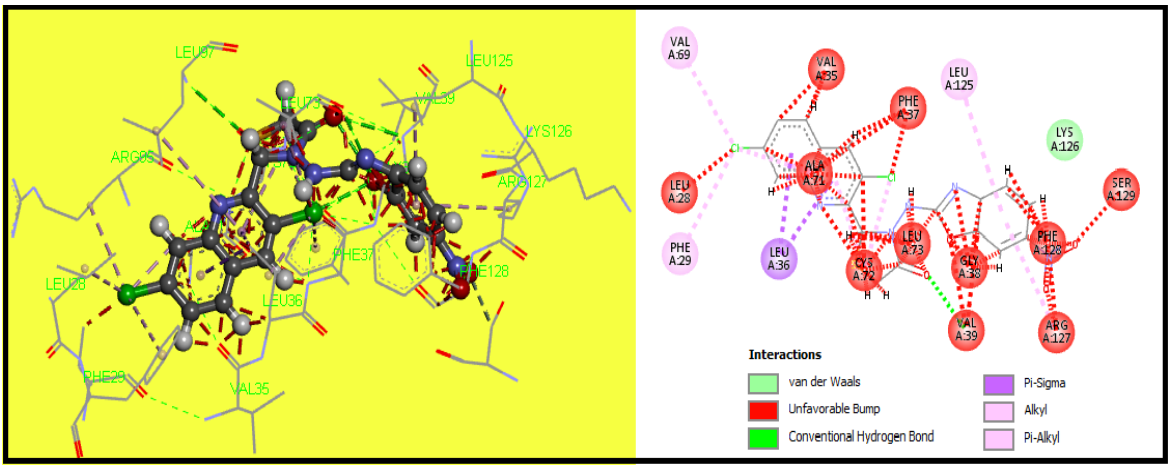

**Structure 6g: 3D & 2D view**




**Structure 6h: 3D & 2D view**




**Structure 6i: 3D & 2D view**




**Structure 6j: 3D & 2D view**

**Figure-3: Three dimensional and two dimensional Interactions of compounds 6(a-j) with the active site of receptor (*PDB*: *3MGN*).**

## 5. CONCLUSIONS

A series of benzoxazole with thiazolidine derivatives were synthesized by the cyclization of substituted Schiff's base with thioglycolic acid. The newly synthesized molecules were characterized by IR, $^{1}$H NMR and mass spectral analysis. To all the compounds, the antibacterial, minimum inhibition concentration, antioxidant activity and molecular docking studies were evaluated. All synthesized benzoxazole with thiazolidine derivatives **6(a-j)** exhibited promising antimicrobial activity against strains. The selected compound **6c, 6f, 6g, 6h, 6i** and **6j** was tested minimum inhibitory concentration, which showed good zone of inhibition at four different concentrations (25 µg/mL, 50 µg/mL, 75 µg/mL and 100 µg/mL) against gram positive and gram negative bacteria. The cytotoxicity of selected benzoxazole derivatives **6c, 6h, 6i** and **6j** displays potent activity against Peripheral Blood Mononuclear Cells. The synthesized compounds were introduced to DPPH free radical scavenging, in which the compounds **6c**, **6e**, **6g, 6h**, **6i** and **6j** showed good free radical scavenging. The synthesized compounds were docked into the plausible target DPPH **(PDB ID: 3MNG).** The docking scores or the interaction binding energies of the target enzyme confirmed the antioxidant of the synthesized molecules. The final conclusion of the present work is benzoxazole linked thiazolidinine with qunoline derivatives such as **6g, 6h**, **6i** and **6j** shows a effective antimicrobial , MIC, antioxidant activity and *in silico* molecular docking studies as compare to other derivatives, it is due to presence of combined effect of benzoxazole linked thiazolidinine with qunoline molecules.

In view of this study, further research to be carried out on the development of new effective anticancer agent by the modification of different functional group in the target compounds.

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Arylidene-2-thioxodihydropyrimidine-4,6(1H,5H)-diones and 3-Thioxo-2,3-dihydro-1H-imidazo[1,5-a]indol-1-ones are LightDependent Tumor Necrosis Factor- Antagonists, Bioorg Med Chem Lett, 2003; 13: 533-541.

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