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Original Article

Synthesis, Characterization and Evaluation of Cytotoxic, antibacterial and Molecular Docking Studies of Fused Heterocyclic 6aH,13H benz[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-b]quinolin-13-one derivatives

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ABSTRACT

The novel heterocyclic compounds 6a*H*,13*H-enz*[4,5]oxazole[2,3,2,3][1,3]thiazino[6,5-*b*]quinolin-13-one derivatives 4-15 have been synthesized by conventional method. The various derivatives of 1,3-benzoxazole-2-thiol were on treating with 2-chloroquinoline-3-carbaldehyde derivatives in DMF yielded target novel molecules 4-15. The obtained products have been characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. The newly synthesized compounds were screened for their *in vitro* cytotoxic, antibacterial and molecular docking studies. The synthesized compounds 9-Chloro-10-nitro-6a*H*,13*Hbenz* [4',5']oxazole [2',3',:2,3] [1,3]thiazino[6,5*b*]quinolin-13 one 6, 2,10-dichloro-6a*H*, 13*H benz* [4',5'] oxazole [2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 9 and 2,8,10-Trichloro-6a*H*,13*Hbenz* [4',5'] oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 15 exhibited potent cytotoxic activity towards Peripheral Blood Mononuclear Cells (PBMCs) with the influence of functional groups attached with central moiety. The cytotoxic results were further supported by molecular interaction by molecular docking studies with receptor PDB ID: 3FLY and showed a minimum binding energy and higher affinity towards the active pocket sites. The study also focused on screening of antibacterial activity and most of the compounds from the series exhibited considerable bacterial inhibition.

Keywords: Thiazino, quinolone, cytotoxic, Peripheral Blood Mononuclear Cells and molecular docking.

1. INTRODUCTION

Benzoxazoles are privileged class of organic compounds of medicinal significance due to their recognized biological chemotherapeutic activities[1,2]. Benzoxazole derivatives exhibit antimicrobial[3-5], antiviral[6,7], multi-drug resistance cancer cell[8] with inhibitory activity on eukaryotic

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topoisomerase II enzyme in cell-free system[9-11]. Recently Anusha and Rao *et al*.,[12] reported the synthesis and biological evaluation of benzoxazole derivatives as new antimicrobial agents. Mary *et al*., reported the vibrational spectroscopic and SAR studies of some benzoxazole derivatives[13,14]. Fighting against bacterial infections has resulted in the development of a wide variety of antibiotics. Infectious diseases are due to Gram- positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), *vancomycin-resistant Enterococcus faecium* (VREF) and penicillin resistant *Streptococcus pneumonia* (PRSP) cause morbidity and mortality today[15]. Besides, during the past 20 years an increase in invasive fungal infection, particularly in immuno suppressed patients, has been observed, which are now considered to be the causes of illness and humanity as well. Therefore, there is still need for new antifungal and antibacterial agents[16].

Quinoline moiety is of great importance to chemists as well as biologists because it is found in a large variety of naturally occurring compounds and also in chemically useful molecules having diverse biological activities. Many quinoline containing compound exhibited a wide spectrum of pharmacological activities such as antibacterial[17], antimalarial, antiplasmodial and anticancer[18]. The pharmacological properties of quinoline and their derivatives had attracted worldwide attention in the last decades because of their wide occurrence in natural products. On the basis of these observations we have planned and synthesized the bioactive benzoxazole fused with quinoline moiety, which showed the comparable biological activities.

Broad therapeutic spectrum of compounds intrinsically possesses cytotoxicity. Structural modification on lead anticancer compounds may eliminate or reduce cytotoxicity to a minimum level. Usually, benzoxazole linked quinolone analogues are known as more selective, less toxic and more active improved leads. Often, substitutions found to be bioactively advantageous on active scaffold on introducing to parent nucleus leading to the enhancement of bioactivity[19-22]. Since the benzoxazole skeleton, which is responsible for selective cytotoxicity of UK-1[23], the synthesis and cytotoxic studies of the C-2 and N-3 substituted benzoxazole derivatives was under taken employing simple and straight forward chemical transformations. In the present study the fused cyclic qunoline with benzoxzoles **4-15** were synthesized. The newly synthesized compounds were tested for antimicrobial activity and cytotoxic study towards Peripheral blood mononuclear cells (PBMC) in molecular docking investigation.

2. EXPERIMENTAL SECTIONS

***(i)* Synthesis of 1,3-benzoxazole-2-thiol 1**

To the solution of methanol (50 ml) and KOH (1.1eq), carbon disulphide (1.1eq) was added slowly at room temperature. To the reaction mass, 2-aminophenol (1.eq) was added with stirring. The reaction mass was refluxed for 6 hr on water bath. Completion of the reaction was monitored by TLC. The reaction mixture was poured to a beaker containing ice cold water and acidified with glacial acetic acid (pH 6). The obtained solid was filtered, dried and recrystallized using ethanol to get the compound 1,3-benzoxazole-2-thiol **1**.

The different derivatives of 1,3-benzoxazole-2-thiol were synthesized by similar method by using various 2-amino phenols.

Colour: white; IR (KBr, cm^{-1}): 3386 cm^{-1} (-SH); ^{1}H NMR (DMSO-d6, δ ppm): 7.3(s, H Ar-H),6.9(dd, H Ar-H), 7.1(dd, H Ar-H) 13.7(s, H-SH); ^{13}C NMR (DMSO-d6, δ ppm): 125-150 (7C, Ar-C); M^+,196.

(ii) Synthesis of 2-chloroquinoline-3-carbaldehyde 2

Acetanilide (2g) was dissolved in DMF (7 mL) and cooled the solution to 0^0C. The cold reaction mixture was stirred for 10 min and slowly $POCl_3$ was added drop by drop to the cold solution. The reaction temperature was maintained to 0^0C and refluxed for 5 hr. The completion of reaction was checked by TLC. The reaction mass was poured onto crushed ice to get solid product. The obtained solid was filtered, dried and recrystallize using ethyl acetate to get compound 2-chloroquinoline-3-carbaldehyde **2**.

The 2,6-Dichloroquinoline-3-carbaldehyde derivative **3** was prepared by similar method using chloro substituted acetophenone.

Colour: white; IR (KBr, cm^{-1}): 1696 cm^{-1} (-C=O); ^{1}H NMR (DMSO-d6, δ ppm): 8.54(s, H Ar-H), 7.31(d, 2H Ar-H), 7.25(m, 2H Ar-H);^{13}C NMR (DMSO-d6, δ ppm): 125-150 (7C, Ar-C); 175 (C, -C=O); M^+,191, M^{+2},193.

***(iii)* Synthesis of 13*H benz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 4**

The compound **1**[24] (0.01mol) was treated with 2-chloroquinoline-3-carbaldehyde **2** (0.01mol) in presence of DMF used as a solvent and refluxed for 8 hr**.** Then the reaction mixture was poured onto crushed ice. The solid product thus obtained was filtered, dried and recrystallized from ethanol to get compound **4.**

The compounds **5-15** have been prepared by following same procedure.

Colour: white; IR (KBr, cm^{-1}): 1670 cm^{-1} (C=O); ^{1}H NMR (DMSO-d6, δ ppm): 8.24(m, 2H Ar-H), 8.07(d, 2H Ar-H), 8.33(s, H Ar-H), 7.52(d, 2H Ar-H), 7.78(m, 2H Ar-H), 6.52(s, H -H); ^{13}CNMR (DMSO-d6, δ ppm): 112-155(17C, Ar-C); 172(1C, C=O); M^+,306.

***(iv)* Synthesis of 10-chloro-6a*H*,13*H benz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5*b*]quinolin-13-one 5**

Colour: cream; IR (KBr, cm^{-1}): 1672 cm^{-1} (C=O); ^{1}H NMR (DMSO-d6, δ ppm): 8.37(s, H Ar-H), 8.02(dd, H Ar-H), 8.25(dd, H Ar-H), 7.88(s, H Ar-H), 7.68(d, 2H Ar-H), 7.29(m, 2H Ar-H), 6.59(s, H -H); ^{13}C NMR (DMSO-d6, δ

ppm): 115-158(17C, Ar-C), 178(1C, C=O); M^{+},340, M^{+2},343.

***(v)* Synthesis of 10-nitro-6a*H*,13*H*benz[4',5']oxazole[2',3',:2,3][1,3]thiazino [6,5*b*]quinolin-13 one 6**

Colour: yellow; IR (KBr, cm^{-1}): 1674 cm^{-1} (C=O); 1220 cm^{-1} (-NO_2); ^{1}H NMR (DMSO-d6, δ ppm): 8.47(s, H Ar-H), 8.26(s, H Ar-H), 8.38(s, H Ar-H), 7.85(d, 2H Ar-H), 7.46(m, 2H Ar-H), 6.63(s, H -H); ^{13}CNMR (DMSO-d6, δ ppm): 111-160(17C, Ar-C), 175(1C, C=O); M^{+},385, M^{+2},388.

***(vi)* Synthesis of 10-methyl-6a*H*,13*H*benz[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*] quinolin-13-one 7**

Colour: white; IR (KBr, cm^{-1}): 1675 cm^{-1} (C=O); 2900 cm^{-1} (-CH_3); ^{1}H NMR (DMSO-d6, δ ppm): 8.24(s, H Ar-H), 8.18(dd, H Ar-H), 7.34(dd, H Ar-H), 7.92(s, H Ar-H), 7.48(d, 2H Ar-H), 7.73(m, 2H Ar-H), 6.59(s, H -H), 2.6(s, 3H, –CH_3); ^{13}C NMR (DMSO-d6, δ ppm): 113-158(7C, Ar-C), 180(1C, C=O), 22-24(1C, CH_3-C); M^{+},320.

***(vii)* Synthesis of 9-nitro-6a*H*, 13*H* *benz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 8**

Colour: dark yellow; IR (KBr, cm^{-1}): 1673 cm^{-1} (C=O), 1120 cm^{-1} (-NO_2); ^{1}H NMR (DMSO-d6, δ ppm): 8.39(s, H Ar-H), 8.52(dd, H Ar-H), 8.26(dd, H Ar-H), 8.09(s, H Ar-H), 7.75(d, 2H Ar-H), 7.62(m, 2H Ar-H), 6.63(s, H -H); ^{13}C NMR (DMSO-d6, δ ppm): 112-158(7C, Ar-C), 176(1C, C=O); M^{+},351.

***(viii)* Synthesis of 8,10-dichloro-6a*H*, 13*H* *benz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 9**

Colour: brown; IR (KBr, cm^{-1}): 1678 cm^{-1} (C=O); ^{1}H NMR (DMSO-d6, δ ppm): 8.37(s, H Ar-H), 8.23(s, H Ar-H), 7.92(s, H Ar-H), 7.72(d, 2H Ar-H), 7.46(m, 2H Ar-H), 6.71(s, H -H);^{13}C NMR (DMSO-d6, δ ppm): 113-154(7C, Ar-C), 177(1C, C=O); M^{+},375, M^{+2},377, M^{+4},379.

***(x)* Synthesis of 2-chloro-6a*H*,13*H* *benz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5*b*]quinolin-13-one 10**

Colour: grey; IR (KBr, cm^{-1}): 1678 cm^{-1} (C=O); the ^{1}H NMR (DMSO-d6, δ ppm): 8.53(m, 2H Ar-H), 8.37(d, 2H Ar-H), 8.18(s, H Ar-H), 7.97(s, H Ar-H), 7.62(dd, H Ar-H), 7.74(dd, H Ar-H), 6.55(s, H -H); ^{13}C NMR (DMSO-d6, δ ppm): 112-158(7C, Ar-C), 178(1C, C=O); M^{+},341, M^{+2},343.

***(ix)* Synthesis of 2,10-dichloro-6a*H*, 13*H* *benz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 11**

Colour: brown; IR (KBr, cm^{-1}): 1675 cm^{-1} (C=O);^{1}H NMR (DMSO-d6, δ ppm):8.42(s, H Ar-H), 8.21(dd, H Ar-H), 8.12(dd, H Ar-H), 7.26(s, H Ar-H), 7.47(s, H Ar-H), 7.64(dd, H Ar-H), 7.52(dd, H Ar-H), 6.63(s, H -H); ^{13}CNMR (DMSO-d6, δ ppm): 112-158(7C, Ar-C), 182(1C, C=O); M^{+},375, M^{+2},377, M^{+4},379.

***(x)* Synthesis of 2,9-dichloro-10-nitro-6a*H*,13*H*benz[4',5']oxazole[2',3',:2,3][1,3]thiazine [6,5*b*]quinolin-13-one 12**

Colour: yellow; IR (KBr, cm^{-1}): 1678 cm^{-1} (C=O); 1124 cm^{-1} (-NO_2); ^{1}H NMR (DMSO-d6, δppm): 8.38(s, H Ar-H), 8.23(s, H Ar-H), 8.06(s, H Ar-H), 7.95(s, H Ar-H), 7.74(dd, H Ar-H), 7.62(dd, H Ar-H), 6.56(s, H -H); ^{13}C NMR (DMSO-d6, δ ppm): 112-158(7C, Ar-C), 187(1C, C=O); M^{+},420, M^{+2},422, M^{+4}, 424.

***(xi)* Synthesis of 2-chloro-10-methyl-6a*H*,13*H*benz[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 13**

Colour: light brown; IR (KBr, cm^{-1}): 1671 cm^{-1} (C=O);^{1}H NMR (DMSO-d6, δ ppm): 8.21(s, H Ar-H), 7.98(dd, H Ar-H), 7.77 (dd, H Ar-H), 8.32(s, H Ar-H), 7.73(s, H Ar-H), 7.46(dd, 2H Ar-H), 7.35(dd, 2H Ar-H), 6.65(s, H -H), 2.45(s, 3H -CH_3); ^{13}C NMR (DMSO-d6, δ ppm): 112-158(7C, Ar-C), 173(1C, C=O); M^{+},355, M^{+2},357.

***(xii)* Synthesis of 2-chloro-9-Nitro -6a*H*, 13*H* *benz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 14**

Colour: yellowish; IR (KBr, cm^{-1}): 1675 cm^{-1} (C=O); 1124 cm^{-1} (-NO_2); ^{1}H NMR (DMSO-d6, δ ppm): 8.27(s, H Ar-H), 8.20 (dd, H Ar-H), 7.95(dd, H Ar-H), 7.98(s, H Ar-H), 7.84(s, H Ar-H), 7.73(dd, H Ar-H), 7.88(dd, H Ar-H), 6.54(s, H -H); ^{13}C NMR (DMSO-d6, δ ppm): 112-158(7C, Ar-C), 177(1C, C=O); M^{+},386, M^{+2},388.

***(xiii)* Synthesis of 2,8,10-trichloro-6a*H*, 13*H* *benz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5-*b*]quinolin-13-one 15**

Colour: pale brown; IR (KBr, cm^{-1}): 1673 cm^{-1} (C=O); ^{1}HNMR (DMSO-d6, δ ppm): 8.56(s, H Ar-H), 8.29(s, H Ar-H), 7.97(s, H Ar-H), 7.33(s, H Ar-H), 7.71(dd, H Ar-H), 7.79(dd, H Ar-H), 6.75(s, H -H); ^{13}C NMR (DMSO-d6, δ ppm): 112-158(7C, Ar-C), 175(1C, C=O); M^{+},409, M^{+2},411, M^{+3},413, M^{+6},415.

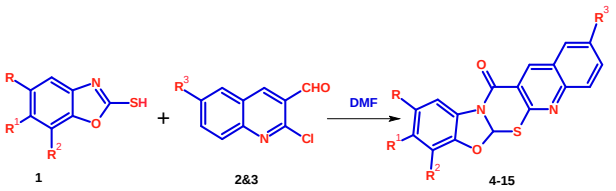
3. RESULTS AND DISCUSSION

Chemistry

Previously, impressive endeavours have been made in the synthesis of different derivatives of 2-amino phenol were treated with carbon disulphide and potassium hydroxide in presence of ethanol as solvent to get the compounds1,3-benzoxazole-2-thiol **1**.The compound **1** was characterized by ^{1}H NMR, which exhibited one singlet at δ 13.7 for –SH(D_2O exchangeable), which is used for the synthesis of targeted molecule **4-15**.

The derivative**1**was treated with 2-chloroquinoline-3-carbaldehyde **2** in presence of DMF as solvent to get the respective derivatives of oxazole, thiazino and quinoline containing molecules **4-15**, which was confirmed by IR, ^{1}H NMR, mass and elemental analysis. The molecule **4**, IR spectrum of **4** showed 1670 cm^{-1} for (C=O) and ^{1}H NMR showedδ6.91(m, 2H Ar-H),7.24(s, 2H Ar-H), 8.53(s, H Ar-

H), 7.32(d, 2H Ar-H), 7.28(m, 2H Ar-H), 6.54(s, H -H).It confirmed the disappearance of –SH functionality at (δ 13.7). The mass spectrum was in concurrence with molecular weights of the compound, the physical data of the target molecules were tabulated in **Table-1**.



Scheme 1: Synthetic route for the preparation of compound 4-15

	R	R^1	R^2	R^3
4	H	H	H	H
5	Cl	H	H	H
6	NO_2	Cl	H	H
7	CH_3	H	H	H
8	H	NO_2	H	H
9	Cl	H	Cl	H
10	H	H	H	Cl
11	Cl	H	H	Cl
12	NO_2	Cl	H	Cl
13	CH_3	H	H	Cl
14	H	NO_2	H	Cl
15	Cl	H	Cl	Cl

Table 1: Physical data of the synthesized compound 4-15

Comp.	Mole. formula	Mole. weight	M.P.^{0}C	Yield %	C, H& N Analysis		
					C	H	N
4	$C_{17}H_{10}N_2O_2S$	306.33	283-284	82%	Calc: 66.65 Obs: 66.62	Calc: 3.29 Obs: 3.26	Calc: 9.14 Obs: 9.10
5	$C_{17}H_9ClN_2O_2S$	340.78	256-257	76%	Calc: 59.92 Obs: 59.88	Calc: 2.66 Obs: 2.61	Calc: 8.22 Obs: 8.19
6	$C_{17}H_8ClN_3O_4S$	385.78	264-267	79%	Calc: 52.93 Obs: 52.90	Calc: 2.09 Obs: 2.05	Calc: 10.89 Obs: 10.60
7	$C_{18}H_{12}N_2O_2S$	320.36	213-215	87%	Calc: 67.48 Obs:67.42	Calc: 3.78 Obs: 3.76	Calc: 8.74 Obs: 8.70
8	$C_{17}H_9N_3O_4S$	351.33	245-247	75%	Calc: 58.12 Obs: 58.08	Calc: 2.58 Obs: 2.52	Calc: 11.96 Obs: 11.90
9	$C_{17}H_8Cl_2N_2O_2S$	375.22	272-273	78%	Calc: 54.42 Obs: 54.35	Calc: 2.15 Obs:2.09	Calc: 7.47 Obs: 7.38
10	$C_{17}H_9ClN_2O_2S$	340.98	282-285	82%	Calc: 59.92 Obs: 59.88	Calc: 2.66 Obs: 2.61	Calc: 8.22 Obs: 8.16
11	$C_{17}H_8Cl_2N_2O_2S$	375.22	267-269	86%	Calc: 54.42 Obs: 54,37	Calc: 2.15 Obs: 2.11	Calc: 7.47 Obs: 7.41
12	$C_{17}H_7Cl_2N_3O_4S$	420.22	255-257	78%	Calc: 48.59 Obs: 48.53	Calc: 1.68 Obs: 1.61	Calc: 10.00 Obs: 9.96
13	$C_{18}H_{11}ClN_2O_2S$	354.81	295-296	85%	Calc: 60.93 Obs: 60.85	Calc: 3.12 Obs: 3.09	Calc: 7.90 Obs: 7.86
14	$C_{17}H_8ClN_3O_4S$	385.78	257-260	88%	Calc: 52.93 Obs: 52.88	Calc: 2.09 Obs: 2.05	Calc: 10.89 Obs: 10.86
15	$C_{17}H_7Cl_3N_2O_2S$	409.67	224-226	84%	Calc: 49.84 Obs: 49.78	Calc: 1.72 Obs: 1.67	Calc: 6.84 Obs: 6.79

The derivatives **4-15** have been screened for antibacterial, cytotoxic and molecular docking studies. In the antibacterial study, few compounds have shown potent zone of inhibition **(Table-2 and Figure-1)**. In the synthesized compounds, marked zone of inhibition of bacteria was observed to compounds **5, 6, 8, 9, 11, 13** and **15,** while least activity was observed to compounds **4, 7, 10, 12** and **14** with standard drug Chloramphenicol. Compound **4-15** were evaluated for their cytotoxic effect on PBMCs cell lines (**Table-3**), to determine their anticancer potential and selectivity. The activity of the tested compounds was influenced considerably by the nature of functional group. Compound **6, 8, 11, 12, 13** and **15** were found more than 70 percent of dead cell viability in three different concentrations (10 µg/mL, 50 µg/mL and 100 µg/mL) against PBMCs cell lines. It was found that methyl and nitro substituent did not show upright cytotoxic activity, whereas chloro, dicholo, tricholoro and chloro with nitro substituted benzoxazole derivatives exhibited effective activity irrespective of concentration. The most effective cytotoxic agents against PBMCs cancer cell lines was found in compound 2,8,10-Trichloro6a*H*,13*Hbenz*[4',5']oxazole[2',3',:2,3][1,3]thiazino [6,5-*b*]quinolin-13-one **15** at 10µg/mL concentration showed 77.2 % dead cell viability, whereas the compound 9-Chloro-10-nitro-6a*H*,13*Hbenz*[4',5']oxazole[2',3',:2,3][1,3]thiazino[6,5*b*] quinolin-13 one **6** at 100µg/mL concentration displayed

5.26 % dead cell viability. On the other hand, compounds **8, 11, 12,** and **13** showed a significant cytotoxicity at the concentration used for the PBMCs cancer cell lines, which were supported by molecular docking studies. The synthesized compounds interact with receptor **PDB ID: 3FLY** amino acids, which displayed the higher binding energy for the derivatives**6**, **11, 12, 13** and **15** as compared to **4**, **5, 7, 8, 9, 10** and 1**4** derivatives.

4. CONCLUSION

A series of fused benzoxazole with quinoline derivatives were synthesized by the cyclization of substituted 1,3-benzoxazole-2-thiol with substituted 2-chloroquinoline-3-carbaldehyde. The newly synthesized molecules were characterized by IR, ^{1}H NMR and mass spectral analysis. To all the compounds, the cytotoxic activities against Peripheral Blood Mononuclear Cells with antibacterial and molecular docking studies were evaluated. All synthesized benzoxazole fused quinoline derivatives **4-15** exhibited promising cytotoxicity against PBMCs cell lines. Compound **6**, **8, 11, 12, 13**and **15** were exhibited effective anticancer activity against PBMCs, it was also supported by the *in vitro* antibacterial activity results. The synthesized compounds were docked into the plausible target PBMCs **(PDB ID: 3FLY).** The docking scores or the interaction binding energies of the target enzyme confirmed the cytotoxic activity of the selected synthesized 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.

4.1 Antibacterial Activity

The newly synthesized benzoxazole fused quinoline derivatives were tested for antibacterial activity against bacterial strains, *Escherichia coli*(ATTC-8739), *Staphylococcus aureus*(ATTC-6538), *Pseudomonas aeruginosa*(ATTC-9027), *Bacillus subtilis*(ATTC-6633), *Bacillus cereus*(ATTC-11778), *Staphylococcus epidermidis*(ATTC-12228) and *Salmonella typhimurium*(ATTC-23564) by agar well diffusion method[25]. The 24 hr old Mueller-Hinton broth culture of test bacteria were swabbed on sterile Mueller-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, 1mg/mL of sterile distilled water), compounds **4-15** (20mg/mL of 10% DMSO), and control (10% DMSO) were added to the respectively labelled wells. The plates were allowed to stand for 30 min and were incubated at 37 ^{0}C for 24 hr in upright position and the zone of inhibition was recorded and tabulated in **Table-2** and graphically represented in **figure-1**.

Table 2: Antibacterial activity of compounds 4-15

Compounds	Zone of inhibition in mm						
	S.aureus	*S.epidermis*	*S. typhi*	*E. coli*	*B.subtilis*	*B. cereus*	*P.aeruginosa*
4	17	14	16	18	17	16	14
5	**21**	**20**	**22**	**19**	**21**	**19**	**18**
6	**20**	**22**	**20**	**19**	**19**	**20**	**20**
7	18	17	15	17	19	18	16
8	**20**	**19**	**21**	**19**	**20**	**19**	**19**
9	**18**	**20**	**19**	**21**	**19**	**20**	**21**
10	19	18	16	21	18	17	17
11	**22**	**19**	**21**	**19**	**20**	**19**	**18**
12	18	20	19	18	16	21	18
13	**20**	**17**	**22**	**19**	**21**	**19**	**22**
14	20	15	19	17	18	21	19
15	**18**	**21**	**17**	**20**	**19**	**21**	**18**
DMSO	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Std	**25**	**24**	**26**	**25**	**25**	**24**	**25**

Std: Chloramphenicol
Solvent: DMSO

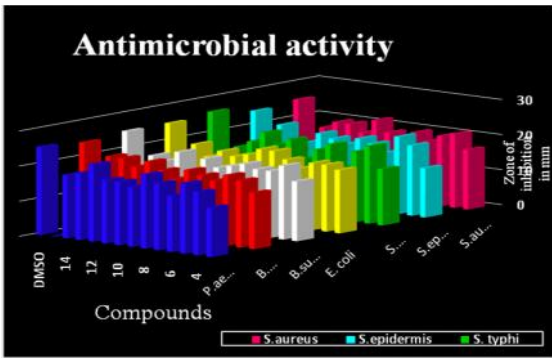


Fig 1: Antibacterial activity of compounds 4-15

4.2 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, Amphoteric in B and 0.9% normal saline]. This mixture (4mL) was dispensed into falcon tubes, 30µL of Phytohemagglutin (Invitrogen) and 200µL of PBMCs were incubated at the atmosphere of 95% air and 5% CO_2 at 37°C for 4 hr [26].

About **10 µg/mL**, **50 µg/mL** and **100 µg/mL** of the compounds **4-15** (1mg/mL) were added to the respectively

labelled 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 [27].

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 thiazino derivatives against PBMCs.

Sample	Total cells	Live cells	Dead cells	% of Cells viability	%of cells non-viability
4-10µg/mL	72	28	44	38.8	61.2
4-50µg/mL	212	60	142	28.3	66.98
4-100µg/mL	131	69	62	52.67	47.32
5-10µg/mL	84	41	43	48.8	51.19
5-50µg/mL	131	51	80	38.93	61.06
5-100µg/mL	96	68	28	70.8	29.16
6-10µg/mL	78	29	49	37.1	62.8
6-50µg/mL	82	38	44	46.3	53.7
6-100µg/mL	**186**	**46**	**140**	**24.73**	**75.26**
7-10µg/mL	173	63	110	36.4	63.6
7-50µg/mL	68	22	46	32.35	67.64
7-100µg/mL	85	40	45	47.05	52.94
8-10µg/mL	**153**	**45**	**108**	**29.41**	**70.59**
8-50µg/mL	136	48	88	35.3	64.7
8-100µg/mL	95	43	52	45.35	54.75
9-10µg/mL	168	69	99	41.08	58.92
9-50µg/mL	85	52	33	61.18	38.82
9-100µg/mL	128	66	62	51.57	48.43
10-10µg/mL	143	52	91	36.37	63.63
10-50µg/mL	177	58	119	32.77	67.23
10-100µg/mL	115	63	48	58.27	41.73
11-10µg/mL	**162**	**42**	**120**	**25.92**	**74**
11-50µg/mL	188	84	104	44.68	55.32
11-100µg/mL	88	45	43	51.13	48.86
12-10µg/mL	171	63	108	36.8	63.2
12-50µg/mL	**163**	**48**	**115**	**29.4**	**70.6**
12-100µg/mL	96	57	39	59.38	40.62
13-10µg/mL	154	61	93	39.6	60.4
13-50µg/mL	119	48	71	40.33	59.66
13-100µg/mL	**136**	**40**	**96**	**29.4**	**70.6**
14-10µg/mL	199	67	132	33.7	66.3
14-50µg/mL	124	51	73	41.1	59
14-100µg/mL	78	42	36	53.85	46.15
15-10µg/mL	**184**	**41**	**143**	**23.2**	**77.8**
15-50µg/mL	129	58	71	44.97	55.03
15-100µg/mL	93	54	39	58.06	41.93
Control	118	17	101	14.4	85.5

4.3 Molecular docking studies

Molecular docking study was performed with the Hex molecular modelling package version 8.0.[28]. Docking study of the synthesized compounds **4-15** were evaluated against Peripheral Blood Mononuclear Cells (PBMCs) (PDB ID: 3FLY). In the present study, an effort was made to evaluate their anti-cancer behaviour, we have selected Peripheral Blood Mononuclear Cells (PDB ID: 3FLY) to obtained docking scores (binding interaction energy). The results were tabulated in **Table-4** and graphically presented in **figure-2**. The synthesized molecules **4-15** binds with various amino acid receptor (PDB ID: 3FLY) in the active pocket sites and given a molecular interaction energy (E-total value) at -219.91 to -265.00 (Kcal/mol). The compounds **6, 11, 12, 13** and **15** showed higher binding energy as compared with the compounds **4, 5, 7, 8, 9, 10** and **14**. The estimated binding affinity of molecules **4-15** with the complex hydrogen network and other interactions with amino acids were MET78, LEU74, ILE84, ILE166, ASN155, LYS152, ASN155, ASP150, ASN155, LEU167, ASN155, GLY170, HIS148, SER208, TYR188, ILE212, SER208, LYS152 and APS150, which were presented in active sites of PBMCs respectively. It explained the role of hydrogen bond formation and other interactions for effective enzyme binding.

Table 4: Docking result of synthesized compounds in the binding site of Peripheral Blood Mononuclear Cells (PDB ID: 3FLY)

Entry	Receptor PDB code	ΔG (Kcal/mol)
4	**3FLY**	**261.54**
5	**3FLY**	**265.00**
6	3FLY	219.91
7	3FLY	255.98
8	**3FLY**	**265.00**
9	3FLY	249.84
10	**3FLY**	**263.76**
11	3FLY	244.67
12	3FLY	246.54
13	3FLY	243.84
14	**3FLY**	**262.10**
15	3FLY	231.52

Fig 2: Molecular docking results of compound 4-15

Three dimensional and two dimensional interactions of compounds **4-15** with the active sites of Peripheral Blood Mononuclear Cells (PDB ID: 3FLY).

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

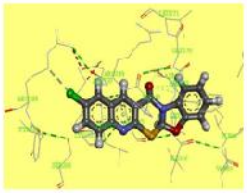
(b) Two dimensional interactions of synthesized compounds with receptor (**PDB ID: 3FLY**).

a. 4: 3D

Figure-2(continued)

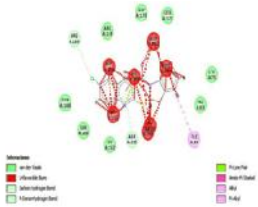
b. 4: 2D

Figure-2(continued)



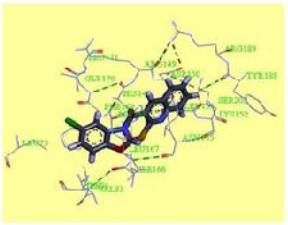
a. 10:3D

Figure-2(continuing)



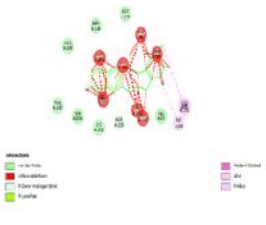
b. 10:2D

Figure-2(continuing)



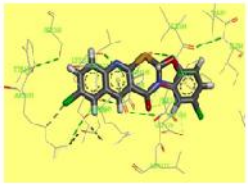
a. 5:3D

Figure-2(continued)



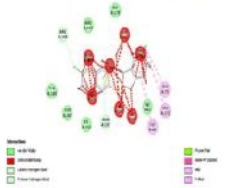
b. 5:2D

Figure-2(continued)



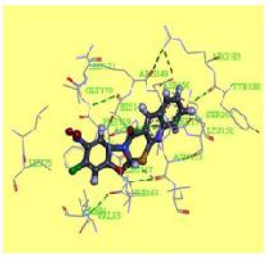
a. 11:3D

Figure-2(continued)



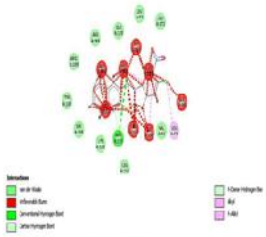
b.11:2D

Figure-2(continued)



a.6:3D

Figure-2(continued)

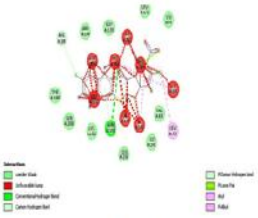


b. 6:2D

Figure-2(continued)

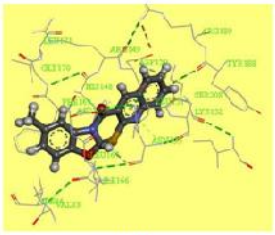
a. 12:3D

Figure-2(continued)



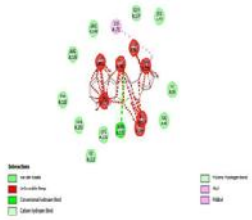
b. 12:2D

Figure-2(continued)



a. 7: 3D

Figure-2 (continued)

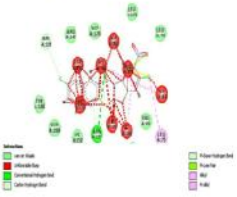


b. 7:2D

Figure-2 (continued)

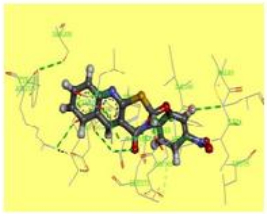
a.13:3D

Figure-2(continued)



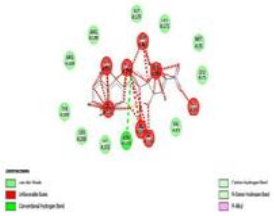
b. 13:2D

Figure-2(continued)



a. 8:3D

Figure-2 (continued)

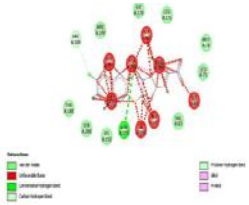


b. 8:2D

Figure-2 (continued)

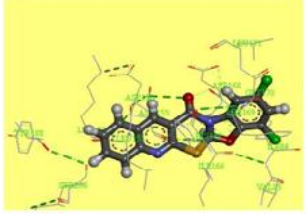
a. 14:3D

Figure-2(continued)



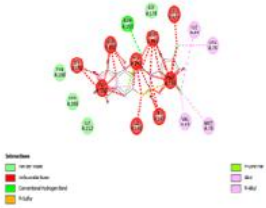
b. 14:2D

Figure-2(continued)



a. 9:3D

Figure-2(continued)

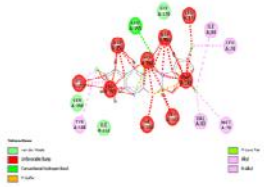


b. 9:2D

Figure-2(continued)

a. 15:3D

Figure-2



b. 15:2D

Figure-2

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