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The *in vitro* Cytotoxic and Molecular Docking Studies of Newly Synthesized Fused Benzoxazole-Triazole Derivatives

SH Shreedhara[1], HM Vagdevi[1*], ND Jayanna[1], R Raghavendra[2], P Kiranmayee[3], Prabhu Das[3] and Mohammed Shafeeulla R[1]

[1]Department of Chemistry, Sahyadri Science College (Autonomous), Shimoga, India
[2]Department of Microbiology and Cell Biology, Indian Institute of Science Bangalore, India
[3]Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka, India

ABSTRACT

The present study describes the synthesis of benzoxazole molecule associated with triazole moiety 6-13. Among these derivatives, 3-[(2-bromoethyl)sulfanyl]-7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazole 7, Dichloro[(7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)sulfanyl] acetic acid 8, Ethyl[(7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)sulfanyl] acetate 11 and [(7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)sulfanyl]acetyl chloride 12 exhibited potent cytotoxic activity towards Peripheral Blood Mononuclear Cells (PBMCs) with the influence of functional groups attached with central moiety. Some of the synthesized compounds displayed a broad spectrum of antibacterial activity and remaining displays moderate results. The cytotoxic results were further supported by molecular docking studies of synthesized compounds with the interaction of receptor (PDB ID: 3FLY). It showed minimum binding energy and good affinity towards the active pocket of receptor.

Keywords: Benzoxazole; Triazole; Peripheral blood mononuclear cells (PBMCs); Cytotoxic; Molecular docking

INTRODUCTION

Benzoxazoles are active and medicinally significant compounds [1]. The azole nucleus is an important class of molecules, it is a common heterocyclic scaffold in a variety of natural products and are important target molecules in drug discovery. In recent years there have been some interesting developments in the pharmacology of benzoxazole derivatives [2]. The substituted benzoxazole have been shown to exhibit antitumor [3], anticancer [4], antibacterial [5], anti HIV-1 [6], antioxidant [7], cyclooxygenase inhibitory [8], antifungal [9], antitubercular [10], 5HT 3 receptor antagonists [11], anti-inflammatory, analgesic and cyclin dependent kinase inhibitory [12], 5-lipoxygenase inhibitory [13], and melatonin receptor agonist [14], activities. 1,2,4-Triazole derivatives have consistently attracted scientific and practical interest because of their widely varying chemical properties, synthetic versatility and pharmacological activities, such as antibacterial [15-21], antifungal [18-22], anti-tubercular [23-25], analgesic [26,27], anti-inflammatory [27-29], anticancer [30,31], anticonvulsant [32,33], antiviral [34,35], insecticidal [36] and antidepressant [37] properties. Moreover, the 1,2,4-triazole compounds carrying sulfone moiety or imine bond have been reported as antibacterial, antifungal, antihypertensive, analgesic and anti-inflammatory or antitumor agents [38-46].

Keeping in view, the significance of benzoxazoles and triazoles as potential pharmacophores and in continuation of our research work on the synthesis of novel series of biologically active heterocyclic derivatives like fused benzoxazole and triazole derivatives, the *in vitro* anticancer activity against Peripheral Blood Mononuclear human cells (PBMCs) have been evaluated at three different concentrations. The salient features of the procedure described here are taking short reaction time, not requiring elevated temperatures, the use of cheap reagents and easily available starting materials. The cytotoxic activity of PBMC was augmented by famotidine at a concentration of 10 µg/mL, which is equivalent to the serum level achieved by the intravenous administration of a dose of 20 mg. This response to famotidine was seen only in cancer patients. On intensive literature survey, the work has been presented here to extend the combined effect of benzoxazole nucleus fused with triazole moiety, which imparts cytotoxic activity and antibacterial effect. The molecular docking also helps to recognize the binding capacity of the drug to particular site.

EXPERIMENTAL SECTION

Chemistry

Synthesis of 6-nitro-1,3-benzoxazole-2-thiol 2:

To the solution of methanol (50 ml) and KOH (1.1 eq), carbon disulphide (1.1 eq) was added slowly at room temperature. To the reaction mass, 2-amino-5-nitrophenol (1.eq) was added with stirring. The reaction mass was refluxed for 6 h on water bath. Completion of the reaction was monitored by TLC. The reaction mixture was poured to 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 2.

Color: yellow; 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.

Synthesis of 2-(ethylsulfanyl)-6-nitro-1, 3-benzoxazole 3:

Equimolar quantity 6-nitro-1,3-benzoxazole-2-thiol 2 (0.01 mol) and ethyliodide (0.01 mol) in dry DMSO (40 ml) in presence of anhydrous sodium hydroxide (0.01 mol) was stirred in cold condition (0-15°C) for 2 hr. Then the reaction mixture was discharged into crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol to get the compound 3.

Color: Brown; IR (KBr, cm^{-1}): 2911 cm^{-1} ($-CH_3$); ^{1}H NMR (DMSO-d6, δ ppm): 1.4 (t, 3H, $-CH_3$), 3.4(q, 2H, S-CH_2), 7.4(s, H, Ar-H), 7.0(dd, H, Ar-H), 7.26(dd, H, Ar-H); ^{13}C NMR (DMSO-d6, δ ppm):120-160 (7C, Ar-C), 13.2 (1C, CH_3-C),14.4 (1C, CH_2-C); M^+,224.

Synthesis of 2-hydrazinyl-6-nitro-1, 3-benzoxazole 4:

Compound 3 (0.01 mol) was taken in a round bottomed flask and treated with hydrazine hydrate (0.015 ml) in 20 ml of ethanol and refluxed for 3 hr. The reaction mixture was cooled and filtered. The obtained solid was recrystallized from ethanol to obtain the compound 4.

Color: wine read; IR (KBr, cm^{-1}): 3349 cm^{-1} ($-NH_2$), 3270 cm^{-1} (-NH); ^{1}H-NMR (DMSO-d6, δ ppm): 4.6 (bs, 2H, -NH_2), 9.3 (bs, H, -NH) (D_2O exchangeable); 6.9 (s, H, Ar-H), 7.22 (dd, H, Ar-H), 7.1 (dd, H, Ar-H); ^{13}C-NMR (DMSO-d6, δ ppm): 115-165 (7C, Ar-C); M^+,194.

Synthesis of 7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazole-3-thiol 5:

To the mixture of ethanol (20 ml) and NaOH (1.1 eq), carbon disulphide (1.1 eq) was added drop by drop at room temperature. To the reaction mass, 2-hydrazinyl-6-nitro-1,3-benzoxazole 4 (1. eq) was added with stirring. The reaction mixture was refluxed for 8 hr on water bath. This was poured onto 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 5.

Color: light orange; IR (KBr, cm^{-1}): 3394 cm^{-1} (-SH); ^{1}H NMR (DMSO-d6, δ ppm): 7.02 (s, H, Ar-H), 7.18(dd, H Ar-H), 7.32(dd, H Ar-H), 13.9(s, H, -SH); ^{13}C NMR (DMSO-d6, δ ppm): 120-160 (8C Ar-C); M^+, 236.

Synthesis of 3-(Ethylsulfanyl)-7-nitro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole 6:

The compound 5 (0.01 mol) in 30 ml of DMSO with iodoethane (1.2 eq) in presence of NaOH (1.1 eq) as a base was refluxed for 4 hr and cooled. The solid separated was filtered, washed with water, dried and recrystallized from ethanol to get the compound 6.

Color: pale yellow; IR (KBr, cm^{-1}): 2912 cm^{-1} ($-CH_3$); ^{1}H NMR (DMSO-d6, δ ppm): 6.93(s, H, Ar-H), 7.13(dd, H, Ar-H), 7.34(dd, H, Ar-H), 3.4(q, 2H $-CH_2$), 1.3(t, 3H $-CH_3$); the ^{13}C NMR (DMSO-d6, δ ppm): 120-160 (8C, Ar-C), 12.4 (1C, CH_3-C), 14.27 (1C, CH_2-C); M^{+1},264.

Synthesis of 3-[(2-bromoethyl)sulfanyl]-7-nitro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole 7:

The compound 5 (0.01 mol) was dissolved in DMSO (20 ml) and dibromoethane (1.2 eq) was added slowly in presence of NaOH (1.1 eq) as a base with constant stirring and refluxed for 6 hr. The completion of the reaction was

monitored by TLC. Then the reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethylacetate to get compound 7.
Color: brown; IR (KBr, cm^{-1}): 787 cm^{-1} (C-Br); ^{1}H NMR (DMSO-d6, δ ppm): 6.88(s, H, Ar-H), 7.0 (dd, H, Ar-H), 7.25(dd, H, Ar-H), 3.8(t, 2H S-CH_2), 2.5(t, 2H Br-CH_2); ^{13}C NMR (DMSO-d6, δ ppm): 120-160 (8C, Ar-C), 16 (C, CH_2-C),18 (2C, CH_2-C); M^+,343, M^{+2},347.

Synthesis of dichloro[(7-nitro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazol-3-yl)sulfanyl]acetic acid 8:
The mixture of trichloroacetic acid (1.1eq) and compound 5 (0.01 mol) was stirred with 20 ml of DMF in the presence of K_2CO_3 (1 eq) as a catalyst for ten minute and refluxed for 8 hr. Then the reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol to get compound 8.
Color: pale brown; IR (KBr, cm^{-1}): 1716 cm^{-1} (C=O); 3528 cm^{-1} (-OH); ^{1}H NMR (DMSO-d6, δ ppm): 6.92(s, H, Ar-H), 7.21 (dd, H, Ar-H), 7.33(dd, H, Ar-H),13.4 (s, 1H COOH); ^{13}C NMR (DMSO-d6, δ ppm): 120-155 (8C, Ar-C), 32 (1C, CCl-), 184(1C, -C=O); M^+,363, M^{+2},367 M^{+3},369.

Synthesis of [(7-nitro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazol-3-yl)sulfanyl]acetic acid 9:
The solution of compound 5 (0.01 mol) and 20 ml DMF was refluxed for 7 hr with chloroacetic acid (1.1 eq) in the presence of K_2CO_3 (1.1 eq) as a catalyst. Then the reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, washed, dried and recrystallized from ethanol to get compound 9.
Color: brown; IR (KBr, cm^{-1}): 1720 cm^{-1} (C=O); 3522 cm^{-1} (C-OH); ^{1}H NMR (DMSO-d6, δ ppm): 7.08(s, H, Ar-H), 7.2 (dd, H, Ar-H), 7.32(dd, H, Ar-H),, 4.9(s, 2H CH_2), 13.2(s, H COOH); ^{13}C NMR (DMSO-d6, δ ppm): 125-165(8C, Ar-C), 53(1C, CH_2-C), 182(1C, -C=O); M^+,294.

Synthesis of Ethyl S-(7-nitro [1,2,4]triazolo[3,4-*b*][1,3]benzoxazol-3-yl) carbonothioate 10:
The compound 5 (0.01 mol) was refluxed for 8 hr with ethyl chloroformate (1.2 eq) in ethanol and pinch K_2CO_3 as catalyst. The completion of the reaction was confirmed by TCL. Then the reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol to get compound 10.
Color: yellowish ;IR (KBr, cm^{-1}): 1737 cm^{-1} (O=C-O); ^{1}H NMR (DMSO-d6, δ ppm) 6.93(s, H, Ar-H), 7.16 (dd, H, Ar-H), 7.38(dd, H, Ar-H), 4.2(q, 2H CH_2),1.3(t, 3H CH_3); ^{13}C NMR (DMSO-d6, δ ppm): 120-160(8C, Ar-C), 172(1C COO-C), 52(C, CH_2-C), 22(1C, CH_3-C); M^+, 308.

Synthesis of ethyl [(7-nitro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazol-3-yl)sulfanyl]acetate 11:
The mixture of compound 5 (0.01 mol) and ethylchloroacetate (1.2 eq) was refluxed in with pinch of K_2CO_3 as a catalyst. Then it was refluxed for 5 hr. The reaction mixture was poured onto crushed ice after the completion of the reaction. Solid product thus obtained was filtered, dried and recrystallized from ethanol to get compound 11.
Color: dark yellow; IR (KBr, cm^{-1}): 1735 cm^{-1} (O=C-O); ^{1}H-NMR (DMSO-d6, δ ppm): 6.94(s, H, Ar-H), 7.15 (dd, H, Ar-H), 7.32(dd, H, Ar-H), 4.3(s, 2H, CH_2),4.1(q, 2H, CH_2),1.2(t, 3H, CH_3); ^{13}C-NMR (DMSO-d6, δ ppm): 118-162(8C, Ar-C), 175(C, COO-C), 51(C, CH_2-C), 54(C, S-CH_2), 19(1C, CH_3-C); M^+,322.

Synthesis of [(7-nitro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazol-3-yl)sulfanyl]acetyl chloride 12:
The compound 5 (0.01 mol) was refluxed for 8 hr in 20 ml of acetone with ethylchloroacetylchloride (1.2 eq) and pinch of K_2CO_3 as a catalyst. Then the reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol to get compound 12.
Color: pale brown; IR (KBr, cm^{-1}): 1734 cm^{-1} (C=O); ^{1}H NMR (DMSO-d6, δ ppm): 6.93(s, H, Ar-H), 7.06 (dd, H, Ar-H), 7.33(dd, H, Ar-H), 4.4(s, 2H, CH_2); ^{13}C NMR (DMSO-d6, δ ppm): 120-160(8C, Ar-C), 35(1C, CH_2-C), 176(1C, C=O-C); M^+,312, M^{+2}, 314.

Synthesis of 1-[(7-nitro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazol-3-yl)sulfanyl]propan-2-one 13:
The compound 5 (0.01 mol) and ethyl chloroacetone (1.1 eq) was refluxed in acetone with pinch K_2CO_3 as a catalyst. Then the reaction mixture was poured onto crushed ice. The solid product thus obtained was filtered, dried and recrystallized from ethanol to get compound 13.
Color: yellow; IR (KBr, cm^{-1}): 1731 cm^{-1} (C=O); ^{1}H NMR (DMSO-d6, δ ppm): 7.12(s, H, Ar-H), 7.24 (dd, H, Ar-H), 7.32(dd, H, Ar-H), 4.2(s, 2H, CH_2); 2.2(s, 3H, CH_3); ^{13}C NMR (DMSO-d6, δ ppm): 120-160(8C, Ar-C), 174(1C, CO-C), 32(1C, CH_2-C), 28(1C, CH_3-C); M^+,292.

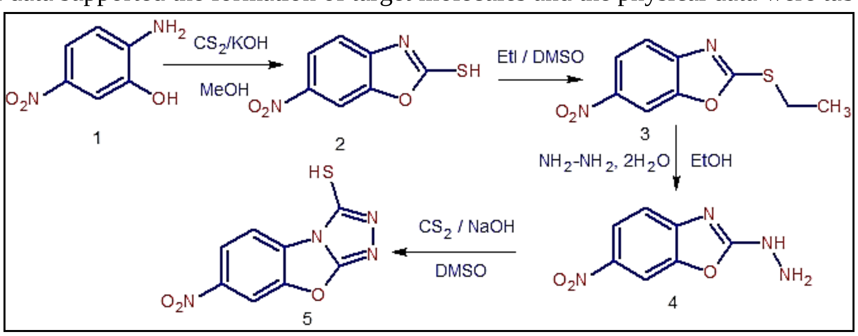
RESULTS AND DISCUSSION

The compound 2-hydrazinyl-6-nitro-1,3-benzoxazole 4 was prepared [47], further the target molecules 6-13 were synthesized using the intermediate 7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazole-3-thiol 5 (Scheme 1). The compound 4 was treated with carbon disulphide in the presence of potassium hydroxide and DMSO as a solvent to get the intermediate compound 5. It is characterized by IR, ^{1}H NMR and Mass analysis. The ^{1}H NMR exhibited singlet at 13.97 for –SH and disappearance of two singlets at δ 4.6 and δ 9.3 for $–NH_2$ and –NH protons respectively (D_2O exchangeable) are confirmed the formation of compound 5.

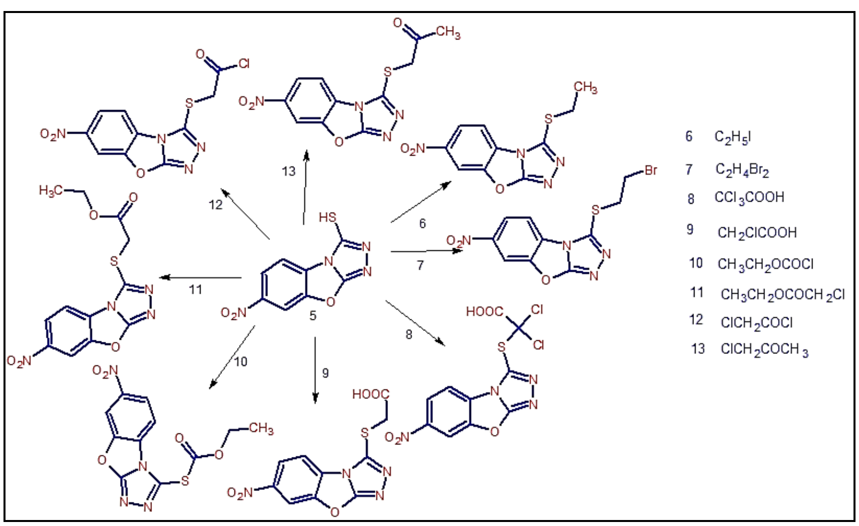
The compound 5 was treated with various reagents in presence of ethanol to get target triazole molecules 6-13 (Scheme 2) and confirmed by spectral and elemental analysis. The compound 5 was reacted with ethyliodide and dibromoethane to form respective compounds 6 and 7. In the ^{1}H NMR of compound 6, the disappearance of –SH proton and appearance of methyl and methylene protons supported the formation of product. Similarly two methylene protons existed in compound 7 as triplet of triplet. The mass peak of compounds 6 shows at M^+, 264 and for compound 7 M^+-343, M^{+2}-346, which is matching with their molecular weights and further confirmed the halogen atom as bromine.

The construction of compounds 8-13 followed similar method of preparation. The compound 5 is treated with trichloroacetic acid and chloroacetic acid yielded compounds 8 and 9, which were confirmed by observing ^{1}H NMR signals at δ 13.4 for acid proton and mass spectrum displayed at M^+-363, M^{+2}-365 and M^{+4}-367. The differentiation of products was shown by the appearance of singlet at δ 3.7 for methylene proton in compound 9. In case of compounds 10 and 11, ester derivatives showed quartet for $-CH_2$ and triplet for $-CH_3$ group in both the compounds and also confirmed by mass spectrum.

The singlets for methylene protons in both 12 and 13 compounds at the region of δ 4.4 and δ 4.2 value were exhibited in ^{1}H NMR respectively. The methyl proton of compound 13 appeared as a singlet at δ 1.9. All the remaining spectral data supported the formation of target molecules and the physical data were tabulated in Table 1.



Scheme 1: Synthetic route for the preparation of compound 5



Scheme 2: Synthetic route for the synthesis of compounds 6-13

Table 1: Physical data of the synthesized compound 6-13

Comp.	Mol. formula	Mol. weight	M.P.^{0}C	Yield %	C, H and N Analysis		
					C	H	N
2	$C_7H_4N_2O_3S$	196	183-186	85.5	Calc: 42.86	Calc: 2.06	Calc: 14.28
					Obs:42.79	Obs:2.02	Obs:14.12
3	$C_9H_8N_2O_3S$	224	78-80	78.4	Calc: 48.21	Calc: 3.60	Calc:12.49
					Obs:48.15	Obs:3.55	Obs:12.43
4	$C_7H_6N_4O_3$	194	145-148	80.5	Calc: 43.30	Calc: 3.11	Calc:28.86
					Obs:43.26	Obs:3.02	Obs:28.79
5	$C_8H_4N_4O_3S$	236	213-215	83.5	Calc: 40.68	Calc: 1.71	Calc:23.72
					Obs:40.61	Obs:1.68	Obs:23.68
6	$C_{10}H_8N_4O_3S$	264	275-279	81.5	Calc: 45.45	Calc: 3.05	Calc: 21.20
					Obs: 45.41	Obs:2.98	Obs: ,21.15
7	$C_{10}H_7BrN_4O_3S$	343	215-218	78	Calc: 35.00	Calc: 2.06	Calc: 16.33
					Obs:34.84	Obs:1.94	Obs:16.21
8	$C_{10}H_4Cl_2N_4O_5S$	363	210-212	81	Calc: 33.08	Calc:1.11	Calc:15.43
					Obs:33.02	Obs:1.08	Obs:15.36
9	$C_{10}H_6N_4O_5S$	294	245-247	83	Calc: 40.82	Calc: 2.06	Calc:19.04
					Obs:40.78	Obs:2.02	Obs:19.01
10	$C_{11}H_8N_4O_5S$	308	196-198	88	Calc: 42.86	Calc:2.62	Calc:18.17
					Obs:42.78	Obs:2.58	Obs:18.12
11	$C_{12}H_{10}N_4O_5S$	322	232-234	86	Calc: 44.72	Calc: 3.13	Calc: 17.38
					Obs:44.67	Obs:3.32	Obs:17.35
12	$C_{10}H_5ClN_4O_5S$	314	238-240	82	Calc: 38.41	Calc: 1.61	Calc: 17.92
					Obs:38.37	Obs:1.55	Obs:17.85
13	$C_{11}H_8N_4O_4S$	292	242-243	85	Calc: 45.20	Calc: 1.55	Calc: 19.17
					Obs:40.15	Obs:1.48	Obs: 19.12

The compounds 6-13 were screened for antibacterial, cytotoxic and molecular docking studies. In the antibacterial study, the compounds have shown inhibition of tested bacteria (Table 2). Among the synthesized compounds 7, 8, 9, 11 and 12 showed very higher zone of inhibition, while least activity was observed to the compounds 6, 10 and 13. The synthesized benzoxazole molecule associated with triazole moiety (6-13) were shown promising source of antibacterial activity. The derivatives 7, 8, 11 and 12 exhibits potent cytotoxic activity (Table 3) towards Peripheral Blood Mononuclear Cells (PBMCs). The compound 8 at lower concentration also possess higher cell viability. At 10m/mL the compound 8 destroyed the cells and it continued by increasing the concentration. In case of Molecular docking studies, the synthesized compounds interacted with receptor 3FLY amino acids, the binding energy (Table 4) of synthesized derivatives 9, 11, 10 and 12 have been recorded. The compounds with higher binding energy emerged as potent drug molecule with Peripheral Blood Mononuclear Cells (PBMCs). Hence further study of the activity associated with different functional group, cultivation conditions and investigation of the active functionality of benzoxazole molecules associated with triazole moiety of 6, 7, 8 and 13 may provide useful information in future.

Antibacterial Activity

The newly synthesized triazole 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 [48], 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, 1mg/mL of sterile distilled water), compounds 6-13 (20mg/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 37°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 the compound 6-13

Zone of inhibition in mm							
Compounds	***S.aureus***	***S.epidermis***	***S. typhi***	***E. coli***	***B.subtilis***	***B. cereus***	***P.aeruginosa***
6	14	17	14	14	16	18	17
7	22	21	20	19	22	21	22
8	20	27	20	22	21	20	19
9	20	19	21	20	19	20	21
10	18	17	18	17	18	16	18
11	19	23	19	22	19	20	21
12	21	24	19	20	21	21	18
13	18	17	19	17	18	16	19
DMSO	0	0.0.	0.0.	0.0.	0	0	0
Std	25	30	24	24	25	23	25

Std-Chloramphenicol; Solvent- DMSO

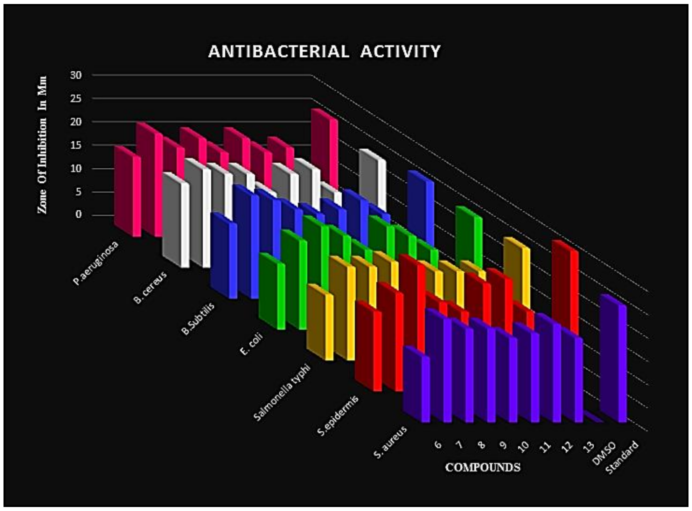


Figure 1: Antibacterial activity of compounds 6-13

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. Removed plasma down to 1 cm above buffy coat, 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 200 µL antimycotic (Antibiotic antimycotic solution with Streptomycin 10 mg/20mL, 10,000 U Penicillin, Amphotericin B and 0.9% normal saline). About 4 mL of this mixture was dispensed into falcon tubes, 30 µL of Phytohemagglutin (Invitrogen) and 150-200 µL of PBMCs were incubated at the atmosphere of 95% air and 5% CO_2 at 37°C for 4 hours [49].

About 10 µg/mL, 50 µg/mL and 100 µg/mL of the compounds 6-13 (1 mg/mL) 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 [50].

Trypan blue exclusion test cells were clarified by centrifuging at 1000 rpm for 30 min at room temperature. The supernatant was discarded and to the 10 µL of PBMCs, 10 µL of tryphan blue was added and incubated for 10 min at room temperature. About 10 µL of incubated sample was loaded on previously cleaned Haemocytometer and counted the number of live, 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. The graphical representation was presented in Figure 2.

Table 3: Cytotoxic activity of newly synthesized triazole derivatives against PBMCs

Sample	Total cells	Live cells	Dead cells	Percent of Cells viability	percent of cells non-viability
6(10 µg/mL)	270	99	171	36.7	63.3
6(50 µg/mL)	163	65	98	40	60.1
6(100 µg/mL)	65	22	43	33.8	66.1
7(10 µg/mL)	171	63	108	36.8	63.2
7(50 µg/mL)	202	35	167	17.3	82.7
7(100 µg/mL)	70	17	53	24.3	75.7
8(10 µg/mL)	199	67	132	33.7	66.3
8(50 µg/mL)	124	51	73	41.1	59
8(100 µg/mL)	67	15	52	23.4	83.8
9(10 µg/mL)	173	63	110	36.4	63.6
9(50 µg/mL)	186	77	109	41.4	58.6
9(100 µg/mL)	88	45	43	51.13	48.86
10(10 µg/mL)	140	53	87	37.85	62.14
10(50 µg/mL)	128	60	68	46.88	53.12
10(100 µg/mL)	125	67	58	53.6	46.4
11(10 µg/mL)	185	42	143	22.7	77.3
11(50 µg/mL)	129	58	71	44.97	55.03
11(100 µg/mL)	93	54	39	58.06	41.93
12(10 µg/mL)	213	75	138	35.52	64.78
12(50 µg/mL)	192	67	125	34.89	65.1
12(100 µg/mL)	147	33	114	22.44	77.55
13(10 µg/mL)	154	61	93	39.6	60.4
13(50 µg/mL)	119	48	71	40.33	59.66
13(100 µg/mL)	72	28	44	38.8	61.2
Control	233	19	214	8.15	92

Molecular Docking Studies

Molecular docking study was performed with the Hex molecular modeling package version 8.0 [51]. Docking study of the synthesized compounds 6-13 were evaluated against Peripheral Blood Mononuclear Cells (PBMCs) or Buffy Coat (PDB ID: 3FLY). In the present study, an effort was made to evaluate their anti-cancer property, we have selected Peripheral Blood Mononuclear Cells (PBMCs) to get Buffy Coat (PDB ID: 3FLY) and obtained docking scores (binding interaction energy). The results were tabulated in Table 4 and graphically presented in Figure 3. With respect to cytotoxicity, the synthesized molecules 6-13 binds with various amino acid receptor (PDB ID: 3FLY) in the active pocket sites and gives a molecular interaction energy (E-total value) at -201.76 to -231.36 (Kcal/mol). The compounds 6, 7, 8, 9, 11 and 13 showed higher binding energy as compared with the compounds 10 and 12. The estimated binding affinity of molecules 6-13 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, gives a reason the importance of hydrogen bond formation and other interactions for effective enzyme binding.

Table 4: Docking results of synthesized compounds in the binding site of Peripheral Blood Mononuclear Cells (PBMCs)

Entry	Receptor PDB code	ΔG (Kcal/mol)
6	3FLY	-220.71
7	3FLY	-220.92
8	3FLY	-201.76
9	3FLY	-222.26
10	3FLY	-231.36
11	3FLY	-221.77
12	3FLY	-230.44
13	3FLY	213.85

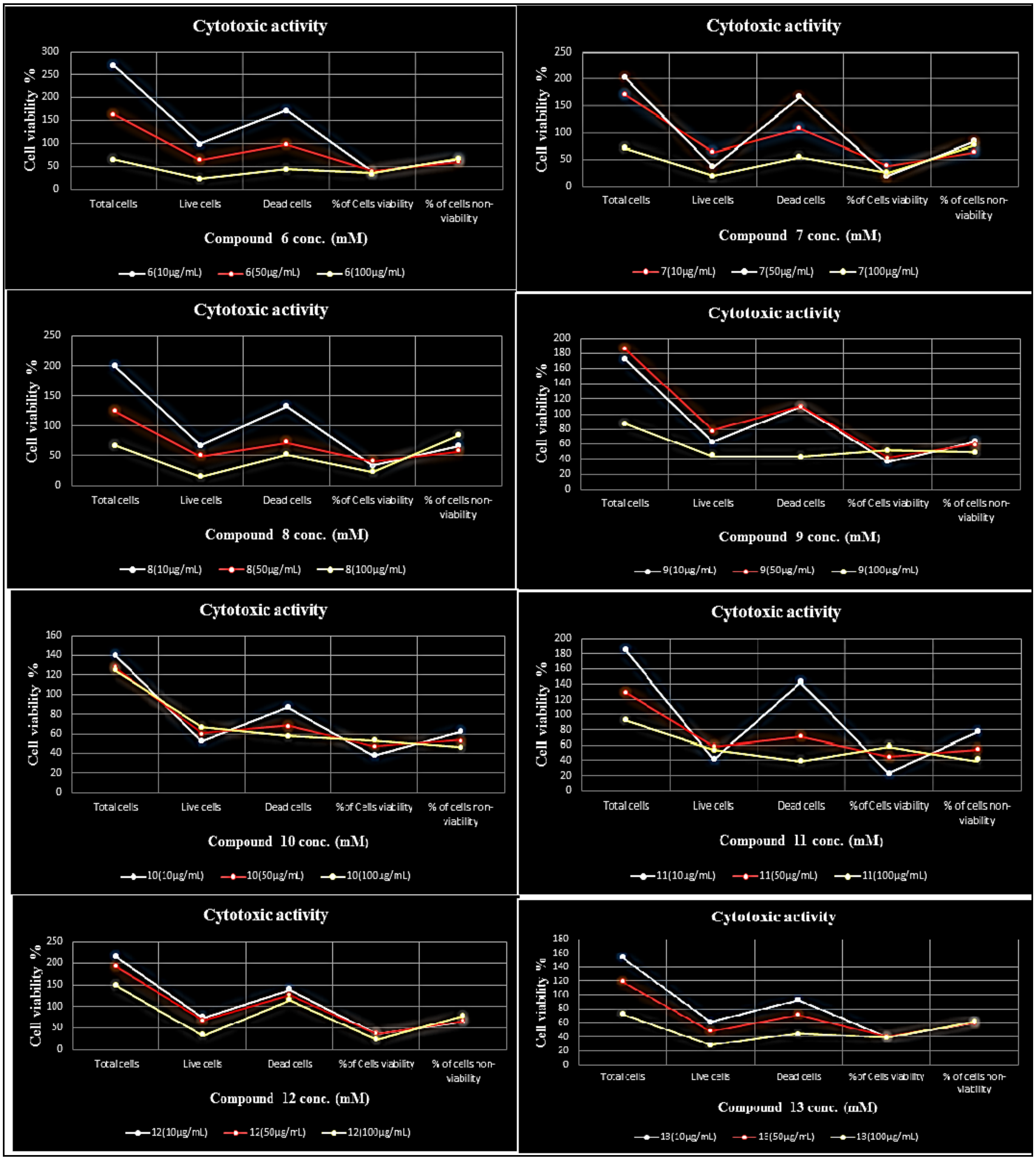
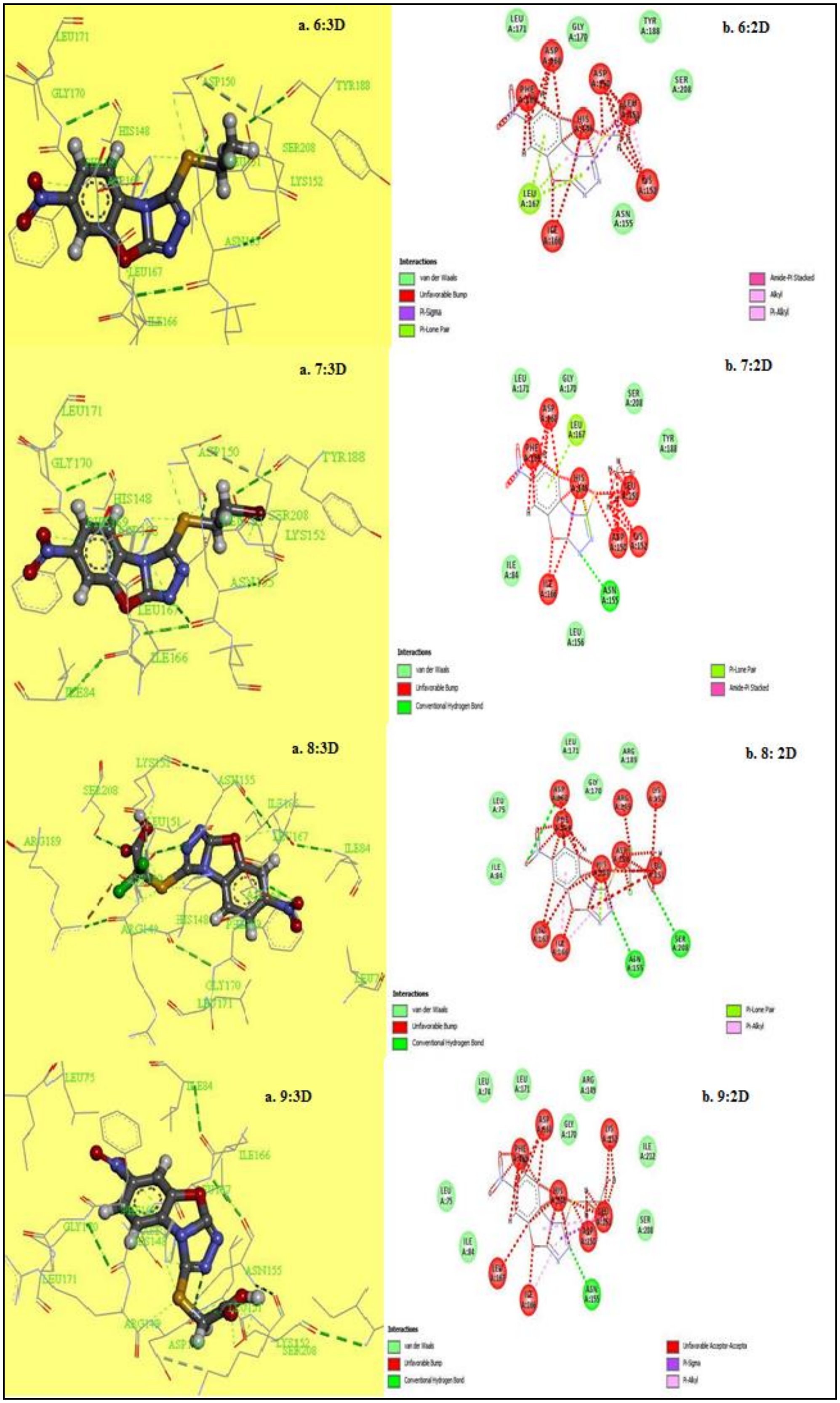


Figure 2: Cytotoxic effect of compounds 6-13 at varying concentrations (10 µg/mL, 50 µg/mL and 100 µg/mL) against the Peripheral Blood Mononuclear Cells was graphically represented. Dose response effect of the synthesized compounds 7, 8, 11 and 12 shows cell viability of PBMCs


a. 6:3D
b. 6:2D
Interactions
van der Waals
Unfavorable Bump
Pi-Sigma
Pi-Lone Pair
Amide-Pi Stacked
Alkyl
Pi-Alkyl
a. 7:3D
b. 7:2D
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Pi-Lone Pair
Amide-Pi Stacked
a. 8:3D
b. 8: 2D
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Pi-Lone Pair
Pi-Alkyl
a. 9:3D
b. 9:2D
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Pi-Sigma
Pi-Alkyl

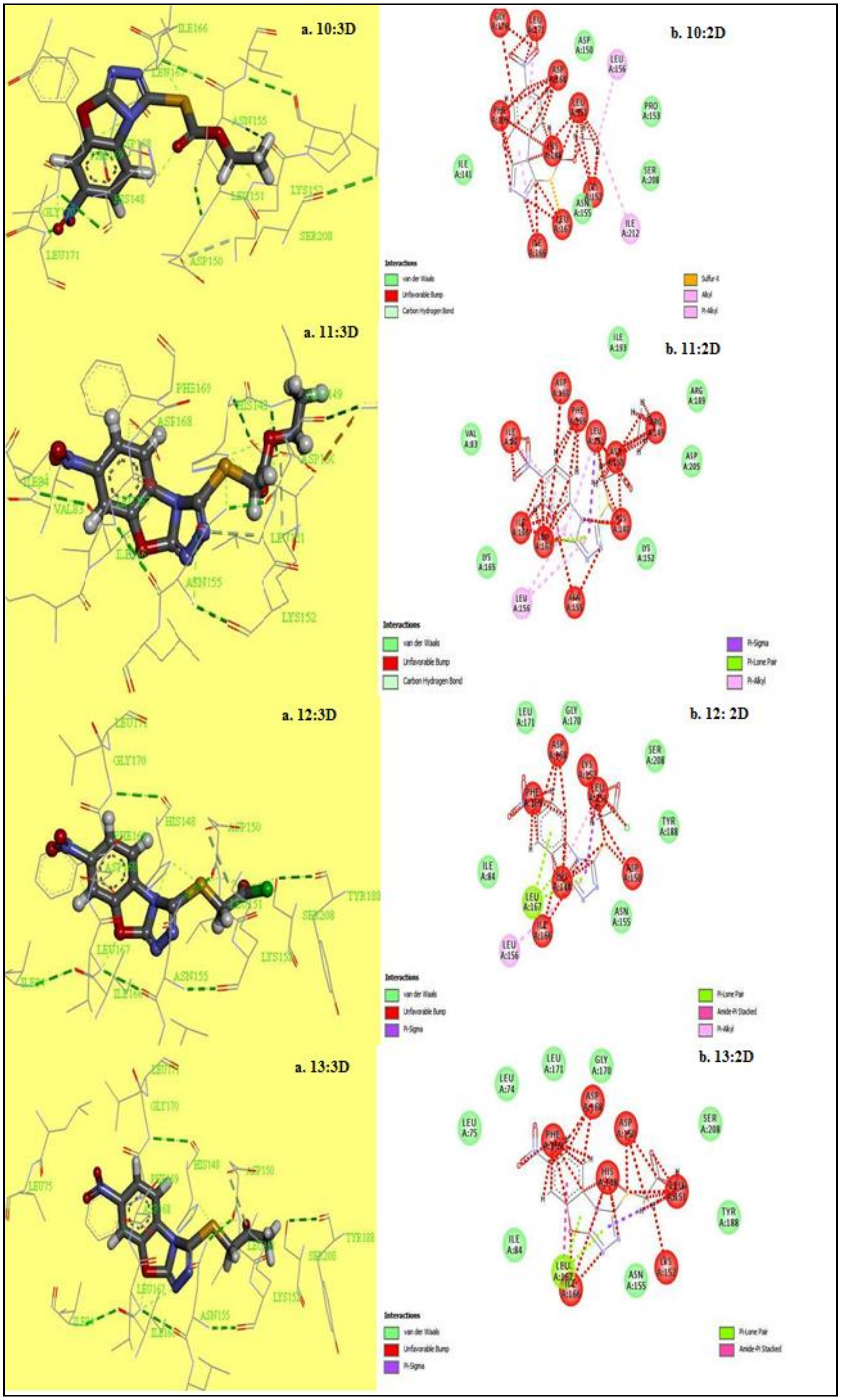


Figure 3: Three dimensional and two dimensional Interactions of compounds 6-13 with the active site of Peripheral Blood Mononuclear Cells (PDB ID: 3FLY); (a) A close-up three dimensional view of the docked pose of compounds structure were shown in the surface model and the ligand have been shown in the ball and stick model (colors by atom).; (b) Two dimensional interaction of synthesized compounds with receptor PDB ID: 3FLY

CONCLUSION

The fused 7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazole-3-thiol derivatives 6-13 were synthesized, characterized by IR, ^{1}H NMR and Mass spectral analysis and investigated for their cytotoxic effect on Peripheral Blood Mononuclear Cells (PBMCs) cell lines, antibacterial and molecular docking study. Detailed investigation of compounds 6-13 against PBMCs cell lines were showed upright cell viability. The compound dichloro[(7-nitro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazol-3-yl)sulfanyl]acetic acid 8 was the most effective anticancer agent against PBMCs by showing greater percentage of dead cell viability and 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 supported the cytotoxic activity of the compounds 8 followed by the compounds 10 and 12 respectively. All these results could be useful to evaluate the cytotoxic inhibitors and can be considered as lead molecules for the better development of drugs.

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REFERENCES

[1] P Lokwani; BP Nagori; N Batra; A Goyal; S Gupta; N Singh. *J Chem Pharm Res.* **2011**, 3(3), 302-311.
[2] PS Laliteshwar; V Chawla; P; Chawla; SK Saraf. *Der Pharm Chem.* **2010**, 2(4), 206-212.
[3] JJX Tang; W Dou; H Zhang; W Liu; C Wang; J Zheng. *J Inorg Biochem.* **2010**, 104, 583-591.
[4] A Kamal; KS Reddy; M Naseer; A Khan; VC Rajesh; C Shetti; MJ Ramaiah. *Bioorg Med Chem Let*. **2010**, 18, 4747-4761.
[5] ND Jayanna; HM Vagdevi; JC Dharshan; R Raghavendra; SB Telkar. *Med Chem Re.* **2013**, 22, 5814-5822.
[6] SM Rida; FA Ashour, AM Soad; E Hawash; M Mona; E Semary. *Eur J Med Chem*. **2005**, 40, 949-959.
[7] RV Satyendra; KA Vishnumurthy; HM Vagdevi; KP Rajesh; H Manjunatha; A Shruthi. *Eur J Med Chem*. **2011**, 46, 3078-3084.
[8] R Paramashivappa; PP Kumar; PVS Rao; AS Rao. *Bioorg Med Chem Let*. **2003**, 13, 657-660.
[9] C Ramalingan; S Balasubramanian; S Kabilan; M Vasudevan. *Eur J Med Chem*. **2004**, 39, 527-533.
[10] V Klimesova; J Koc; K Waisser; J Kaustova; U Mollmann. *Eur J Med Chem*. **2009**, 44, 2286-2293.
[11] Z Yang; DJ Fairfax; JH Maeng; L Masih; A Usyatinsky; C Hassler. *Bioorg Med Chem Let*. **2010**, 20, 6538-6541.
[12] M Sham; SN Singh; A Kumar; O Lozach; L Meijer. *Bioorg Med Chem Let*. **2006**, 14, 3758-3765.
[13] H Song; SR Oh; HK Lee; G Han; JH Kim; HW Chang; KE Doh. *Bioorg Med Chem Let*. **2010**, 18, 7580-7585.
[14] LQ Sun; J Chen; M Bruce; JA Deskus; JR Epperson; KTG Johnson. *Bioorg Med Chem Let*. **2004**, 14, 3799-3802.
[15] H Bayrak; A Demirbas; N Demirbas; S Alpay-Karaoglu. *Eur J Med Chem*. **2009**, 44, 4362-4366.
[16] BS Holla; R Gonsalves; S Shenoy. *Eur J Med Chem*. **2000**, 35, 267-271.
[17] A Shafiee; A Sayadi; MH Roozbahani; A Foroumadi; F Kamal. *Arch Pharm Med Chem*. **2002,** 10, 495-499.
[18] S Sharma; S Gangal; A Rauf; M Zahin. *Arch Pharm Chem Life Sci*. **2008**, 341, 714-720.
[19] NN Gulerman; HN Dogan; S Rollas; C Johansson; C Celik. *Il Farmaco*. **2001**, 56, 953-958.
[20] S Papakonstantinou-Garoufalias; N Pouli; P Marakos; A Chytyroglou-Ladas. *IlFarmaco*. **2002**, 57, 973-977.
[21] G Turan-Zitouni; ZA Kaplacikli; MT Yildiz; P Chevallet; D Kaya. *Eur J Med Chem*. **2005**, 40**,** 607-613.
[22] AR Jalilian; S Sattari; M Bineshmarevasti; A Shafiee; M Daneshtalab. *Arch Pharm Pharm Med Chem.* **2000**, 333, 347-354.
[23] I Küçükgüzel; sg Küçükgüzel; S Rollas; M Kiraz. *Bioorg Med Chem Lett*. **2001**, 11(13), 1703-1707.
[24] L Zahajska; V Klimesova; J Koci; K Waisser; J Kaustova. *Arch Pharm Pharm Med Chem*. **2004**, 337, 549-555.

[25] A Foroumadi; Z Kiani; F Soltani. *Il Farmaco*. **2003**, 58, 1073-1076.
[26] G Turan-Zitouni; M Sivaci; FS Kilic; K Erol. *Eur J Med Chem*. **2001**, 36, 685-689.
[27] B Tozkoparan; E Küpeli; E Yeşilada; M Ertan. *Bioorgan Med Chem*. **2007**, 15(4), 1808-1814.
[28] SM Rabea; NA El-Koussi; HY Hassan; T Aboul-Fadl. *Arch Pharm Chem Life Sci*. **2006**, 339, 32-40
[29] L Labanauskas; E Udrenaite; P Gaidelis; A Brukstus. *Il Farmaco*. **2004**, 59, 255-259.
[30] BS Holla; B Veerendra; MK Shivananda; B Poojary. *Eur J Med Chem*. **2003**, 38, 759-767.
[31] A Duran; HN Dogan; S Rollas. *Il Farmaco*. **2002**, 57, 559-564.
[32] ASA Almasirad; M Tabatabai; A Faizi; N Kebriaeezadeh; A Mehrabi; A Dalvandi A Shafiee. *Bioorg Med Chem Lett*. **2004**, 14, 6057-6059.
[33] I Küçükgüzel; ŞG Küçükgüzel; S Rollas; G Ötük-Sanış; O Özdemir; İ Bayrak; T Altuğ; JP Stables. *Il Farmaco*. **2004**, 59(11), 893-901.
[34] M Kritsanida; A Mouroutsou; P Marakos; N Pouli; S Papakonstantinou- Garoufalias; C Pannecouque; M Witvrouw; E De Clercq. *Il Farmaco*. **2002**, 57, 253-257.
[35] MT Abdel-Aal; WA El-Sayed; SM El-Kosy; ESH El-Ashry. *Arch Pharm Chem Life Sci*. **2008**, 341, 307-313.
[36] B Chai; X Qian; S Cao; H Liu; G Song. *Arkivoc* **2003**, 1, 141-145.
[37] A Varvaresou; TA Tsotinis; A Tsantili-Kakoulidou; A Vamvakides. *Il Farmaco*. **1998**, 53, 320-326.
[38] V Padmavathi; P Thriveni; SG Reddy; D Deepti. *Eur J Med Chem*. **2008**, 43, 917-924.
[39] WT Ashton; CL Cantone; LL Chang; SM Hutchine; RA Strelitz; M Mac Coea; RSL Chang; VJ Lotti; KA Faust; B Tsing-Chen; P Bunting; TW Schorn, SD Kivlighn; PKS Sieglt. *J Med Chem*. **1993**, 36, 591-609.
[40] B Tozkoparan; E Kupeli; E Yesilada; M Ertan. *Bioorg Med Chem*. **2007**, 15, 1808-1814.
[41] N Demirbas; S Karaoglu-Alpay; A Demirbas; K Sancak. *Eur J Med Chem.* **2004**, 39, 793-804.
[42] N Demirbas; R Ugurluoglu; A Demirbas. *Bioorg Med Chem*. **2002**, 10, 3717-3723.
[43] H Bektas; N Karaali; D Sahin; A Demirbas; S Alpay-Karaoglu; N Demirbas. *Molecules*. **2010**, 15, 2427-2438.
[44] H Bektas; A Demirbas; N Demirbas; S Alpay-Karaoˇglu. *Med Chem Res*. **2011**, 21, 212-222.
[45] N Demirbas; A Demirbas; S Alpay-Karaoglu. *Russian J Bioorg Chem*. **2005**, 31, 387-397.
[46] H Bektas;A Demirbas; N Demirbas; S Alpay-Karaoglu. *Turk J Chem*. **2010**, 34, 165-180.
[47] ND Jayanna; HM Vagdevi; JC Dharshan; R Raghavendra; BS Telkar. *Med Chem Res*. **2013**, 22, 5814-5822.
[48] ND Jayanna; HM Vagdevi; JC Dharshan; TR PrashithKekuda. *Int J Chem Sci.* **2012**, 10(3), 1529-1540.
[49] C Robert; X Lu; A Law; TC Freeman; DA Hume. *Immunobiology*. **2011**, 216(11), 1203-1208.
[50] W Strober. *Curr Protoc Immunol*. **2001**.
[51] JC Dharshan; KA Vishnumurthy; YD Bodke; ND Jayanna; R Mohammed Shafeeulla. *Asian J Org Med Chem*. **2016**, 1, 10-16.