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In vitro cytotoxic, Antimicrobial and Antioxidant activity of 6-Chloro-2,3-dihydro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole Derivatives.

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ABSTRACT

The novel compounds 6-Chloro-2,3 dihydro[1,2,4]triazolo[3,4-*b*][1,3]benzoxazole derivatives **4-19** have been synthesized by conventional method. The cyclization of the compounds **3(a-b)** with different aromatic benzoic acid derivatives in $POCl_3$ resulted in the formation of the compounds **4-19.** The structures of the compounds were elucidated by spectral and elemental analysis. Most of the compounds were exhibited potent activity in the antibacterial study and were enriched by the MIC results. Some of the synthesized derivatives exhibited potent cytotoxic activity towards Peripheral Blood Mononuclear Cells (PBMCs) with the influence of functional groups attached with central moiety. The antioxidant screening was also done for the same compounds to obtain promising results from the synthesized compounds.

Keywords: Benzoxazole, Triazoles, Benzoic acid, $POCl_3$, PBMCs.

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INTRODUCTION

The rising prevalence of multi-drug-resistant microbial strains such as methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermis* and *vancomycin-resistant Enterococcus* etc is a problem of ever-increasing significance [1]. Although antimicrobial resistance was recognized soon after the deployment of sulfonamides and penicillin [2], it now appears that the emergence of antibiotic-resistant bacteria is inevitable to almost every new drug. As a consequence, new efforts to develop new antibacterial agents are highly needed. Benzoxazole ring is one of the most common heterocycles in medicinal chemistry. Previous reports revealed that substituted benzoxazoles possess diverse chemotherapeutic activities including 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], antifungal [16], anti-tubercular [17], analgesic [18], anti-inflammatory [18], anticancer [20], anticonvulsant [21], antiviral [22], insecticidal [24] and antidepressant [25] properties. Keeping in view of the significance of benzoxazoles and triazolesas potential pharmacophores and in continuation of our research work on the synthesis of novel series of biologically active heterocyclic derivatives, it was planned to synthesize some molecules having both benzoxazole and triazole nucleus together, which may imparts cytotoxic activity and antibacterial effect. The compounds were screened for their antimicrobial activity, minimum inhibition concentration evaluated at four different concentrations.

EXPERIMENTAL SECTIONS

*(i)*Synthesis of 4-(6-chloro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)aniline 4

The compounds **[26] 3(a-b)** (0.01mol) were dissolved in $pocl_3$ (10ml), followed by treated with benzoic acid (0.01mol) and refluxed for 8 hr. then the reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystalized from ethanol to get compound **4.**
The compounds **5-19** can be prepared by following similar procedure.

Yield: 0.3821 (70 %), m.p. 253-255^0 C; IR (KBr v_{max} cm^{-1}): 3006.16, (Ar C-H str), 1485.35 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.80-7.88(m, 7H, Ar-H), 3.55 (s, 2H, NH_2).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 156.52, 155.23, 152.14, 150.41, 149.36, 148.35, 146.19, 143.65, 141.31, 137.08, 135.65, 131.19, 129.62, 77,28, 55.38. elemental analysis: calculated (%) for $C_{14}H_9ClN_4O$; C,59.06; H,3.19; N,19.68; observed C,59.02; H,3.15; N,19.61; M^{+1}, 285; M^{+2},287.

*(ii)*Synthesis of 6-chloro-3-(2,4-difluorophenyl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole 5

Yield: 0.2712 (76 %), m.p. 225-227^0 C; IR (KBr v_{max} cm^{-1}): 3004.59, (Ar C-H str), 1488.14 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.88-7.92(m, 6H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.10, 153.29, 151.08, 150.60, 147.91, 146.46, 144.16, 141.09, 139.63, 137.56, 134.48, 130.19, 125.31, 77.62. elemental analysis: calculated (%) for $C_{14}H_6ClF_2N_3O$; C,55.01; H,1.98; N,13.75; observed C,54.95, H,1.92; N,13.69; M^{+1},306; M^{+2},308, M^{+4},310, M^{+6},312.

*(iii)*Synthesis of 6-chloro-3-(furan-2-yl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole 6

Yield: 0.3216 (81 %), m.p. 188-190^0 C; IR (KBr v_{max} cm^{-1}): 3006.49, (Ar C-H str), 1484 .59 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.76-7.85(m, 6H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 154.10, 152.67, 149.54, 146.18, 144.39, 142.15, 139.49, 135.39, 133.92, 129.38, 76.94, 56.75. elemental analysis: calculated (%) for $C_{12}H_6ClN_3O_2$; C,55.51, H,2.33, N,16.18; observed C,55.47; H,2.028; N,16.14; M^{+1},260; M^{+2},262.

*(iv)*Synthesis of 6-chloro-3-(pyridin-3-yl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole 7

Yield: 0.2947 (73 %), m.p. 212-214^0 C; IR (KBr v_{max} cm^{-1}): 3001.26, (Ar C-H str), 1482.66 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.82-7.96 (m, 7H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 154.27, 153.16,

152.67, 151.68, 148.51, 146.81, 145.38, 142.43, 133.03, 129.85, 125.19, 78.40, 55.76. elemental analysis: calculated (%) for $C_{13}H_9ClN_4O$; C,57.26; H,3.33; N,20.55; observed C,57.14; H,3.22; N,20.18; M^{+1},271; M^{+2},272.

***(v)*Synthesis of 1-[4-(6-chloro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl) phenyl]methanedi amine 8**

Yield: 0.3651 (81 %), m.p. 281-283^0 C; IR (KBr v_{max} cm^{-1}): 3003.86, (Ar C-H str), 1483.50 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.84-7.86(m, 7H, Ar-H), 3.86 (s, 4H, NH_2).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.68, 154.18, 151.48, 148.06, 146.26, 145.87, 143.14, 140.63, 138.56, 133.19, 129.25, 125.43, 77.28, 52.46. elemental analysis: calculated (%) for $C_{15}H_{14}ClN_5O$; C,57.42, H,3.86, N,22.32; observed C,57.38; H,3.81; N,22.27; M^{+1}, 314; M^{+2},316.

***(vi)* Synthesis of 6-chloro-3-(4-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole 9**

Yield: 0.3416 (86 %), m.p. 221-223^0 C; IR (KBr v_{max} cm^{-1}): 3005.36, (Ar C-H str), 1477.02 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.80-7.87 (m, 7H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.46, 154.76, 150.17, 148.15, 145.35, 143.16, 140.28, 137.92, 133.47, 128.08, 122.94, 119.53, 78.40, 53.19. elemental analysis: calculated (%) for $C_{14}H_7ClN_4O_3$; C,53.43; H,2.24; N,17.80; observed C,53.39; H,2.21; N,17.74; M^{+1},315; M^{+2},317.

***(vii)* Synthesis of 6-chloro-3-(pyridin-2-yl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole 10**

Yield: 0.2965 (83 %), m.p. 261-263^0 C; IR (KBr v_{max} cm^{-1}): 3001.63, (Ar C-H str), 1482.46 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.82-7.79 (m, 7H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 154.67, 152.16, 149.38, 147.48, 144.12, 142.36, 139.85, 135.41, 132.34, 125.18, 122.72, 76.83, 56.48. elemental analysis: calculated (%) for $C_{13}H_7ClN_4O$; C,57.69; H,2.61; N,20.70; observed C,57.65; H,2.58; N,20.64; M^{+1},271; M^{+2},273.

***(viii)* Synthesis of 3-(6-chloro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)phenol 11**

Yield: 0.3208 (80 %), m.p. 238-240^0 C; IR (KBr v_{max} cm^{-1}): 3004.63, (Ar C-H str), 1485.71 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.80-7.96 (m, 7H, Ar-H), 5.67 (s, 1H, OH).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 153.13, 152.85, 150.64, 148.37, 145.71, 143.49, 142.87, 137.60, 135.05, 133.53, 127.33, 125.66, 77.52, 55.43. elemental analysis: calculated (%) for $C_{14}H_8ClN_3O_2$; C,58.86; H,2.82; N,14.71; observed C,58.82; H,2.78; N,14.68; M^{+1},286; M^{+2},288.

(ix) synthesis of 4-(7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)aniline 12

Yield: 0.3617 (85 %), m.p. 226-228^0 C; IR (KBr v_{max} cm^{-1}): 3009.46, (Ar C-H str), 1488.63 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.76-7.92(m, 7H, Ar-H), 3.68 (s, 2H, NH_2).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.29, 154.32, 151.83, 149.68, 147.63, 145.33, 143.85, 141.13, 139.39, 137.79, 132.57, 130.77, 77.28, 55.62. Elemental analysis: calculated (%) for $C_{14}H_9N_5O_3$; C,56.95; H,3.07; N,23.72; observed C, 56.92; H, 3.03; N,23.68; M^{+1}, 295.

***(x)*Synthesis of 3-(2,4-difluorophenyl)-7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazole 13**

Yield: 0.2286 (76 %), m.p. 264-266^0 C; IR (KBr v_{max} cm^{-1}): 3004.16, (Ar C-H str), 1478.46 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.88-7.98(m, 6H, Ar-H), 3.65 (s, 2H, NH_2).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 154.35, 152.28, 150.44, 148.11, 146.30, 144.05, 142.61, 140.36, 138.89, 136.50, 133.22, 127.14, 79.16, 56.93. Elemental analysis: calculated (%) for $C_{14}H_6F_2N_4O_3$; C,53.18; H,1.91; N,17.72; observed C, 53.15; H, 1.88; N,17.68; M^{+1}, 316, M^{+2}, 318, M^{+4}, 320.

***(xi)* Synthesis of 3-(furan-2-yl)-7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazole 14**

Yield: 0.2826 (74 %), m.p. 216-218^0 C; IR (KBr v_{max} cm^{-1}): 3011.65, (Ar C-H str), 1491.63 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.75-7.95(m, 6H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.43, 153.85,

151.68, 148.53, 143.71, 141.36, 138.79, 134.13, 132.92, 126.37, 77.56, 56.16. elemental analysis: calculated (%) for $C_{12}H_6N_4O_4$; C,53.34, H,2.24, N,20.74; observed C,53.31; H,2.21; N,20.70; M^{+1},270.

***(xii)*Synthesis of 7-nitro-3-(pyridin-3-yl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole 15**

Yield: 0.2634 (72 %), m.p. 246-248^0 C; IR (KBr v_{max} cm^{-1}): 3009.32, (Ar C-H str), 1489.41 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.84-7.91(m, 7H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 156.12, 154.82, 150.67, 148.55, 144.83, 140.60, 137.46, 134.13, 133.15, 130.03, 124.70, 78.42, 55.61. elemental analysis: calculated (%) for $C_{13}H_7N_5O_3$; C,55.52, H,2.51, N,24.90; observed C,55.48; H,2.48; N,24.84; M^{+1},281.

***(xiii)* Synthesis of 1-[4-(7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)phenyl]methanedi amine 16**

Yield: 0.3218 (78 %), m.p. 291-293^0 C; IR (KBr v_{max} cm^{-1}): 3008.24, (Ar C-H str), 1483.83 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.78-7.94(m, 7H, Ar-H), 3.94 (s, 4H, NH_2).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 153.46, 152.71, 150.62, 148.49, 145.16, 144.20, 142.31, 139.48, 137.72, 134.65, 130.15, 126.37, 78.13, 55.18, 45.63. elemental analysis: calculated (%) for $C_{15}H_{12}N_6O_3$; C,55.55, H,3.73, N,25.91; observed C,55.50; H,3.69; N,25.88; M^{+1}, 324.

***(xiv)* Synthesis of 7-nitro-3-(4-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole 17**

Yield: 0.3751 (88 %), m.p. 204-206^0 C; IR (KBr v_{max} cm^{-1}): 3003.26, (Ar C-H str), 1482.18 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.76-7.95 (m, 7H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 154.28, 152.49, 150.72, 149.62, 146.38, 144.06, 138.13, 134.14, 133.47, 128.08, 124.94, 122.02, 77.96, 55.38. elemental analysis: calculated (%) for $C_{14}H_7N_5O_5$; C,51.70; H,2.17; N,21.53; observed C,51.67; H,2.13; N,21.49; M^{+1},325.

***(xv)* Synthesis of 7-nitro-3-(pyridin-2-yl)[1,2,4]triazolo[3,4-b][1,3]benzoxazole 18**

Yield: 0.3193 (78 %), m.p. 287-289^0 C; IR (KBr v_{max} cm^{-1}): 3005.72, (Ar C-H str), 1485.81 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.77-7.93 (m, 7H, Ar-H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.14, 153.44, 148.63, 146.77, 143.12, 142.97, 140.18, 136.28, 131.67, 126.72, 123.68, 77.28, 56.71. elemental analysis: calculated (%) for $C_{13}H_7N_5O_3$; C,55.52; H,2.51; N,24.90; observed C,55.48; H,2.47; N,24.88;M^{+1},281.

***(xvi)* Synthesis of 3-(7-nitro[1,2,4]triazolo[3,4-b][1,3]benzoxazol-3-yl)phenol19**

Yield: 0.2618 (83 %), m.p. 233-235^0 C; IR (KBr v_{max} cm^{-1}): 3006.97, (Ar C-H str), 1492.12 (Ar C-C str in ring). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.73-7.86 (m, 7H, Ar-H), 5.72 (s, 1H, OH).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.47, 153.38, 151.82, 147.09, 146.63, 141.87, 139.67, 136.04, 132.86, 131.73, 126.48, 124.35, 78.08, 56.82. elemental analysis: calculated (%) for $C_{14}H_8N_4O_4$; C,56.76; H,2.72; N,18.91; observed C,56.67; H,2.68; N,18.88; M^{+1},286.

RESULTS AND DISCUSSION

Chemistry

All the compounds were synthesized as routed in the **scheme 1**. The chloro and nitro substituted 2-aminophenol were treated with carbon disulphide and potassium hydroxide in the presence of methanol solvent to get substituted 1,3-benzoxazole-2-thiol **1(a-b)** compounds. The compounds **1(a-b)** were treated with iodoethane in the presence of sodium hydroxide in DMSO solvent to get 2-(ethylsulfanyl)-1,3-benzoxazole **2(a-b)** followed by reaction with hydrazine hydride by using ethanol solvent to get intermediate compounds 2-hydrazinyl-1,3-benzoxazole **3(a-b) (Scheme-1)**. The compounds **3(a-b)** were characterized by spectroscopic analysis.

The derivatives **4-19** were synthesized by using 2-hydrazinyl-1,3-benzoxazole **3(a-b**). using different benzoic acids with in $POCl_3$ to forms benxoxazole fused triazole derivatives. The formation of target molecules confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass, and elemental analysis. IR spectrum shows strong absorbance peak at 3006.16 cm^{-1} (Ar C-H str); at 1485.35 (Ar C-C str in ring) groups and ^{1}H NMR spectrum exhibits peak δ 6.80-7.88(m, 8H, Ar-H) protons, which confirms the cyclization of 2-hydrazino benzoxazoles with acid groups and

forms cyclized triazole ring. The Mass spectra's are concurrence with Molecular weights of the target compounds.

The derivatives **4-19** have been screened for *in vitro* cytotoxic, antibacterial and antioxidant activity. For selected compounds minimum inhibition concentration was carried out. The selected compound were evaluated for their cytotoxic effect on PBMCs cell lines (**Table-1** and **Figure-1**), to examine their anticancer potentiality. The activity of the tested compounds was influenced considerably by the nature of functional group. Compound **5**, **6, 7, 10, 13, 14, 16, 18** and **19** 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 amino and nitro substituent did not show upright cytotoxic activity, whereas difloro, pyridine, hydroxyl, furan and methane diamine benzoic acid derivatives exhibited effective activity irrespective of concentration. The antibacterial study, maximum compounds have showed potent zone of inhibition **(Table-2 and Figure-2)**. In the synthesized compounds, marked zone of inhibition of bacteria was observed by the compounds **4, 5, 8, 9, 11, 13, 14, 16, 17** and **19** while moderate activity was observed in compounds **6, 7, 10, 12, 15** and **18** with comparison of standard drug Chloramphenicol. Some of selected Compounds exhibited potent antibacterial activity were evaluated for their minimum inhibition concentration **(Table-3)** to determine their distinct zone of inhibition of bacteria at varied minimum concentration. Compounds **4, 5, 8, 9, 11, 13, 14, 16** and **19** were found fine zone of inhibition in four different concentrations (25µg/mL, 50µg/mL, 75 µg/mL and 100 µg/mL) against gram positive and gram negative bacteria. It was found that benoxazole fused triazole derivatives showed marked zone of inhibition with observation of effective microbial activity results. The antioxidant activity was done with effective free radical scavenging method (**Table-4** and **Figure-3**). The compounds **4, 5, 8, 9, 11, 12, 13, 14, 16, 17** and **19** were possess excellent antioxidant activity compared with Ascorbic acid as standard.

Table-1 Cytotoxic activity of newly synthesized triazole derivatives against PBMCs.

Sample	Total cells	Live cells	Dead cells	% of Cells viability	%of cells non-viability
5-10µg/mL	82	32	50	39.02	60.97
5-50µg/mL	212	60	142	28.3	66.98
5-100µg/mL	**136**	**38**	**98**	**27.94**	**72.05**
6-10µg/mL	90	42	48	46.66	53.33
6-50µg/mL	**125**	**27**	**98**	**21.60**	**78.40**
6-100µg/mL	92	68	24	73.91	26.08
7-10µg/mL	78	29	49	37.1	62.8
7-50µg/mL	82	38	44	46.3	53.7
7-100µg/mL	**186**	**46**	**140**	**24.73**	**75.26**
10-10µg/mL	**153**	**45**	**108**	**29.41**	**70.59**
10-50µg/mL	136	48	88	35.3	64.7
10100µg/mL	95	43	52	45.35	54.75
13-10µg/mL	**162**	**42**	**120**	**25.92**	**74**
13-50µg/mL	188	84	104	44.68	55.32
13-100µg/mL	88	45	43	51.13	48.86
14-10µg/mL	171	63	108	36.8	63.2
14-50µg/mL	**163**	**48**	**115**	**29.4**	**70.6**
14-100µg/mL	96	57	39	59.38	40.62
16-10µg/mL	119	48	71	40.33	59.66
16-50µg/mL	154	61	93	39.6	60.4
16-100µg/mL	**136**	**40**	**96**	**29.4**	**70.6**
18-10µg/mL	124	51	73	41.1	59
18-50µg/mL	**199**	**57**	**142**	**28.64**	**71.35**
18-100µg/mL	78	42	36	53.85	46.15
19-10µg/mL	**184**	**41**	**143**	**23.2**	**77.8**
19-50µg/mL	93	54	39	58.06	41.93
19-100µg/mL	129	58	71	44.97	55.03
Control	118	17	101	14.4	85.5

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Table-2 Antibacterial activity of compounds 4-19

Compounds	Zone of inhibition in mm					
	Gram positive bacteria			*Gram negative bacteria*		
	Saureus	*B.subtilis*	*S.epidermis*	*V.cholerae*	*S.typhi*	*E.coli*
4	20.02±0.26	22.09±0.88	23.09±0.34	24.99±0.98	22.02±0.11	20.09±0.23
5	21.03±0.22	24.00±0.87	23.01±0.22	**26.98±1.91**	**25.21±4.56**	22.32±0.65
6	22.98±0.32	23.02±9.98	24.89±0.22	**26.01±0.33**	**26.99±0.29**	23.78±0.36
7	19.98±1.23	**25.98±0.47**	22.09±2.22	23.78±9.01	22.09±11	32.32±3.09
8	20.09±11	21.78±0.87	20.89±0.45	22.93±0.43	23.01±2.09	22.09±2.54
9	18.09±0.34	19.89±2.09	20.34±0.15	22.08±3.56	21.07±2.22	19.09±4.56
10	22.37±7.90	23.20±2.98	22.89±0.46	**25.03±3.09**	**25.09±1.34**	20.09±1.08
11	19.78±0.88	20.45±0.22	23.98±0.43	21.09±0.23	22.98±1.67	20.38±0.77
12	21.77±0.35	20.67±0.29	19.98±0.88	22.99±0.66	20.09±0.54	21.09±0.54
13	**25.67±0.33**	23.09±0.26	23.90±1.11	**26.09±0.34**	**25.85±0.33**	24.93±0.32
14	24.98±0.22	22.90±0.26	**26.90±0.33**	**25.90±0.22**	24.89±2.98	23.48±0.39
15	21.67±2.09	22.44±0.33	20.77±0.43	23.09±0.25	21.98±9.89	22.09±2.23
16	23.56±8.09	24.98±0.83	23.89±2.09	22.33±0.29	24.77±0.37	**25.90±1.98**
17	20.89±0.56	23.98±2.90	22.44±0.32	23.09±0.21	20.89±2.22	24.98±0.25
18	21.68±20	25.88±0.22	**26.09±0.33**	**25.03±1.09**	24.98±0.65	23.07±0.43
19	20.89±0.21	**25.09±0.45**	22.98±0.43	24.09±0.99	**26.04±0.33**	21.93±0.44
Std	28.99±0.1	29.78±0.43	28.92±0.56	30.09±0.98	29.00±0.22	28.93±0.99

Std: Chorophinocol
Solvent: DMSO

Table-3 Minimum inhibition concentration of selected compounds

Std: Chorophinocol

	Bacterial strains	Conc	Compounds and its zone of inhibition in mm										
			4	5	8	9	11	13	14	16	17	19	
Gram positive bacteria	*S. aureus*	25	19	**20**	18	16	18	17	17	16	21	18	29
		50	20	**21**	20	16	17	19	18	17	21	18	
		75	22	**23**	22	18	18	21	19	17	19	20	
		100	24	**25**	23	19	19	23	20	19	24	21	
	B.cereus	25	19	19	17	19	**20**	16	**19**	19	17	17	30
		50	20	20	19	20	**21**	18	**21**	20	18	19	
		75	23	21	21	22	**23**	19	**23**	21	19	21	
		100	22	23	22	23	**25**	20	**25**	22	20	22	
	S.epidermis	25	18	18	16	17	16	15	14	16	15	**19**	29
		50	19	19	17	19	18	16	16	19	17	**21**	
		75	21	20	20	18	19	18	17	19	18	**23**	
		100	21	22	19	18	21	19	19	20	19	**25**	
Gram negative bacteria	*V. cholerae*	25	16	**21**	**20**	18	16	**20**	16	19	**19**	18	28
		50	17	**23**	**22**	20	19	**22**	16	20	**20**	19	
		75	17	**23**	**23**	22	19	**25**	18	22	**20**	20	
		100	19	**25**	**25**	23	20	**26**	19	24	**25**	21	
	S.typhi	25	**19**	**20**	**19**	**19**	19	**21**	18	**21**	19	18	29
		50	**20**	**23**	**22**	**20**	20	**23**	20	**22**	20	19	
		75	**21**	**26**	**24**	**20**	21	**25**	22	**23**	20	21	
		100	**23**	**24**	**26**	**25**	23	**26**	23	**25**	22	22	
	E.coli	25	16	**21**	14	16	18	18	17	16	17	18	28
		50	19	**22**	15	18	19	19	17	18	19	19	
		75	19	**23**	18	20	20	21	19	18	19	19	
		100	20	**25**	19	19	21	24	21	20	21	22	

Solvent: DMSO

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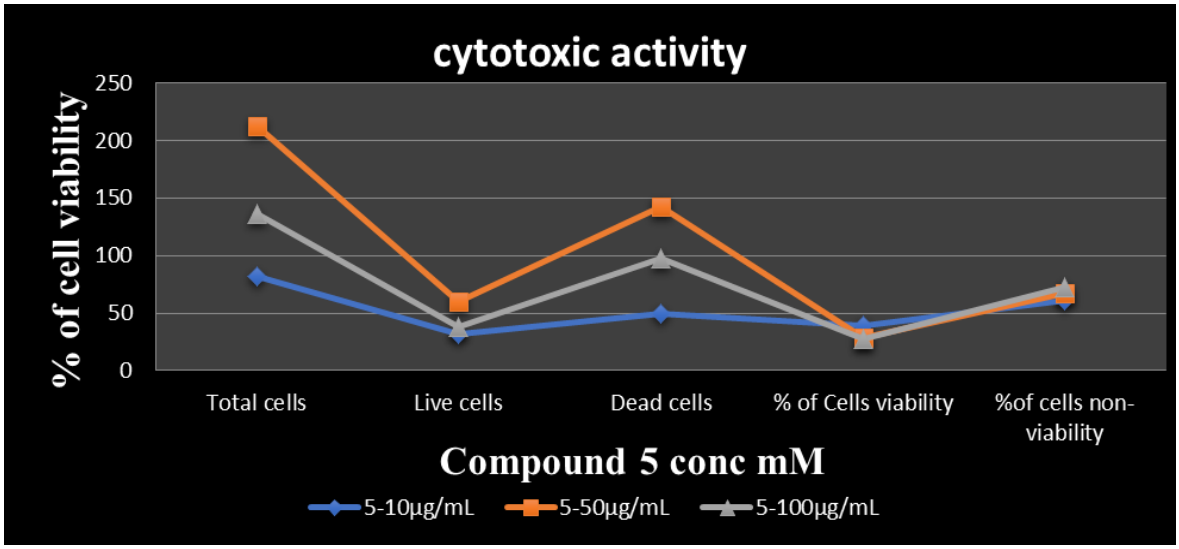
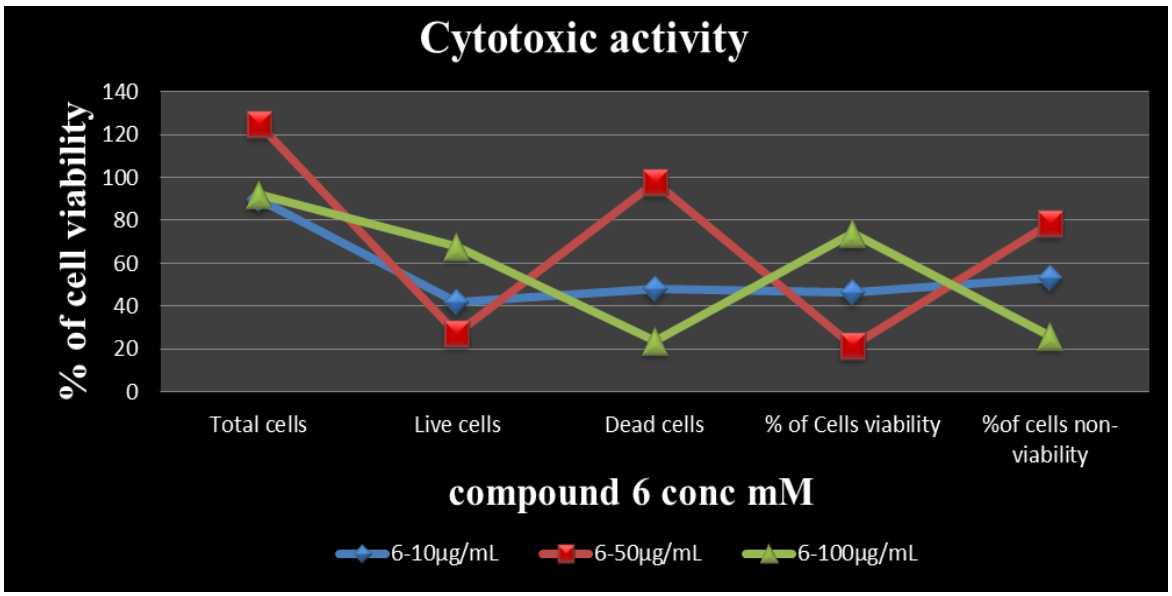
Table-4 Antioxidant activity of synthesized compound 4-19

Compounds	Scavenging activity of different concentration (Mg/mL) in %				
	5	10	15	20	25
4	**62.20**	**64.59**	**68.19**	**70.42**	**73.71**
5	**82.16**	**85.10**	**86.63**	**88.78**	**89.48**
6	50.92	56.18	61.73	63.12	68.15
7	53.67	58.30	61.71	64.38	69.09
8	**74.64**	**77.56**	**79.18**	**81.38**	**84.87**
9	**78.25**	**80.43**	**83.71**	**86.45**	**88.53**
10	48.18	50.56	53.74	58.46	61.89
11	**76.56**	**79.67**	**81.12**	**83.63**	**85.10**
12	**73.47**	**78.16**	**81.61**	**83.42**	**86.53**
13	**81.19**	**83.38**	**85.95**	**88.72**	**90.43**
14	**72.02**	**75.36**	**78.41**	**80.63**	**82.25**
15	50.81	53.00	54.40	58.78	62.38
16	**75.74**	**78.68**	**81.15**	**84.62**	**86.73**
17	**77.92**	**79.54**	**82.33**	**85.16**	**89.43**
18	53.67	58.30	61.71	64.38	69.09
19	**78.95**	**80.53**	**83.56**	**85.79**	**89.12**
Ascorbic acid	**86.71**	**88.57**	**90.04**	**93.62**	**96.33**





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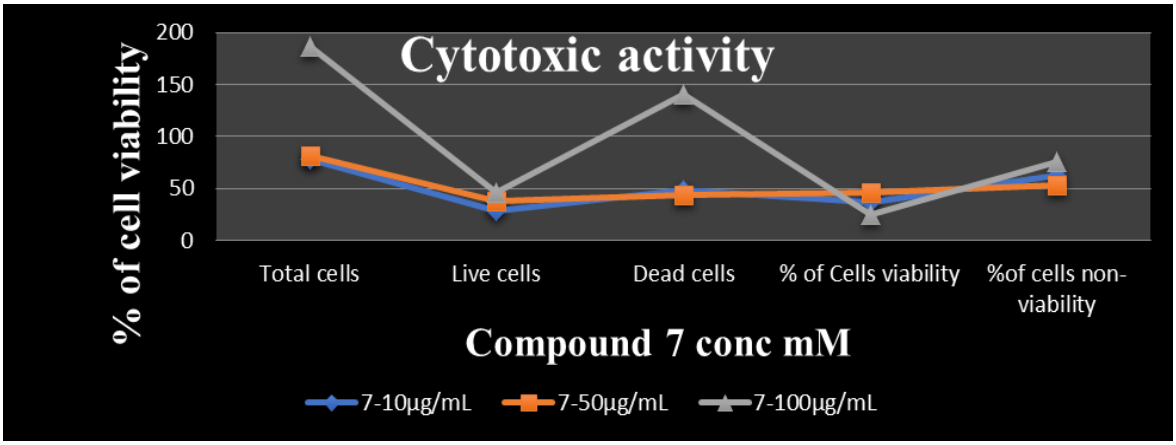

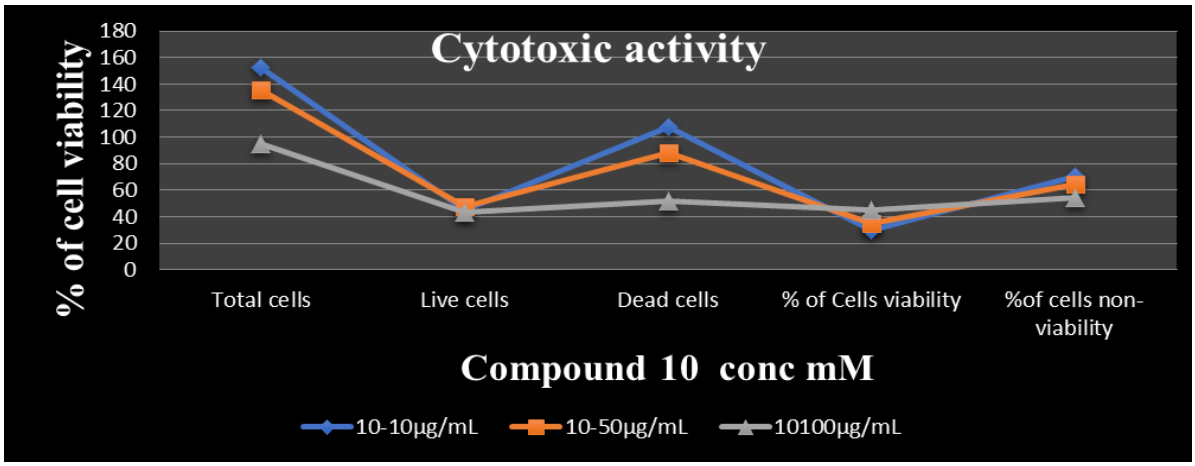

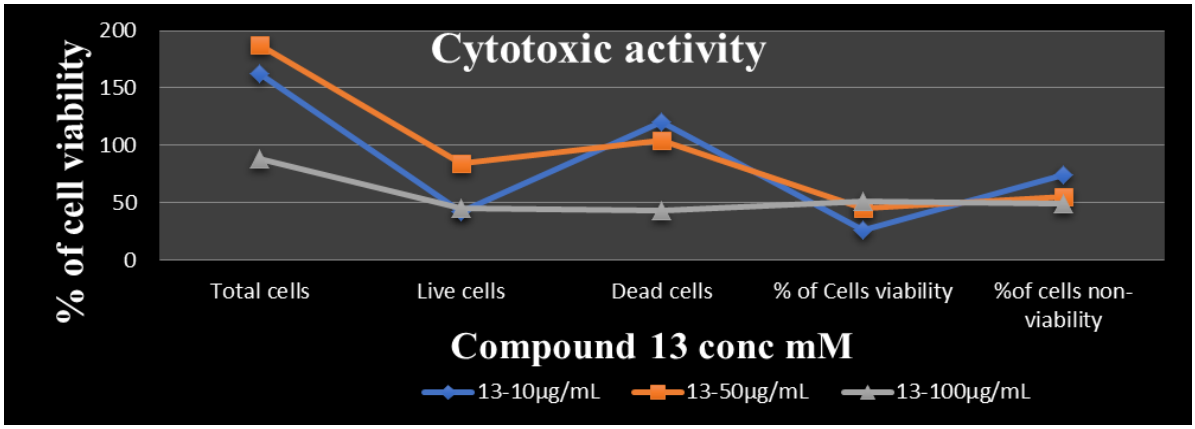

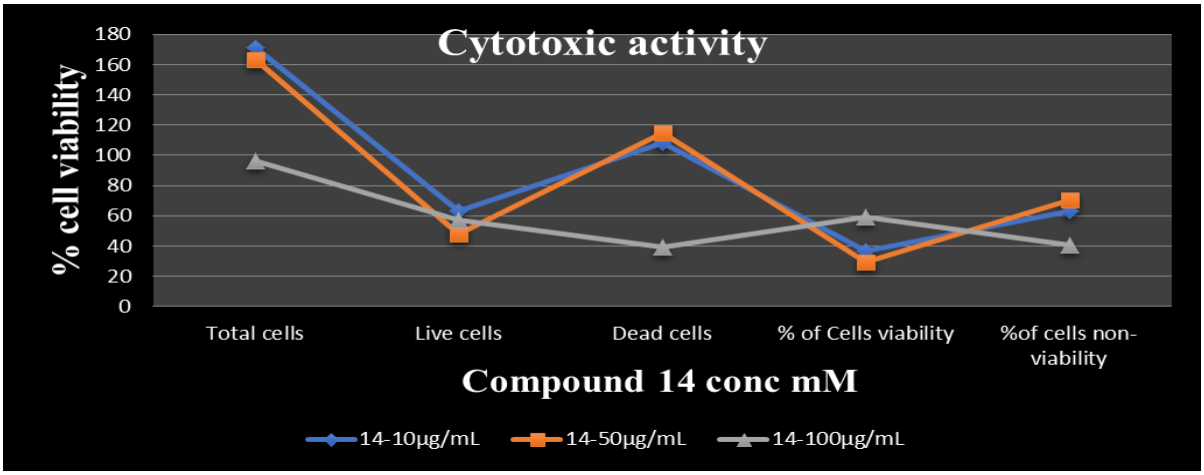

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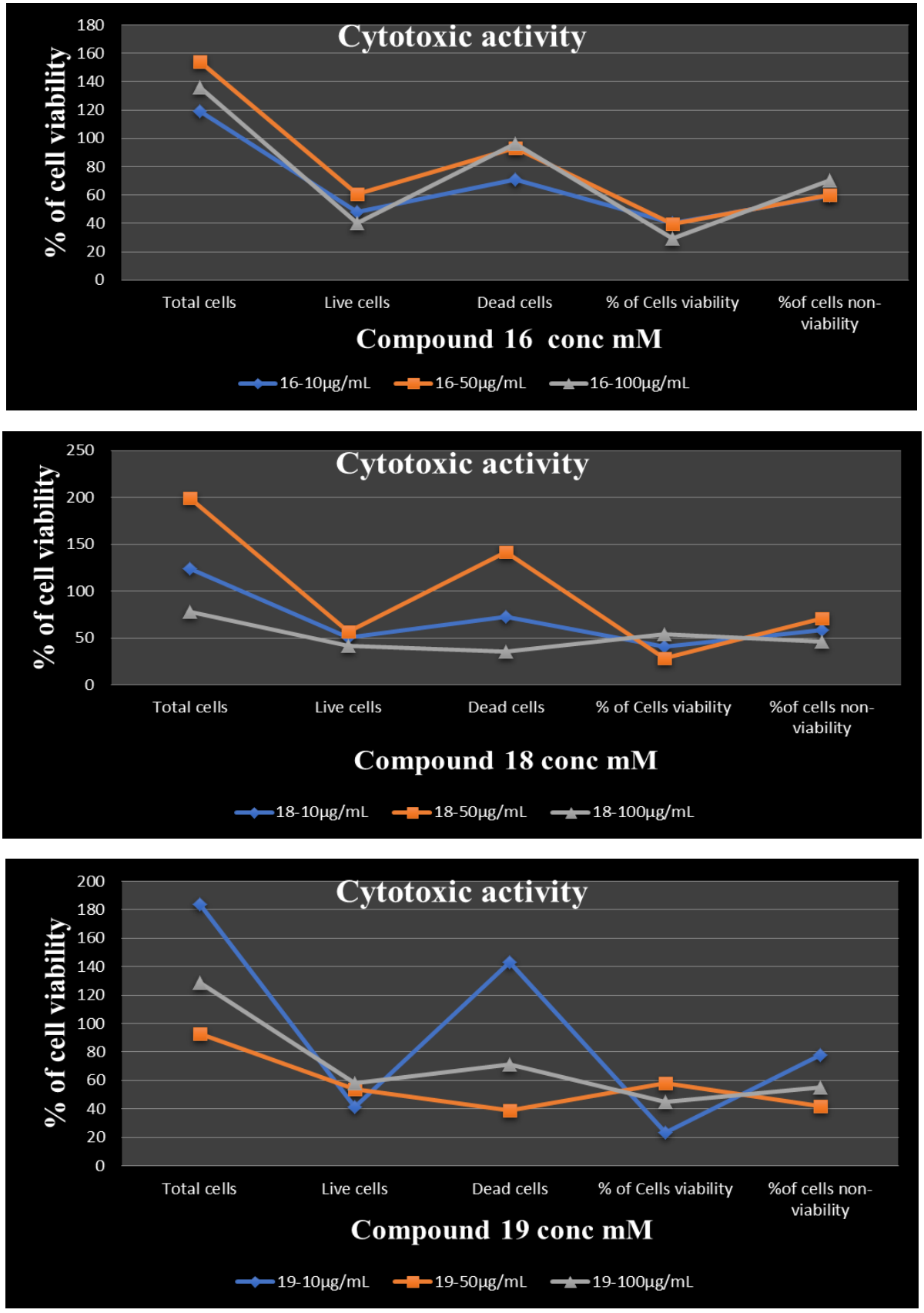

Figure-1. Cytotoxic effect of selected compounds at varying concentrations (10µg/mL, 50µg/mL and 100µg/mL) against Peripheral Blood Mononuclear Cells. Dose-response effect of each synthesized compounds showed in graphical representation.

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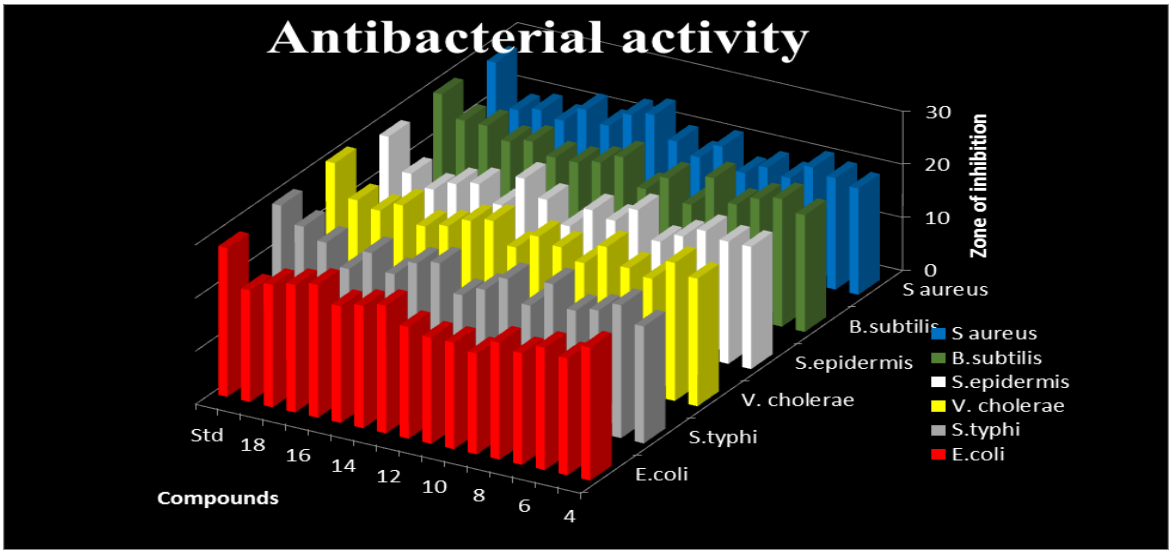


Figure-2 Antibacterial activity of compounds 4-19

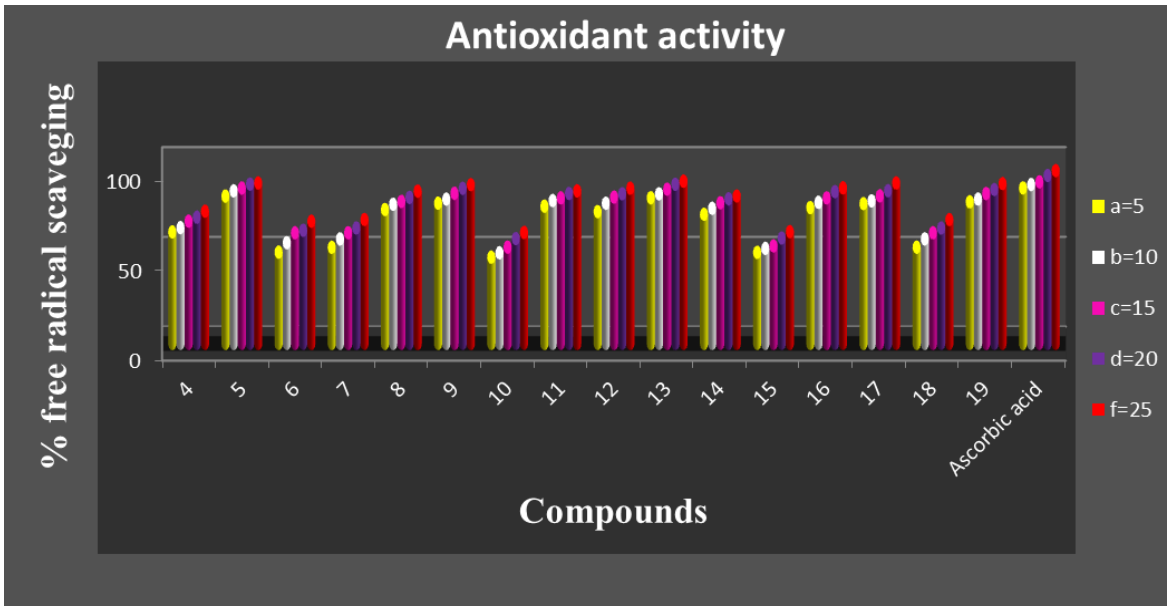
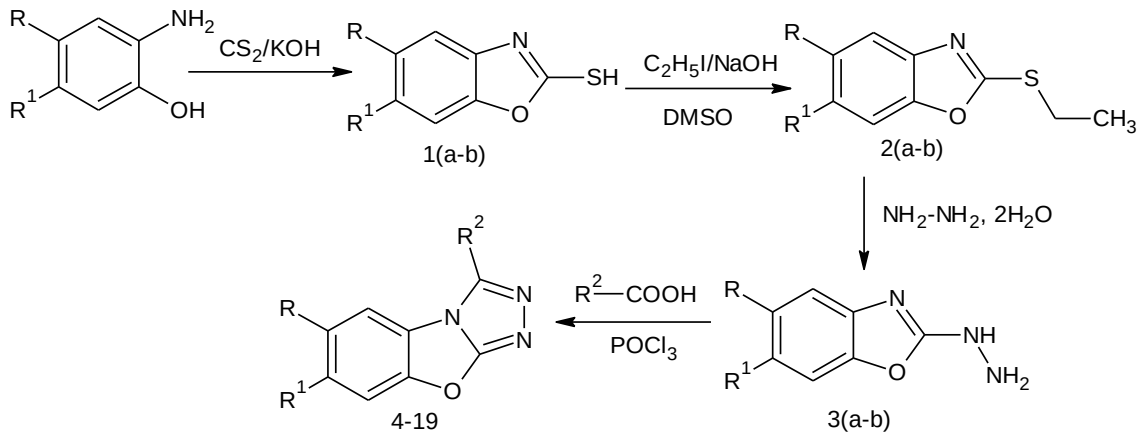


Figure-3 antioxidant activity of synthesized compound 4-19



Scheme 1: Synthetic route for the preparation of compound 4-19

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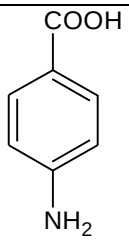
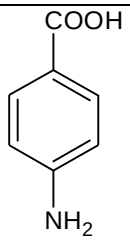
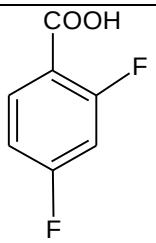
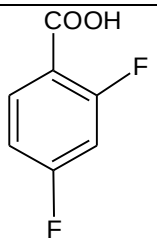
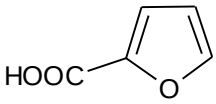
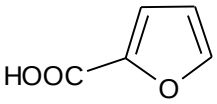
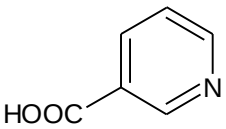
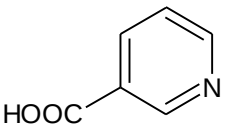
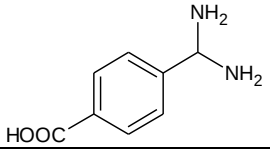
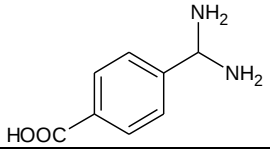
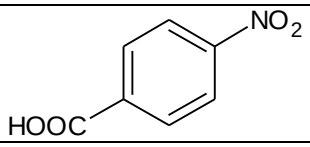
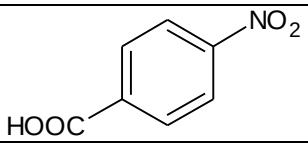
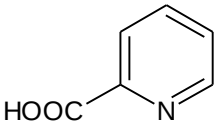
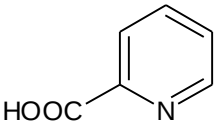
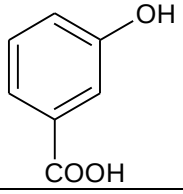
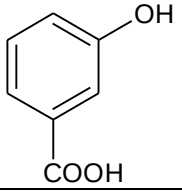
Comp	R	R^1	R^2	Comp	R	R^1	R^2
3a	Cl	H	-	3b	H	NO_2	-
4	Cl	H		12	H	NO_2	
5	Cl	H		13	H	NO_2	
6	Cl	H		14	H	NO_2	
7	Cl	H		15	H	NO_2	
8	Cl	H		16	H	NO_2	
9	Cl	H		17	H	NO_2	
10	Cl	H		18	H	NO_2	
11	Cl	H		19	H	NO_2	

Biological activity of the synthesized compounds 4-19

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 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 **[27].**

About **10 µg/mL**, **50 µg/mL** and **100 µg/mL** of the selected compounds (1mg/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 **[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 10 min 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-1**.The graphical representation was presented in **figure-1**.

Antibacterial Activity

The newly synthesized benzoxazole linked triazole 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 **[29]**. 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 **4-19** (20 mg/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 37^0C for 24 hr in upright position and the zone of inhibition was recorded and tabulated in **Table-2** and graphically represented in **figure-2**.

Minimum Inhibitory Concentration (MIC).

The MIC of the selected compounds **4, 5, 8, 9, 11, 13, 14, 16, 17** and **19** was determined by micro dilution method **[30]**. The respective clinical strain was 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 μg/mL), were loaded into corresponding 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-3.**

Antioxidant activity synthesized compounds 4-19

DPPH Assay.

The radical scavenging ability of synthesized compounds and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on a DPPH free radical. Different concentrations (25, 50, 75 and 100μg/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** μg/mL concentrations of compounds and standard separately and make up 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 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 **[31]**.

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CONCLUSIONS

A series of benzoxazole with triazole derivatives were synthesized by the cyclization of substituted 2-hydrazinyl-1,3-benzoxazole **3(a-b)** with various benzoic acids. The newly synthesized molecules were characterized by IR, ^{1}H NMR and mass spectral analysis. For all the compounds, the in vitro cytotoxic, antibacterial, minimum inhibition concentration and antioxidant activity studies were evaluated. The cytotoxic activity against Peripheral Blood Mononuclear Cells were tested by selected benzoxazole derivatives. The compounds **5, 6, 7, 10, 13, 14, 16, 18** and **19** were exhibited effective anticancer activity against PBMCs. Among the synthesized benzoxazole with triazole derivatives. Compounds **4, 5, 8, 9, 11, 13, 14, 16, 17** and **19** were emerged as potent antibacterial compounds, which were supported by MIC. The synthesized compounds were familiarized for free radical scavenging, in which the compounds **4, 5, 8, 9, 11, 12, 13, 14, 16, 17** and **19** showed good free radical scavenging activity.

ACKNOWLEDGMENTS

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