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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 POCl₃ 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, POCl₃, 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 triazoles as 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 impart 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 recrystallized 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^o C; IR (KBr ν_{max} cm^{-1}): 3006.16, (Ar C-H str), 1485.35 (Ar C-C str in ring). ¹H NMR (DMSO- d_6 , 400MHz) δ : 6.80-7.88(m, 7H, Ar-H), 3.55 (s, 2H, NH₂). ¹³C 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 C₁₄H₉ClN₄O; C,59.06; H,3.19; N,19.68; observed C,59.02; H,3.15; N,19.61; M⁺, 285; M⁺, 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^o C; IR (KBr ν_{max} cm^{-1}): 3004.59, (Ar C-H str), 1488.14 (Ar C-C str in ring). ¹H NMR (DMSO- d_6 , 400MHz) δ : 6.88-7.92(m, 6H, Ar-H). ¹³C 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 C₁₄H₆ClF₂N₃O; C,55.01; H,1.98; N,13.75; observed C,54.95, H,1.92; N,13.69; M⁺,306; M⁺,308, M⁺,310, M⁺,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^o C; IR (KBr ν_{max} cm^{-1}): 3006.49, (Ar C-H str), 1484.59 (Ar C-C str in ring). ¹H NMR (DMSO- d_6 , 400MHz) δ : 6.76-7.85(m, 6H, Ar-H). ¹³C 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 C₁₂H₆ClN₃O₂; C,55.51, H,2.33, N,16.18; observed C,55.47; H,2.028; N,16.14; M⁺,260; M⁺,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^o C; IR (KBr ν_{max} cm^{-1}): 3001.26, (Ar C-H str), 1482.66 (Ar C-C str in ring). ¹H NMR (DMSO- d_6 , 400MHz) δ : 6.82-7.96 (m, 7H, Ar-H). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3003.86, (Ar C-H str), 1483.50 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.84-7.86(m, 7H, Ar-H), 3.86 (s, 4H, NH₂). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3005.36, (Ar C-H str), 1477.02 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.80-7.87 (m, 7H, Ar-H). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3001.63, (Ar C-H str), 1482.46 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.82-7.79 (m, 7H, Ar-H). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3004.63, (Ar C-H str), 1485.71 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.80-7.96 (m, 7H, Ar-H), 5.67 (s, 1H, OH). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3009.46, (Ar C-H str), 1488.63 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.76-7.92(m, 7H, Ar-H), 3.68 (s, 2H, NH₂). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3004.16, (Ar C-H str), 1478.46 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.88-7.98(m, 6H, Ar-H), 3.65 (s, 2H, NH₂). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3011.65, (Ar C-H str), 1491.63 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.75-7.95(m, 6H, Ar-H). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3009.32, (Ar C-H str), 1489.41 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.84-7.91(m, 7H, Ar-H). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3008.24, (Ar C-H str), 1483.83 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.78-7.94(m, 7H, Ar-H), 3.94 (s, 4H, NH₂). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3003.26, (Ar C-H str), 1482.18 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.76-7.95 (m, 7H, Ar-H). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3005.72, (Ar C-H str), 1485.81 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.77-7.93 (m, 7H, Ar-H). ¹³C 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^o C; IR (KBr ν_{max} cm^{-1}): 3006.97, (Ar C-H str), 1492.12 (Ar C-C str in ring). ¹H NMR (DMSO-*d*₆, 400MHz) δ . 6.73-7.86 (m, 7H, Ar-H), 5.72 (s, 1H, OH). ¹³C 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 POCl₃ to forms benzoxazole fused triazole derivatives. The formation of target molecules confirmed by IR, ¹H NMR, ¹³C 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 ¹H 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 benzoxazole 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
10-100µg/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

Table-2 Antibacterial activity of compounds 4-19

Compound s	Zone of inhibition in mm					
	Gram positive bacteria			Gram negative bacteria		
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>S.epidermis</i>	<i>V.cholerae</i>	<i>S.typhi</i>	<i>E.coli</i>
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

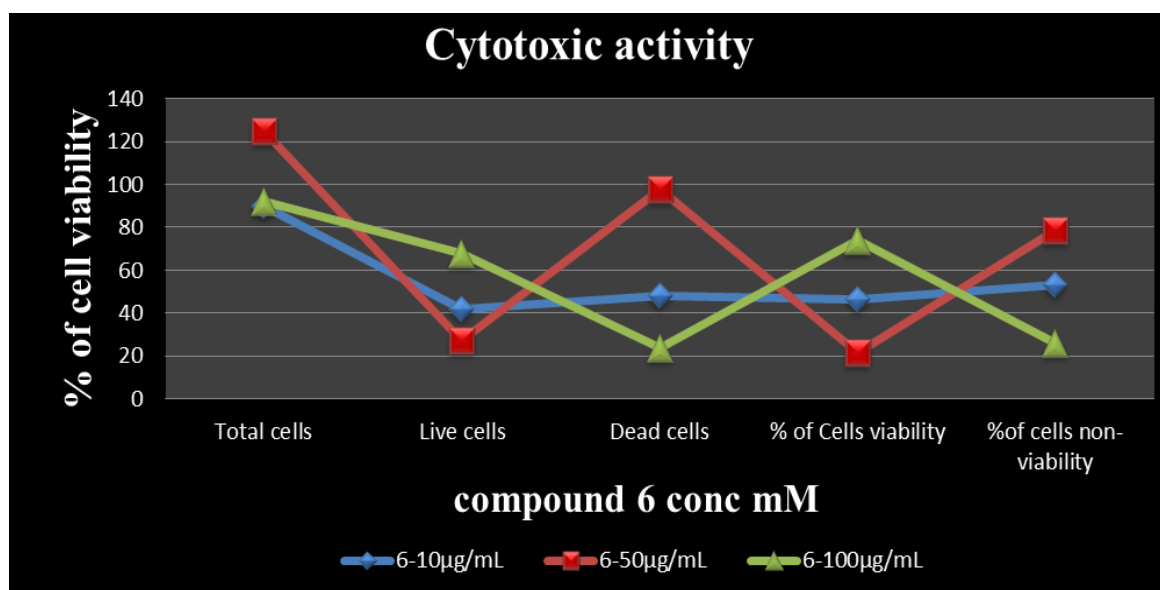
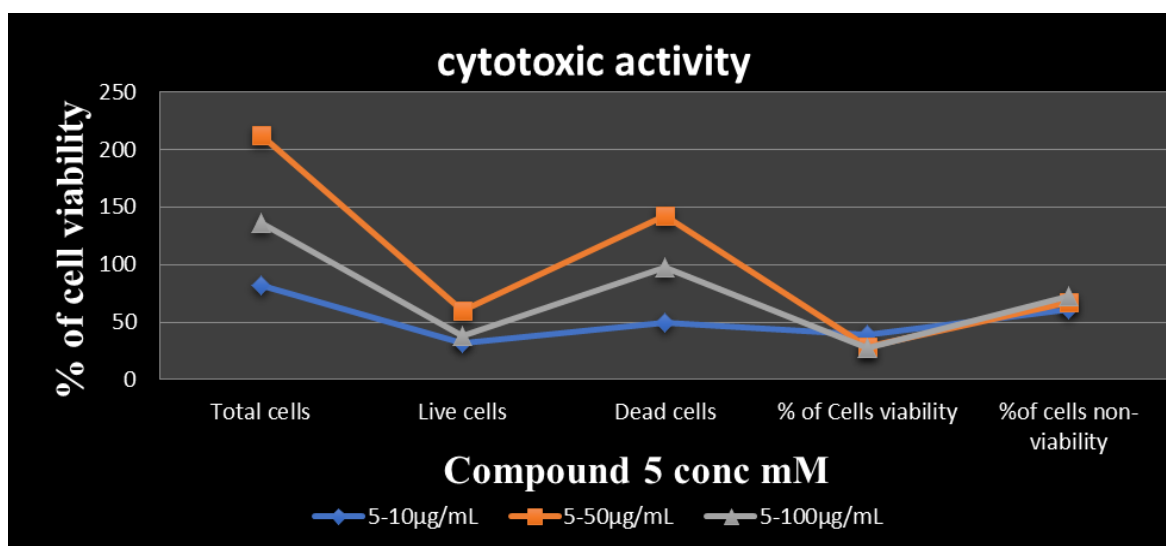
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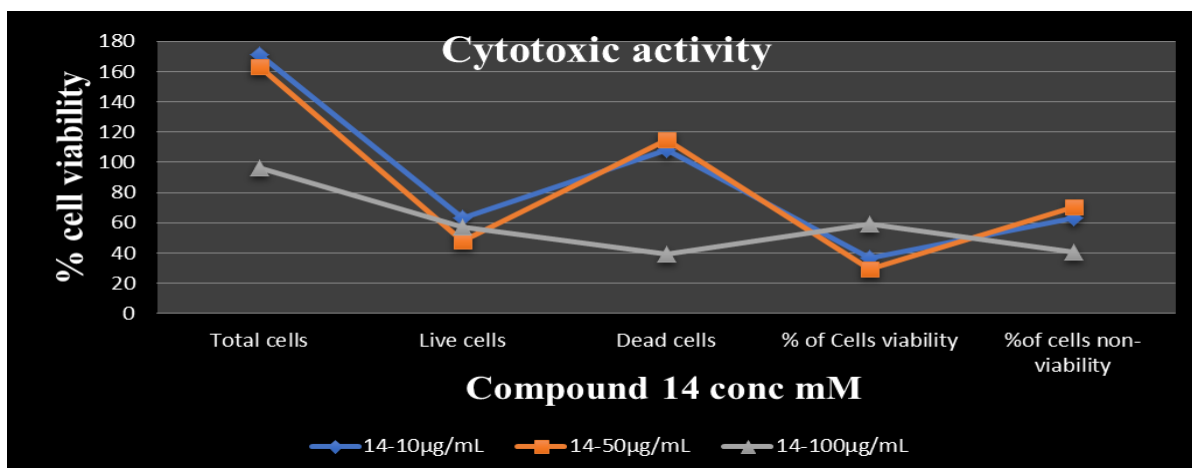
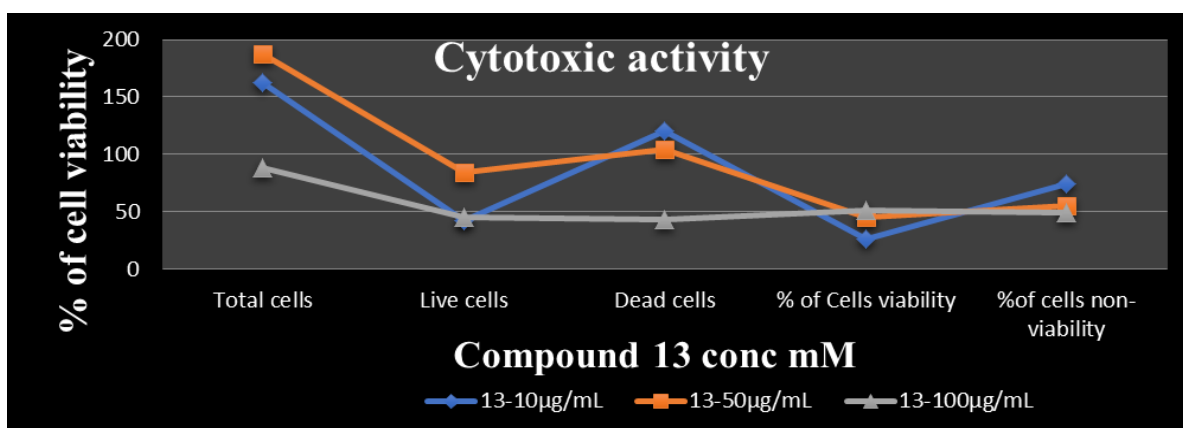
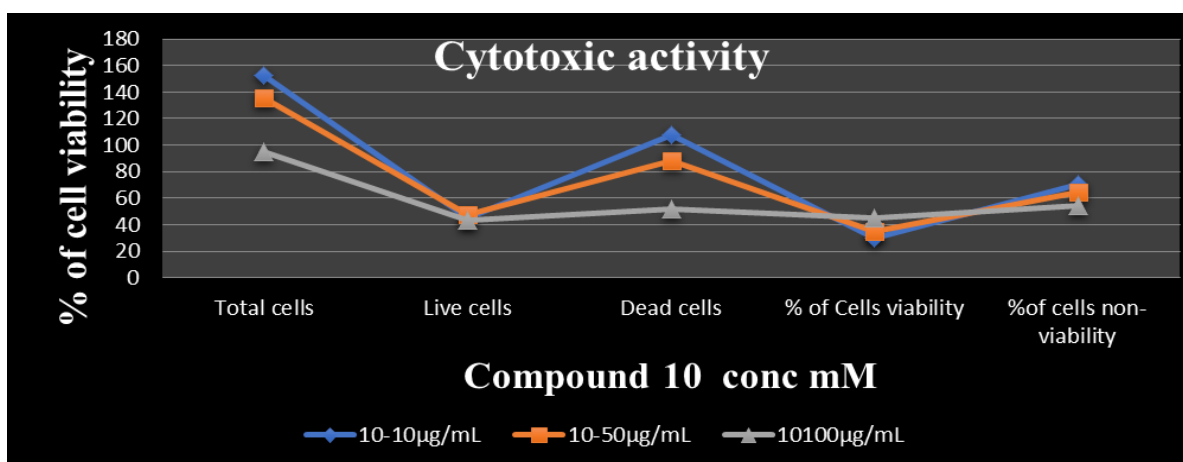
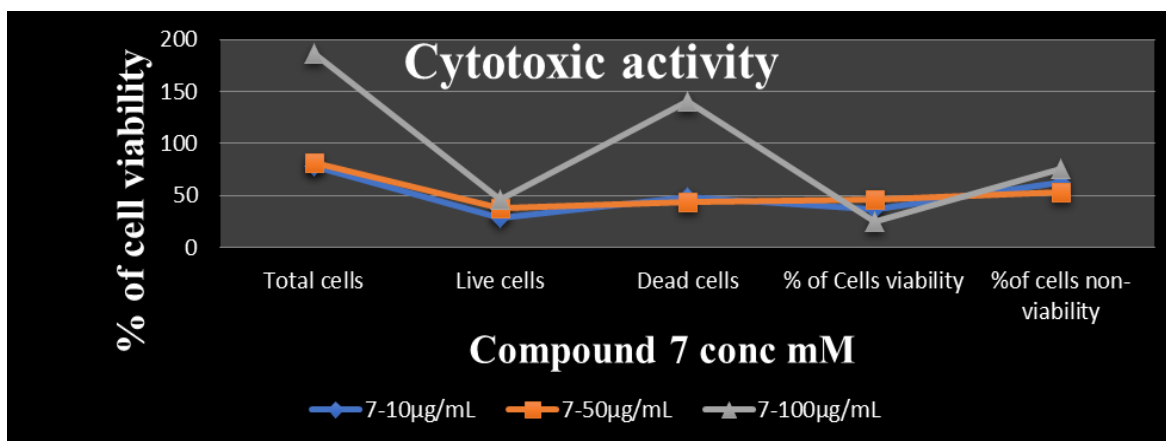
	Bacterial strains	Conc	Compounds and its zone of inhibition in mm										
			4	5	8	9	11	13	14	16	17	19	
Gram positive bacteria	<i>S. aureus</i>	25	19	20	18	16	18	17	17	16	21	18	29
		50	20	21	20	16	17	19	18	17	21	18	
		75	22	23	22	18	18	21	19	17	19	20	
		100	24	25	23	19	19	23	20	19	24	21	
	<i>B.cereus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

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





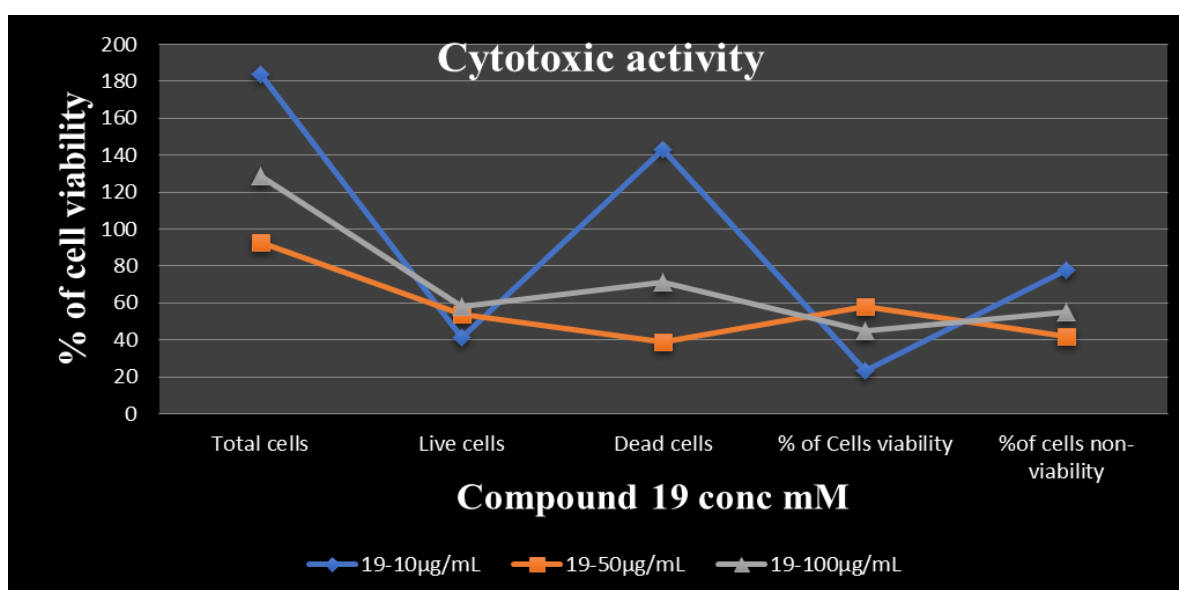
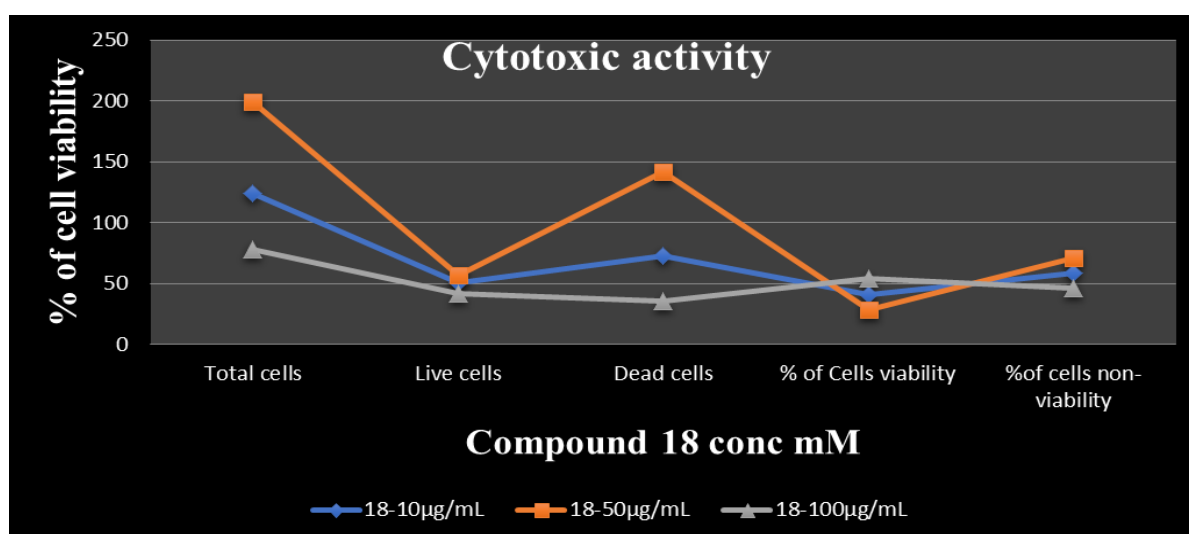
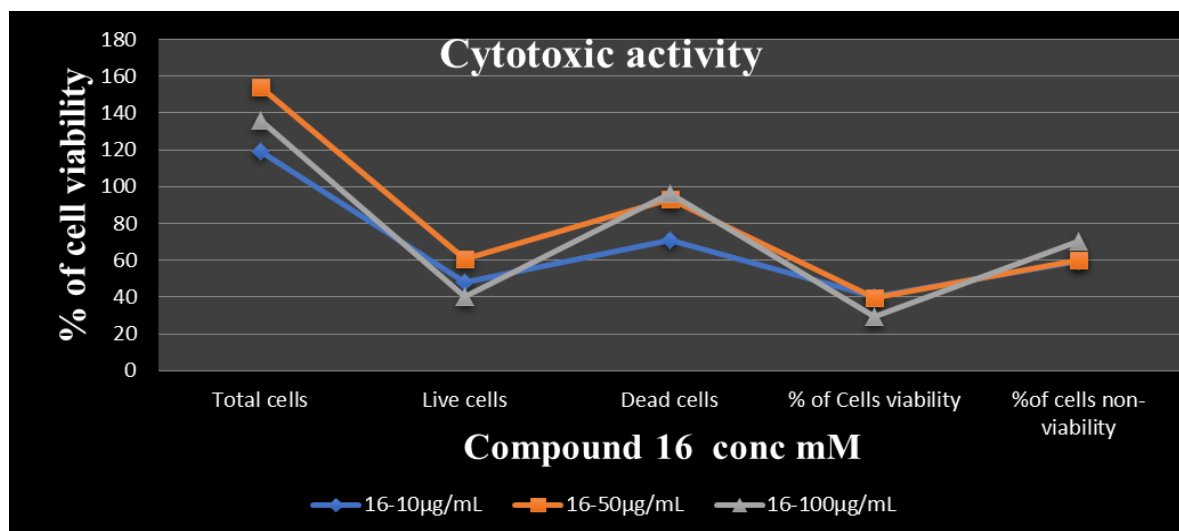


Figure-1. Cytotoxic effect of selected compounds at varying concentrations (10µg/mL, 50µg/mL and 100µg/mL) against Peripheral Blood Mononuclear Cells. Dose-response effect of each synthesized compounds showed in graphical representation.

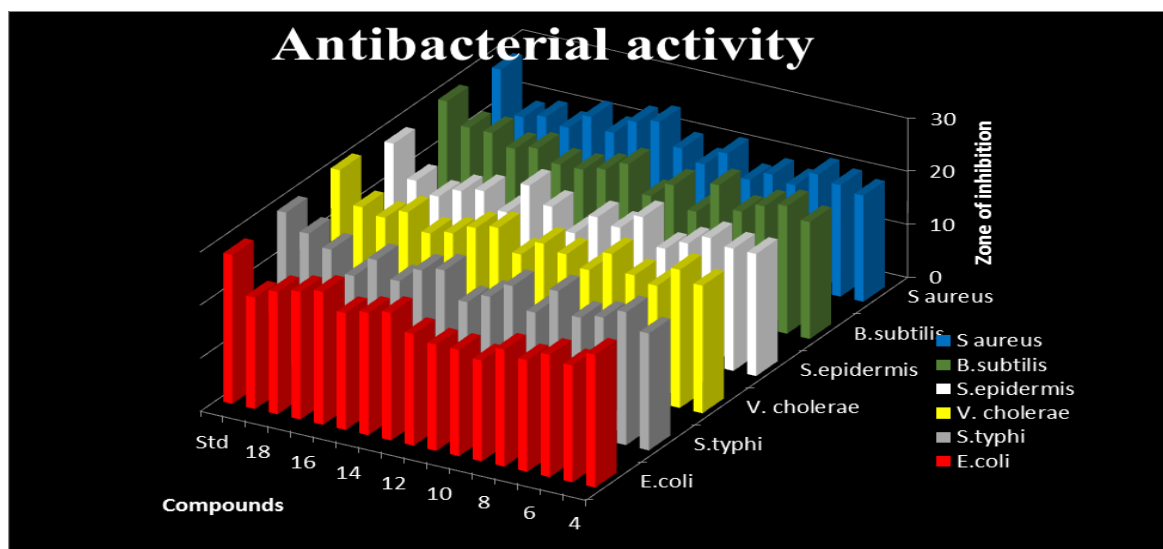


Figure-2 Antibacterial activity of compounds 4-19

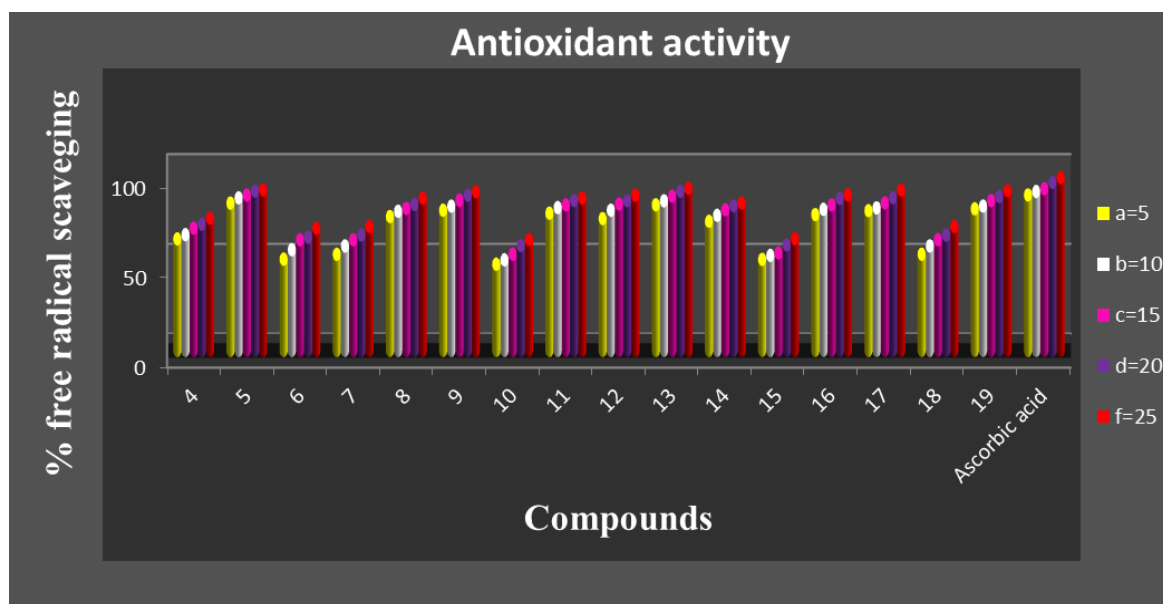
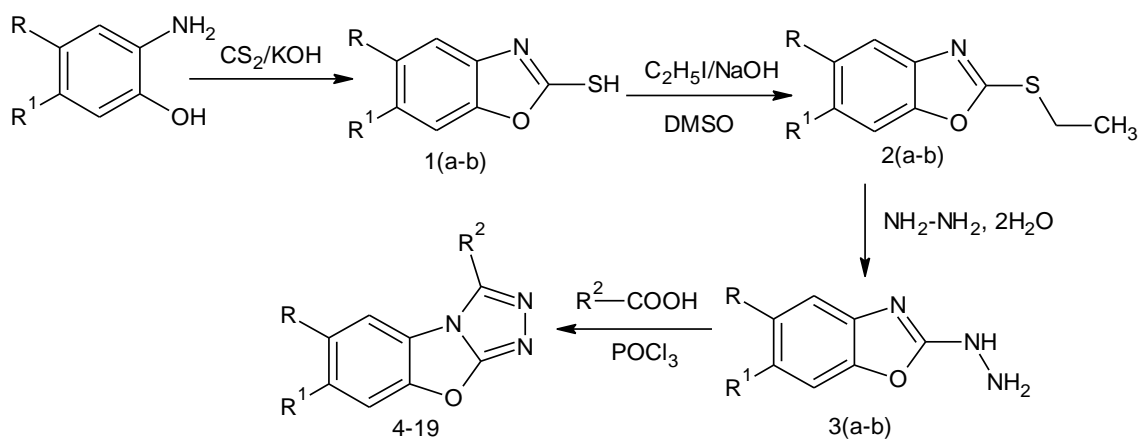
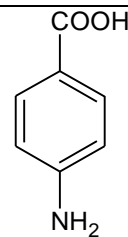
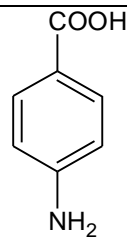
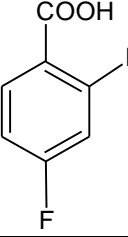
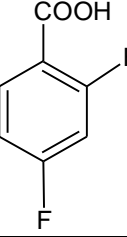
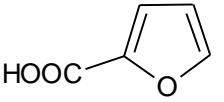
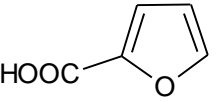
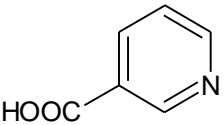
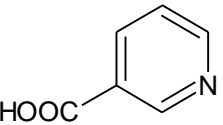
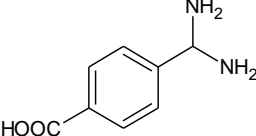
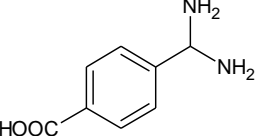
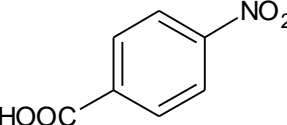
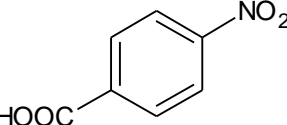
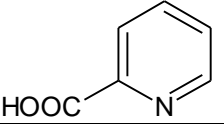
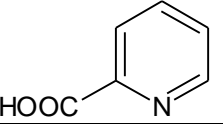
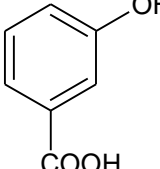
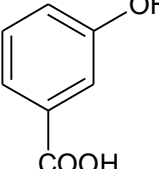


Figure-3 antioxidant activity of synthesized compound 4-19



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

Comp p	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 200 μ L 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, 30 μ L of Phytohemagglutinin (Invitrogen) and 200 μ L of PBMCs were incubated at the atmosphere of 95% air and 5% CO₂ at 37°C for 4 hr [27].

About 10 μ g/mL, 50 μ g/mL and 100 μ g/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 10 μ L of PBMCs, 10 μ L of trypan blue was added and incubated for 10 min at room temperature. About 10 μ L 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 Trinocular 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°C 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 μ g/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 100 μ g/mL) of compounds and standard were prepared in methanol. In clean and labeled test tubes, 3 mL of DPPH solution (0.002% in methanol) was mixed with **00, 05, 10, 15, 20 and 25** μ g/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].

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, ¹H 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.

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