INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

SYNTHESIS, *IN VITRO* CYTOTOXIC, ANTIBACTERIAL AND ANTIOXIDANT EVALUATION OF 2-(1,3-BENZOXAZOL-2-YL)-2,3-DIHYDROPHTHALAZINE-1,4-DIONE DERIVATIVES

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ABSTRACT

The *in vitro* cytotoxic activity of the selected chloro and nitro substituted benzoxazole derivatives 5,6,7,8-tetrabromo-2-(1,3-benzoxazol-2-yl)-2,3,5,6-tetrahydrophthalazine-1,4-dione **7(a-b),** 2-(1,3-benzoxazol-2-yl)-2,3-dihydrobenzophthalazine-1,4-dione **8a,** 3-(1,3-benzoxazol-2-yl)-3,4-dihydro-1H-1,3,4-benzotriazepine-2,5-dione **9(a-b)** and 7-chloro-3-(1,3-benzoxazol-2-yl)-3,4-dihydro-1H-1,3,4-benzotriazepine-2,5-dione **10(a-b)** shows more than 70% of cell viability, followed by the antibacterial, MIC and antioxidant activity. The target derivatives were synthesized by using intermediate chloro and nitro substituted 2-hydrazinyl-1,3-benzoxazole with different anhydrides. Obtained products have been characterized by IR, ^{1}H NMR Mass spectral studies, and evaluated their biological activity.

Keywords: Benzoxazole, pyridazine, PBMCs, anhydrides.

1 INTRODUCTION

The benoxazole framework represents a privileged structural motif of important value in biologically active natural products and pharmaceutical compounds. The benzoxazole core structure is found in a variety of cytotoxic natural products, such as the antimycobacterial pseudopteroxazole,[1] UK- 1,[2] AJI9561,[3] and salvianen.[4] Recent medicinal chemistry applications[5] of benzoxazoles include the cathepsin S inhibitor **1**,[6] 5-HT3 receptor agonist **2**,[7] HIV reverse transcriptase inhibitor L-697, 661,[8] estrogen receptor-_ agonist ERB-041,[9] selective peroxisome proliferator-activated receptor γ antagonist JTP- 426467,[10] anticancer agent NSC-693638,[11] and orexin-1 receptor antagonist SB-334867.[12] Other applications of benzoxazoles include their use as herbicides, such as Fenoxaprop and as fluorescent whitening agent dyes such as bisbenzoxazolyl ethylenes and arenes.[13]

Nitrogen-containing heterocyclic compounds are one of the most fruitful and extensively developing fields of heterocyclic chemistry. These compounds exhibit various kinds of biological activities. During the past decades increasing interest in the synthesis and biological activities of pyridazine derivatives has been observed.[14–16] Pyridazine compounds have been reported to possess varied biological activities such as antimicrobial,[17] antihypertensive,[18] anticancer,[19] anti-inflammatory[20] and antifungal activities.[21] These facts have prompted us to synthesize some novel pyridazine derivatives. Recently, pyridazinone nucleus has been extensively studied in the search for new and selective medicinal agents as drugs acting on the cardiovascular system.[22] Furthermore, a number of thienopyridazines have been claimed to possess interesting biological and pharmacological activities such as,

anticancer,[23] useful as NAD(P)H oxidase inhibitor,[24] and identified as a new allosteric modulator of the adenosine A1 receptor (A1AR).[25] Also Pyrazolo [3,4-b]pyridines comprise a very interesting class of compounds because of their significant and versatile biological, and pharmacological activities, such as antimicrobial,[26] cardiovascular,[27] antiviral,[28] and antileishmanial[29] activities. The pyrazolo [3,4-b] pyridine moieties represent important building blocks in both natural and synthetic bioactive compounds.[30] They show anxiolytic activity along with Xanthine oxidase inhibitors, cholesterol formation-inhibitor, and Anti-Alzheimer.[31] They also act as potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3).[32]

2 RESULT AND DISCUSSION

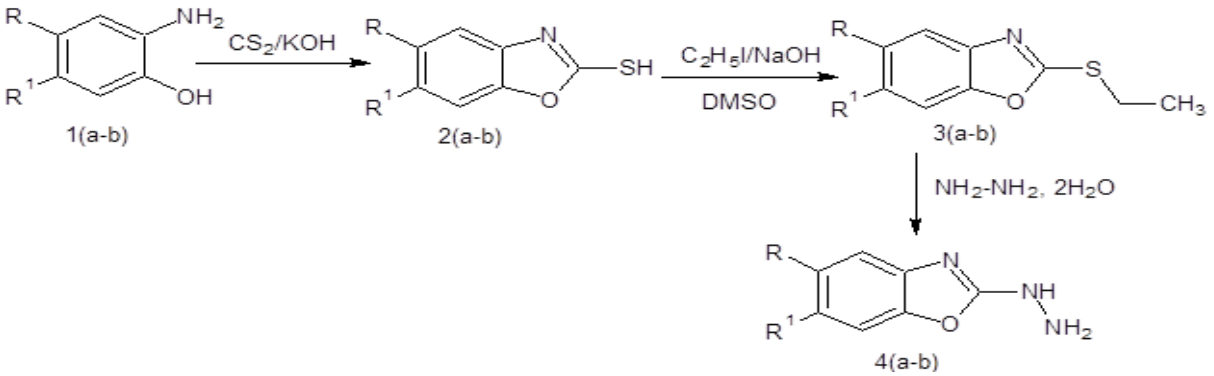
Chemistry

The Chloro and nitro substituted 2-amino-5-phenols **1(a-b)** were treated with carbon disulphide and potassium hydroxide in presence of methanol gives a compound chloro and nitro substituted 1,3-benzoxazole-2-thiol **2(a-b)**. To the compounds **2(a-b),** iodoethane and sodium hydroxide was added in DMSO, to get chloro and nitro substituted 2-(ethylsulfanyl)-1,3-benzoxazole **3(a-b)**. The compounds **3(a-b)** weretreated with hydrazine hydrate to obtain intermediate chloro and nitro substituted 2-hydrazinyl-1,3-benzoxazole derivatives **4(a-b).** The compounds **4(a-b)** were characterized by ^{1}H NMR, which exhibited two singlets at δ 4.6 and δ 9.3 for –NH_2 and –NH protons for chloro substituted and two singlets at δ 4.8 and δ 9.4 for –NH_2 and –NH protons for nitro substituted, respectively (D_2O exchangeable.

The derivatives 2-(5-chloro-1,3-benzoxazol-2-yl)-2,3-dihydrophthalazine-1,4-dione **5a** were synthesized using intermediate **4a** reacting with phthalic anhydride. The newly synthesized molecules exhibited strong absorbance band at 1655 cm^{-1} for -NH and 1692 cm^{-1} for C=O groups in IR spctrum and the ^{1}H NMR showed peak at δ 11.33 (s, -NH) confirms the disappearance of NH_2 proton and formation of new ring by insertion reaction. The mass peak also concurrence with molecular weights of targeted molecules.

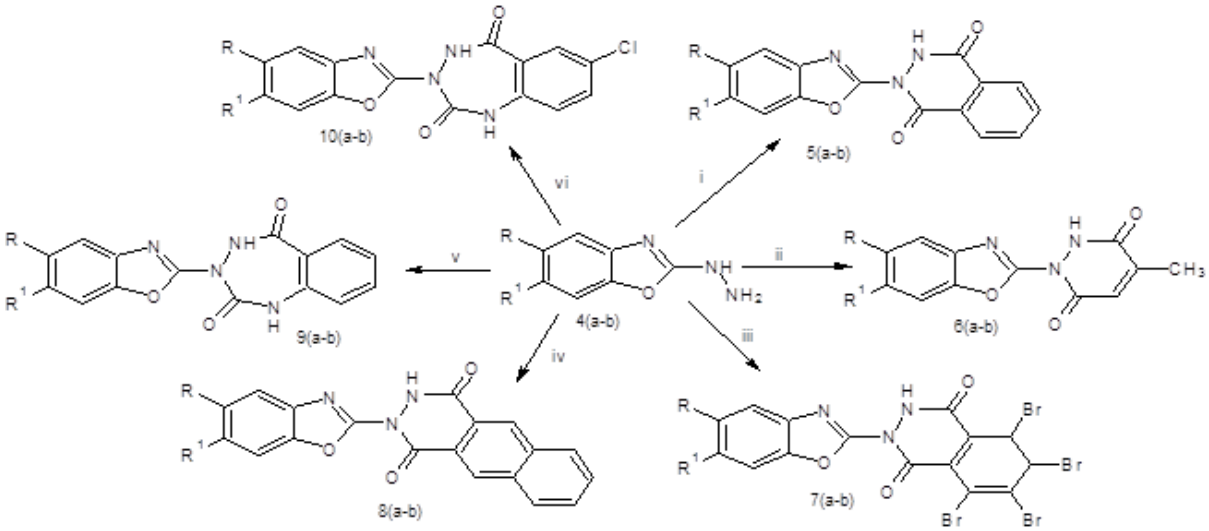
The constructed molecules **5(a-b)-10(a-b)** have been screened for antibacterial, minimum inhibition concentration, cytotoxic and antioxidant activity studies. In antibacterial study, few compounds have showed potent zone of inhibition **(Table-1 and Figure-1)**. Among the synthesized compounds, marked zone of inhibition of bacteria was observed in compounds **7a, 7b, 8a, 9a, 9b, 10a** and **10b** compared with standard drug Chloramphenicol. The selected compounds, which shows good results were evaluated for their minimum inhibitory concentration (**Table-2**) to determine their distinct zone of inhibition of bacteria at different concentration. The compounds **7a, 7b, 8a, 9a, 9b, 10a** and **10b** were found marked 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 tetrabromo substituent derivatives showed marked zone of inhibition with observation of effective microbial activity results. The selected compound were evaluated for their cytotoxic effect on PBMCs cell lines (**Table-3** and **Figure-2**) to determine their anticancer activity. The derivatives **7a, 7b, 8a, 9a, 9b, 10a** and **10b** 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 selected derivatives tetra bromo, naphthalene and triazepine substituted benzoxazole derivatives exhibited effective activity irrespective of concentration. Which was further supported by antioxidant activity, which were done with effective free radical scavenge (**Table-4** and **Figure-3**). The derivatives **7a, 7b, 8a, 9a, 9b, 10a** and **10b** shows potent free radical scavenging as compare with **6a, 6b, 7a, 7b** and **8b**.

Scheme

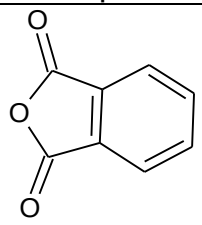
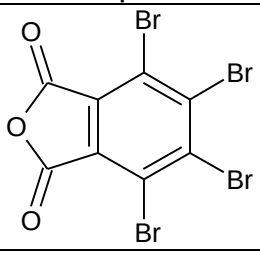
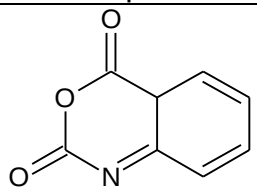
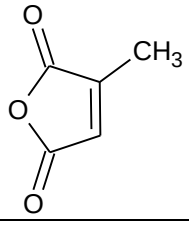
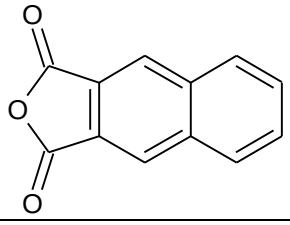
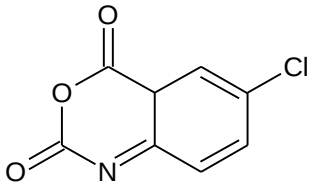


	R	R^1
A	Cl	H
B	H	NO_2

Scheme 1: synthetic route for intermediate



Scheme 2: synthetic route for the compound 5(a-b) - 10(a-b)

Code	Compound	Code	compound	Code	Compound
i		iii		v	
ii		iv		vi	

3 Experimental sections

(i) Synthesis of 2-(5-chloro-1,3-benzoxazol-2-yl)-2,3-dihydrophthalazine-1,4-dione 5a

The compounds [33] **4 a** (0.01mol) was dissolved in acetic acid (10 ml) followed by treated with pthallic anhydride (0.01mol) and refluxed for 6 h. then the reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystalized from ethanol to get compound **5a**.

The compounds **5b** and **6 (a-b)-10 (a-b)** can be prepared by following similar method.

Yield: 0.3518 (85 %), m.p. 236-238 ^{0} C; IR (KBr v_{max} cm^{-1}): 1655.16, (NH-C=O str), 1692.67 (N-C=O str), 3458 (-NH str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 7.01-8.03 (m, 7H, Ar-H), 11.33 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 170.37, 169.40, 153.10, 152.12, 150.75, 149.51 148.48, 145.66, 143.90, 141.94, 136.08, 133.64, 130,50, 125.60, 123.64. elemental analysis: calculated (%) for $C_{15}H_8ClN_3O_3$; C,57.43; H,2.57; N,13.40; observed C,57.39; H,2.53; N,13.35. M^{+1}, 313.69; M^{+2},315.69.

(ii) Synthesis of 2-(6-nitro-1,3-benzoxazol-2-yl)-2,3-dihydrophthalazine-1,4-dione 5b

Yield: 0.3271 (82 %), m.p. 258-260 ^{0} C; IR (KBr v_{max} cm^{-1}): 1658.34, (NH-C=O str), 1694.40 (N-C=O str), 3456.13 (-NH str). ^{1}H

NMR (DMSO-d_6, 400MHz) δ. 6.90-8.25 (m, 7H, Ar-H), 11.38 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.83, 170.38, 154.72, 153.46, 151.12, 150.46 147.70, 144.35, 142.48, 140.95, 137.84, 132.14, 129.31, 123.61, 122.53. elemental analysis: calculated (%) for $C_{15}H_8N_4O_5$; C,55.56; H,2.49; N,17.28; observed C,55.51; H,2.45; N,17.25. M^{+1}, 324.24.

(iii) Synthesis of 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione 6a

Yield: 0.3271 (89 %), m.p. 136-138 ^{0} C; IR (KBr v_{max} cm^{-1}): 1660.23, (NH-C=O str), 1695.13 (N-C=O str), 3454.86 (-NH str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.86-8.97 (m, 4H, Ar-H), 11.42 (s, 1H, -NH), 2.53 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.64, 172.50, 155.78, 152.05, 150.73, 149.46 149.22, 141.67, 136.08, 124.73, 121.16, 22.49. elemental analysis: calculated (%) for $C_{12}H_8ClN_3O_3$; C,51.91; H,2.90; N,15.13; observed C,51.88; H,2.86; N,15.09. M^{+1}, 277.66, M^{+2}, 279.66.

(iv) Synthesis of 1-(6-nitro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione 6b

Yield: 0.2864 (78 %), m.p. 242-244 ^{0} C; IR (KBr v_{max} cm^{-1}): 1661.15, (NH-C=O str), 1694.75 (N-C=O str), 3455.42 (-NH str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.75-8.86 (m, 4H, Ar-H), 11.38(s, 1H, -NH), 2.56 (s, 3H, -CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.19, 170.41, 154.10, 151.16, 149.39, 148.71 146.65, 142.87, 138.35, 126.21, 120.98, 24.92. elemental analysis: calculated (%) for $C_{12}H_8N_4O_5$; C,50.01; H,2.80; N,19.44; observed C,49.97; H,2.76; N,19.41. M^{+1}, 288.21.

(v) Synthesis of 5,6,7,8-tetrabromo-2-(5-chloro-1,3-benzoxazol-2-yl)-2,3,5,6-tetrahydro phthalazine-1,4-dione 7a

Yield: 0.3854 (83 %), m.p. 325-327 ^{0} C; IR (KBr v_{max} cm^{-1}): 1658.61, (NH-C=O str), 1691.18 (N-C=O str), 3453.26 (-NH str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.85-8.92 (m, 3H, Ar-H), 11.52 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.54, 171.28, 156.85, 156.56, 155.71, 155.49 146.65, 144.31, 143.05, 142.60, 142,12, 136.47, 128.75, 124.67, 122.34. elemental analysis: calculated (%) for $C_{15}H_6Br_4ClN_3O_3$; C,28.54; H,0.96; N,6.66; observed C,28.49; H,0.92; N,6.62. M^{+1}, 631.29, M^{+2}, 633.29, M^{+4}, 635.29, M^{+6}, 637.29, M^{+8}, 639.29, M^{+10}, 641.29.

(vi) Synthesis of 5,6,7,8-tetrabromo-2-(6-nitro-1,3-benzoxazol-2-yl)-2,3,5,6-tetrahydro phthalazine-1,4-dione 7b

Yield: 0.3530 (79 %), m.p. 259-261 ^{0} C; IR (KBr v_{max} cm^{-1}): 1660.28, (NH-C=O str), 1689.47 (N-C=O str), 3451.86 (-NH str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.79-8.84 (m, 3H, Ar-H), 11.52 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.42, 173.67, 155.92, 155.36, 155.04, 153.28 147.43, 146.26, 142.84, 142.16, 141.53, 138.16, 132.36, 128.73, 121.62. elemental analysis: calculated (%) for $C_{15}H_6Br_4N_4O_5$; C,28.07; H,0.94; N,8.73; observed C,28.03; H,0.92; N,8.69. M^{+1}, 641.84, M^{+2}, 643.84, M^{+4}, 645.84, M^{+6}, 647.84, M^{+8}, 649.84.

(vii) Synthesis of 2-(5-chloro-1,3-benzoxazol-2-yl)-2,3-dihydrobenzo[g]phthalazine-1,4-dione 8a

Yield: 0.3107 (79 %), m.p. 213-215 ^{0} C; IR (KBr v_{max} cm^{-1}): 1658.97, (NH-C=O str), 1690.68 (N-C=O str), 3450.35 (-NH str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.76-9.23 (m, 9H, Ar-H), 11.49 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.42, 173.67, 155.92, 155.36, 155.04, 153.28 147.43, 146.26, 142.84, 142.16, 141.53, 138.16, 136.46 132.36, 134.48 128.73, 121.62. elemental analysis: calculated (%) for $C_{19}H_{10}ClN_3O_3$; C,62.74; H,2.77; N, 11.55; observed C,62.71; H,2.73; N,11.52. M^{+1}, 363.75, M^{+2}, 365.75.

(viii) Synthesis of 2-(6-nitro-1,3-benzoxazol-2-yl)-2,3-dihydrobenzo[g]phthalazine-1,4-dione 8b

Yield: 0.2861 (76 %), m.p. 268-270 ^{0} C; IR (KBr v_{max} cm^{-1}): 1660.40, (NH-C=O str), 1691.73 (N-C=O str), 3451.19 (-NH str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.80-9.36 (m, 9H, Ar-H), 11.52 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 169.13, 168.78, 153.67, 151.16, 149.31, 148.87 148.14, 146.60, 145.78, 145.05, 143.55, 141.23, 138.63, 135.10, 132.36, 128.73, 121.62. elemental analysis: calculated (%) for $C_{19}H_{10}N_4O_5$; C,60.97; H,2.69; N, 14.97; observed C,60.94; H,2.65; N,14.64. M^{+1}, 374.30.

(ix) Synthesis of 3-(5-chloro-1,3-benzoxazol-2-yl)-3,4-dihydro-1H-1,3,4-benzotriazepine-2,5-dione 9a

Yield: 0.3156 (73 %), m.p. 256-258 ^{0} C; IR (KBr v_{max} cm^{-1}): 1678.60, (NH-C=O str), 1695.36 (N-C=O str), 3455.26 (-NH str), 3463.14 (-NH str).. ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.86-9.70 (m, 7H, Ar-H), 11.25 (s, 1H, -NH), 11.52 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 171.31, 170.14,

154.71, 152.64, 151.63, 149.83 144.98, 143.28, 141.53, 138.75, 138.13, 135.10, 132.36, 128.73, 121.62. elemental analysis: calculated (%) for $C_{15}H_9ClN_4O_3$; C,54.81; H,2.76; N, 17.04; observed C,54.75; H,2.70; N,17.01. M^{+1}, 328.70, M^{+2}, 330.70.

(x) Synthesis of 3-(6-nitro-1,3-benzoxazol-2-yl)-3,4-dihydro-1H-1,3,4-benzotriazepine-2,5-dione 9b

Yield: 0.2867 (78 %), m.p. 246-248 ^{0} C; IR (KBr v_{max} cm^{-1}): 1677.15, (NH-C=O str), 1693.74 (N-C=O str), 3456.46 (-NH str), 3465.38 (-NH str).. ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.74-9.43 (m, 7H, Ar-H), 11.36 (s, 1H, -NH), 11.63 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.46, 171.51, 152.63, 151.35, 151.02, 148.43 147.98, 147.45, 140.30, 139.18, 138.16, 134.65, 131.75, 129.67, 126.80. elemental analysis: calculated (%) for $C_{15}H_9N_5O_5$; C,53.10; H,2.67; N, 20.64; observed C,53.05; H,2.62; N,20.59. M^{+1}, 339.26.

(xi) Synthesis of 7-chloro-3-(5-chloro-1,3-benzoxazol-2-yl)-3,4-dihydro-1H-1,3,4-benzotri azepine-2,5-dione 10a

Yield: 0.3275 (75 %), m.p. 285-287 ^{0} C; IR (KBr v_{max} cm^{-1}): 1674.67, (NH-C=O str), 1691.37 (N-C=O str), 3454.57 (-NH str), 3461.73 (-NH str).. ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.74-9.43 (m, 6H, Ar-H), 11.46 (s, 1H, -NH), 11.60 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.12, 172.35, 152.74, 150.56, 149.41, 149.05, 148.43, 145.48, 141.85, 138.96, 138.32, 133.63, 130.18, 128.17, 125.63. elemental analysis: calculated (%) for $C_{15}H_8Cl_2N_4O_3$; C,49.61; H,2.22; N, 15.43; observed C,49.58; H,2.18; N,15.39. M^{+1}, 363.15, M^{+1}, 365.15.

(xii) Synthesis of 7-chloro-3-(6-nitro-1,3-benzoxazol-2-yl)-3,4-dihydro-1H-1,3,4-benzotri azepine-2,5-dione 10b

Yield: 0.2475 (72 %), m.p. 263-265 ^{0} C; IR (KBr v_{max} cm^{-1}): 1672.43, (NH-C=O str), 1689.26 (N-C=O str), 3452.569 (-NH str), 3462.78 (-NH str). ^{1}H NMR (DMSO-d_6, 400MHz) δ. 6.74-9.43 (m, 6H, Ar-H), 11.38 (s, 1H, -NH), 11.58 (s, 1H, -NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 173.73, 170.12, 150.53, 150.12, 147.46, 145.96, 144.52, 144.03, 140.94, 137.63, 136.17, 132.81, 129.60, 127.05, 124.71. elemental analysis: calculated (%) for $C_{15}H_8ClN_5O_5$; C,48.21; H,2.16; N,18.74; observed C,48.19; H,2.15; N,18.69. M^{+1}, 373.75.

4. Biological activity of the synthesized compounds 5(a-b) to 10(a-b)

4.1 Antibacterial Activity

The newly synthesized benzoxazole fused quinoline derivatives were tested for antibacterial activity againstbacterial strains, *Escherichia coli* (ATTC-8739), *Staphylococcus aureus* (ATTC-6538), (ATTC-9027), *Vibro cholerae* (ATTC-6633), *Bacillus cereus* (ATTC-11778), *Staphylococcus epidermidis* (ATTC-12228) and *Salmonella typhimurium* (ATTC-23564) by agar well diffusion method[34]. 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 **5(a-b) - 10(a-b)** (20mg/mL of 10% DMSO), and control (10% DMSO) were added to the respectively labelled wells. The plates were allowed to stand for 30 minutes and were incubated at 37^{0}C for 24 hr in upright position and the zone of inhibition was recorded and tabulated in **Table-1** and graphically represented in **figure-1**.

4.2 Minimum Inhibitory Concentration (MIC)

The MIC of all the synthesized compounds **5(a-b) - 6(a-b)** was determined by micro dilution method [35]. 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-2**.

4.3 Cytotoxic activity

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

Blood samples from healthy volunteers were collected by venipuncture and transferred into 2 ml heparin coated vacutainers. It was diluted to 1:1 ratio with PBS (Phosphate buffer solution, pH 7.0) layered onto 4 mL Ficol without getting mixed up. It was further separated by centrifuging at 1,000 rpm for 30 min at room temperature. During the centrifugation the PBMCs move from plasma and suspend as the density gradient. Plasma was removed down to 1 cm above buffy coat and discarded the white layer lying on top of the red cells. The buffy coat layer was washed twice with PBS. Roswell Park Memorial Institute (Gibco, Life Technologies) medium was prepared by mixing 10 mL of Fetal bovine

serum (Invitrogen) and 200µL antimycotic [Antibiotic antimycotic solution with Streptomycin (10mg/20mL), 10,000 U Penicillin, Amphotericin B and 0.9% normal saline]. This mixture (4mL) was dispensed into falcon tubes, 30µL 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 [36, 37].

About **10 µg/mL**, **50 µg/mL** and **100 µg/mL** of the selected compounds (1mg/mL)were added to the respectively labelled PBMCs tubes and incubated for 72 hr at the earlier mentioned conditions. After 72 hr, cell viability was determined by the trypan-blue dye exclusion method [38].

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-3**.The graphical representation was presented in **figure-2**.

4.4 Antioxidant Activity

DPPH Assay

The free radical scavenging ability of synthesized compounds **5(a-b) - 10(a-b)**and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on a DPPH free radical. Different concentrations (5, 10, 15, 20 and 25 μg/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 of different concentrations of compounds and standard separately, makeup the solution up to 4 ml by adding methanol. The tubes were incubated at room temperature in dark for 30 minutes, and the optical density was measured at 517 nm using UV-Visible Spectrophotometer. The absorbance of the DPPH control was also noted. The 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 [39].

5. CONCLUSION

A series newly synthesized molecules were characterized by IR, ^{1}H NMR and mass spectral analysis. For compounds, the cytotoxic activity against Peripheral Blood Mononuclear Cells with antibacterial, MIC and antioxidant activity were evaluated. selected chloro and nitro substituted benzoxazole derivatives exhibited promising cytotoxicity against PBMCs cell lines. Compound **7a**, **7b, 8a, 9a, 9b, 10a** and **10b** were exhibited effective anticancer activity against PBMCs, it was also supported by the *in vitro* antibacterial, MIC with antioxidant activity with effective result. By considering effective biological activity, we can conclude that benzoxazole is a potent medicinal value molecule.

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

6. ACKNOWLEDGMENTS

The authors are grateful to the Principal, Sahyadri Science College, Shivamogga, for laboratory facilities to carry out research work. The authors are also thankful to MIT, Manipal and IIT Bombay for providing ^{1}H NMR and ^{13}C NMR spectral facilities. We are also thankful to the Department of Microbiology and Cell Biology IISc Bangalore, India and Department of Cell Biology and Molecular Genetics, Sri DevarajUrs Academy of Higher Education and Research, Tamaka-563103, Kolar, Karnataka, India for their support in carrying out cytotoxic activity.

Table1: Antibacterial activity of synthesized compounds 5(a-b) - 10(a-b)

Compounds	Zone of inhibition in mm					
	S.aureus	***B. cereus***	***S.epidermis***	***V. cholerae***	***S. typhi***	***E. coli***
5a	17.67±0.33	13.67±0.33	15.67±0.33	17.33±0.33	17.33±0.33	16.67±0.67
5b	18.43±0.20	18.00±0.20	19.83±0.46	20.90±0.07	18.90±0.32	19.97±0.62
6a	18.13±0.12	20.07±0.39	20.70±0.55	19.07±0.19	17.10±0.40	19.77±0.09
6b	18.34±0.02	20.34±0.02	19.12±0.21	20.63±0.21	21.12±0.82	18.20±0.21
7a	20.80±0.08	22.97±0.09	23.17±0.39	21.67±0.37	23.13±0.38	17.80±0.34
7b	20.70±0.55	19.00±0.48	22.20±0.87	19.77±0.09	22.57±0.32	20.13±0.26
8a	20.70±0.55	18.93±0.32	22.80±0.11	22.67±0.50	22.90±0.57	19.83±0.17
8b	18.57±0.33	18.73±0.18	20.85±0.07	21.67±0.15	19.37±0.19	20.88±0.44
9a	20.30±0.15	18.03±0.09	21.43±0.27	19.40±0.25	20.60±0.66	22.40±0.29
9b	17.33±0.33	20.97±0.09	23.17±0.39	21.67±0.37	23.13±0.38	19.80±0.34
10a	22.38±0.23	22.47±0.16	19.37±0.19	21.27±0.03	21.28±0.08	22.41±0.05
10b	18.73±0.18	19.59±0.04	26.37±0.03	25.24±0.06	22.37±0.07	18.32±0.05
Std	26.57±0.33	27.73±0.18	28.85±0.07	23.67±0.15	24.37±0.19	25.88±0.44

Std: Chloramphenicol Solvent: DMSO

Table 2: Minimum inhibition concentration of compounds 5(a-b) - 6(a-b)

Comp	Gram positive bacteria												Gram negative bacteria											
	S. aureus				B.cereus				S.epidermis				V. cholerae				S.typhi				E.coli			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
7a	19	20	22	24	**21**	**24**	**23**	**22**	16	18	18	20	15	19	18	19	**19**	**21**	**23**	**25**	18	19	19	22
7b	**20**	**22**	**23**	**25**	19	20	20	22	17	19	19	21	18	19	20	21	18	19	20	21	20	21	22	23
8a	**21**	**22**	**25**	**23**	16	17	17	19	**20**	**22**	**23**	**25**	16	18	19	21	16	19	19	20	**20**	**22**	**23**	**25**
9a	16	17	17	19	19	21	19	20	16	19	19	20	18	19	19	20	**18**	**19**	**25**	**23**	**19**	**20**	**22**	**25**
9a	19	21	22	24	17	18	19	20	15	17	18	19	**21**	**22**	**24**	**25**	**20**	**22**	**26**	**22**	16	18	20	24
10a	14	16	16	18	17	18	19	20	18	19	20	21	**21**	**22**	**23**	**25**	**19**	**21**	**23**	**25**	16	17	17	19
10b	18	18	20	21	17	19	21	22	**19**	**21**	**23**	**25**	18	20	22	23	17	19	21	22	**21**	**23**	**23**	**25**
STD	29				30				29				28				29				28			

Std: Chloramphenicol
Solvent: DMSO

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

Sample	Total cells	Live cells	Dead cells	% of Cells viability	%of cells non-viability
7a-10µg/mL	95	43	52	45.35	54.75
7a-50µg/mL	**136**	**38**	**98**	**27.94**	**72.05**
7a-100µg/mL	**153**	**45**	**108**	**29.41**	**70.59**
7b-10µg/mL	168	69	99	41.08	58.92
7b-50µg/mL	**155**	**43**	**112**	**27.74**	**72.25**
7b-100µg/mL	**168**	**42**	**102**	**24.57**	**75.43**
8a-10µg/mL	143	52	91	36.37	63.63
8a-50µg/mL	**162**	**42**	**120**	**25.92**	**74**
8a-100µg/mL	115	63	48	58.27	41.73
9a-10µg/mL	171	63	108	36.8	63.2
9a-50µg/mL	**163**	**48**	**115**	**29.4**	**70.6**
9a-100µg/mL	96	57	39	59.38	40.62
9b-10µg/mL	154	61	93	39.6	60.4
9b-50µg/mL	119	48	71	40.33	59.66
9b-100µg/mL	**136**	**40**	**96**	**29.4**	**70.6**
10a-10µg/mL	199	67	132	33.7	66.3
10a-50µg/mL	124	51	73	41.1	59
10a-100µg/mL	**186**	**46**	**140**	**24.73**	**75.26**
10b-10µg/mL	**184**	**41**	**143**	**23.2**	**77.8**
10b-50µg/mL	129	58	71	44.97	55.03
10b-100µg/mL	93	54	39	58.06	41.93
Control	118	17	101	14.4	85.5

Table 4: antioxidant activity of the compound 5(a-b) - 10(a-b)

Compounds	Scavenging activity of different concentration (Mg/mL) in %				
	A=5	B=10	C=15	D=20	F=25
5a	28.36	32.59	33.61	38.93	44.67
5b	50.81	53.00	54.40	58.78	62.38
6a	42.38	46.46	48.31	50.75	52.10
6b	50.92	60.18	64.73	67.12	70.15
7a	**75.08**	**78.38**	**81.69**	**82.63**	**85.37**
7b	**78.02**	**84.29**	**87.64**	**89.38**	**92.15**
8a	53.67	58.30	61.71	64.38	69.09
8b	**80.56**	**80.69**	**82.60**	**83.63**	**84.78**
9a	**78.68**	**82.32**	**85.56**	**86.37**	**91.67**
9b	**73.96**	**78.68**	**82.39**	**87.94**	**88.54**
10a	**74.18**	**75.36**	**78.28**	**81.47**	**84.68**
10b	**81.19**	**83.38**	**84.95**	**85.61**	**88.60**
Ascorbic acid	**86.71**	**88.57**	**90.04**	**93.62**	**96.33**

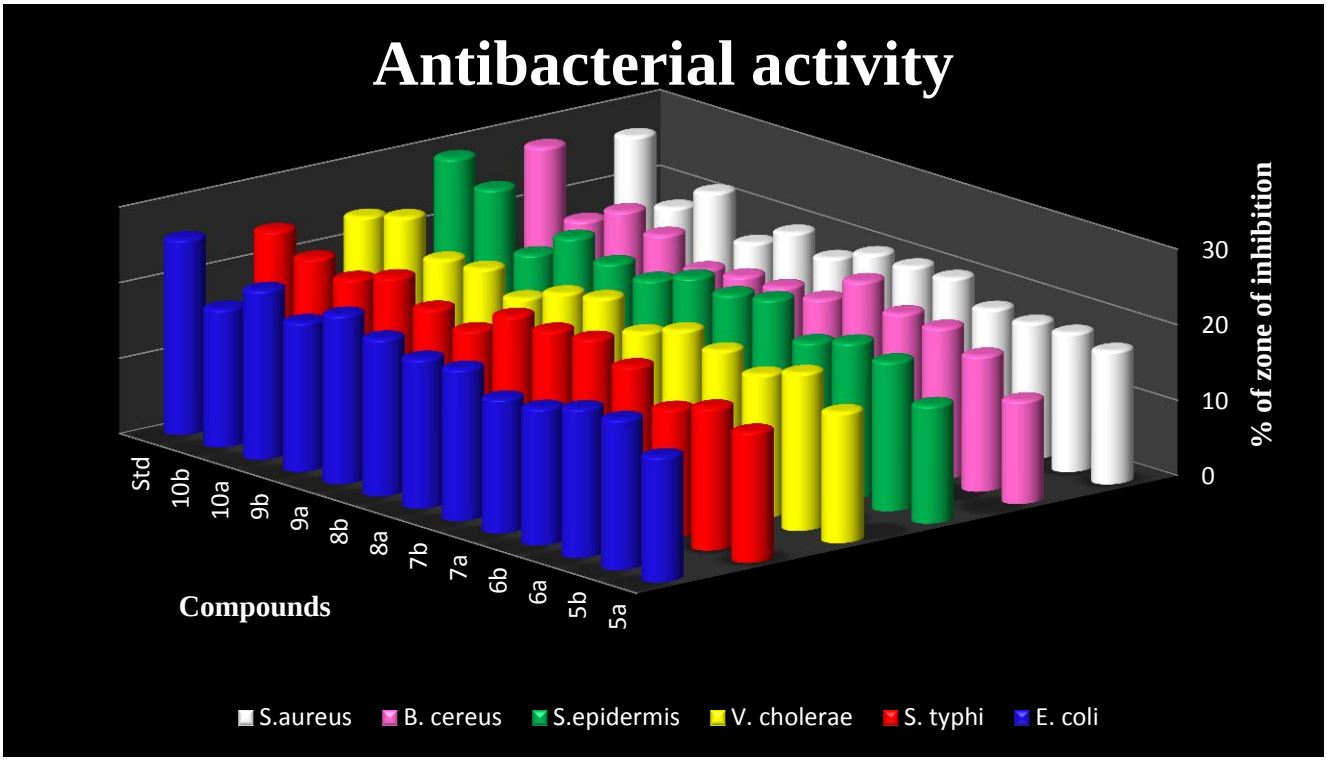


Fig. 1: Antibacterial activity of synthesized compounds 5(a-b) - 10(a-b)

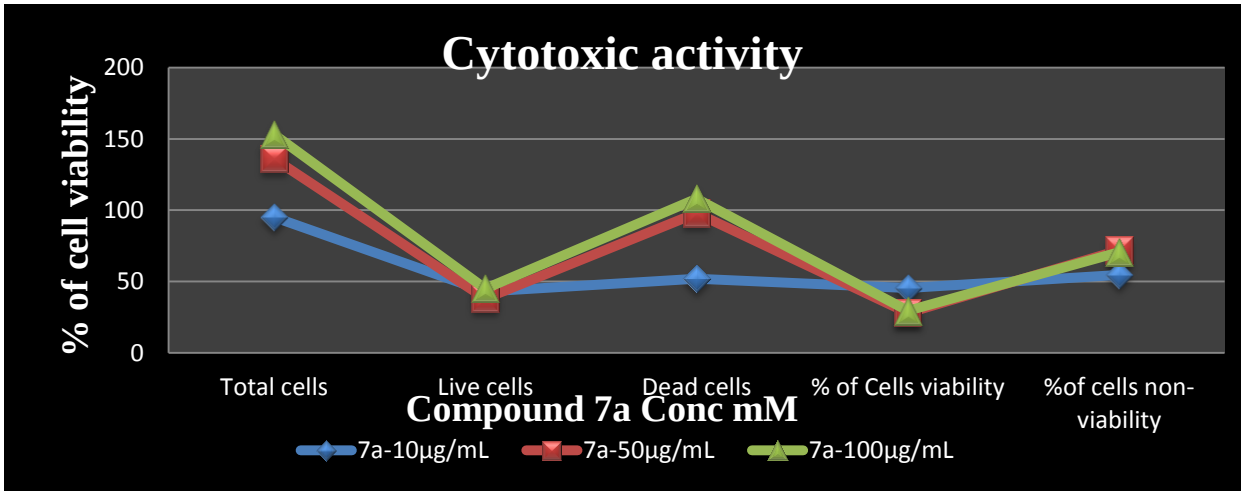
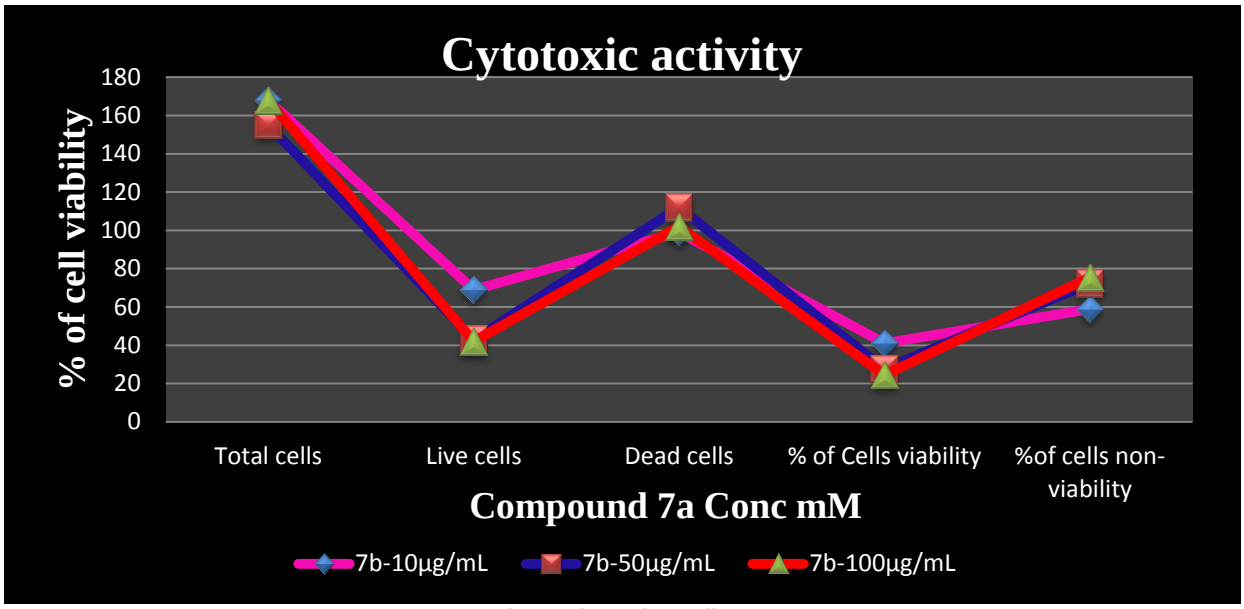


Fig. 2: (continued)



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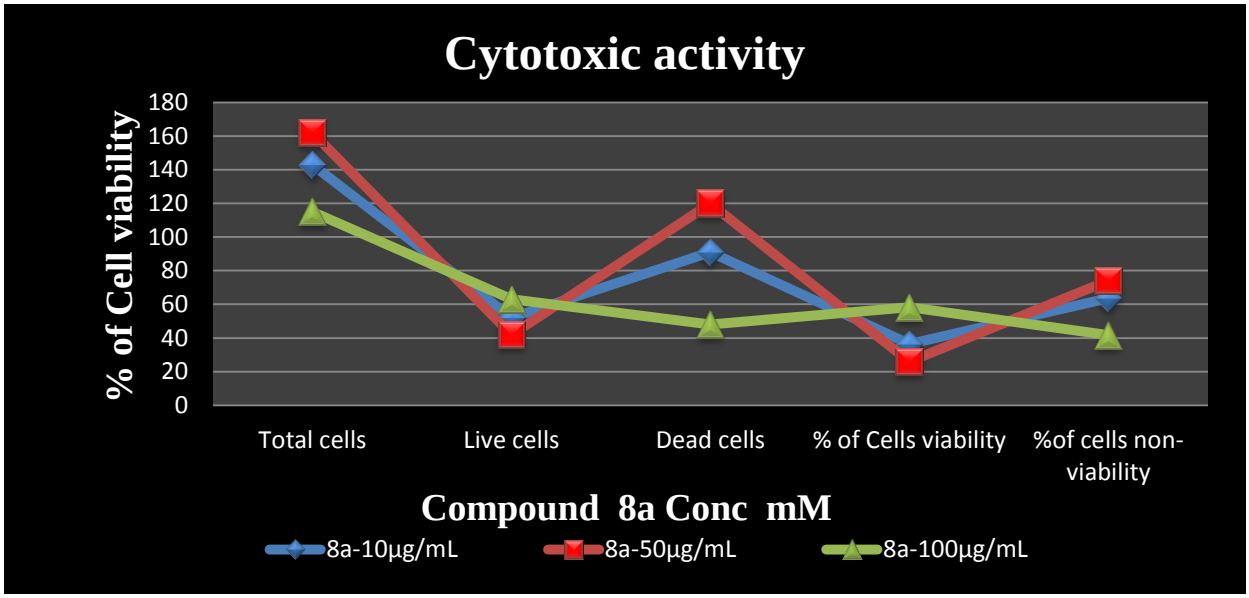

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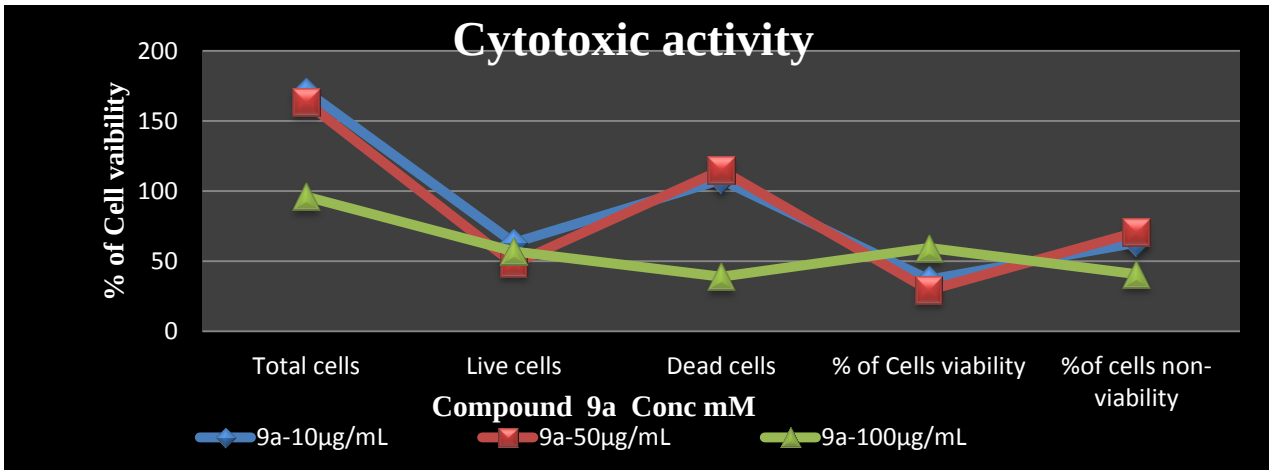

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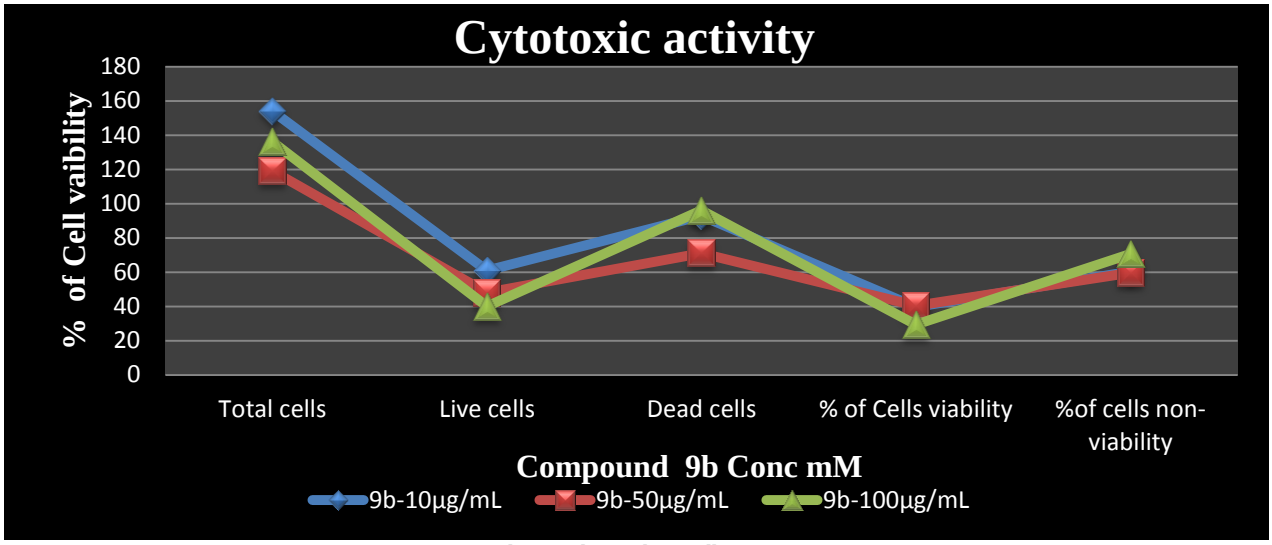

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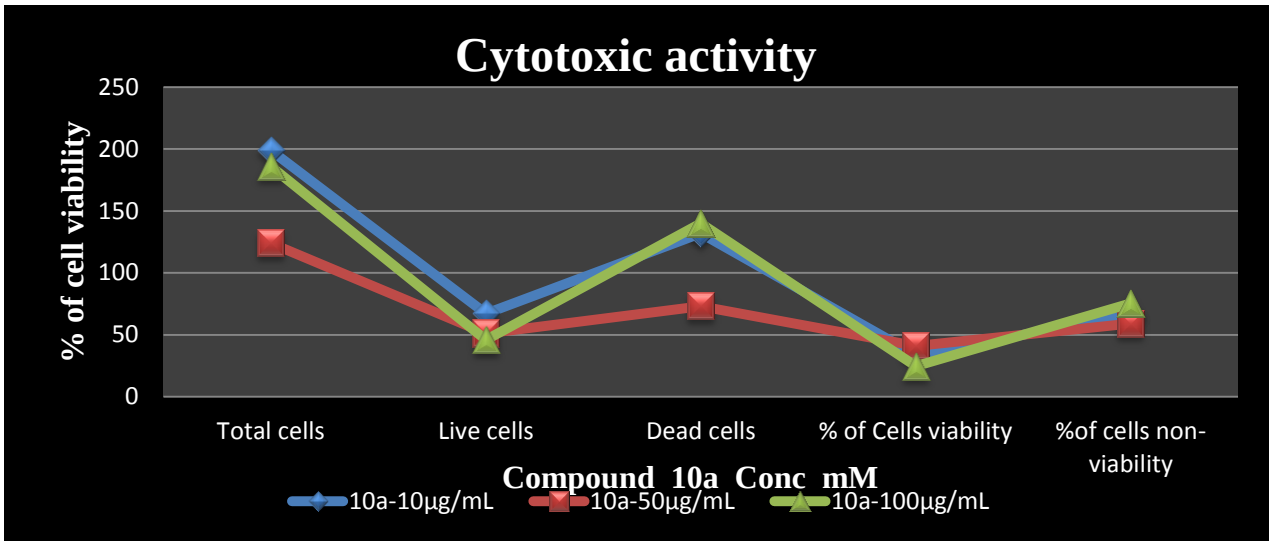

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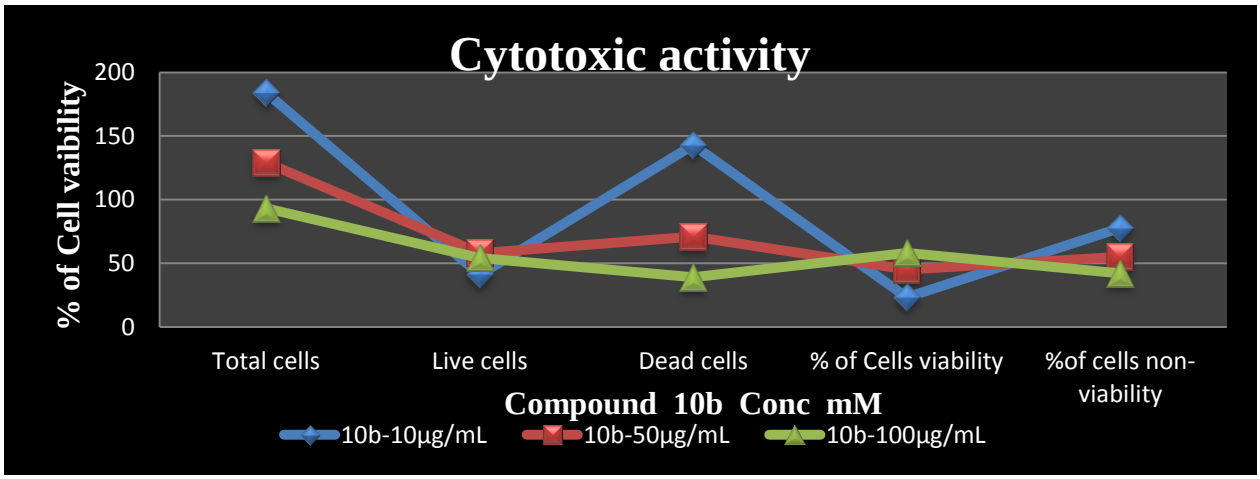

Fig. 2: Cytotoxic effect of selected compounds at varying concentrations (10µg/mL, 50µg/mL and 100µg/mL) against Peripheral Blood Mononuclear Cells. Dose-response effect of each synthesized compounds showed in graphical representation

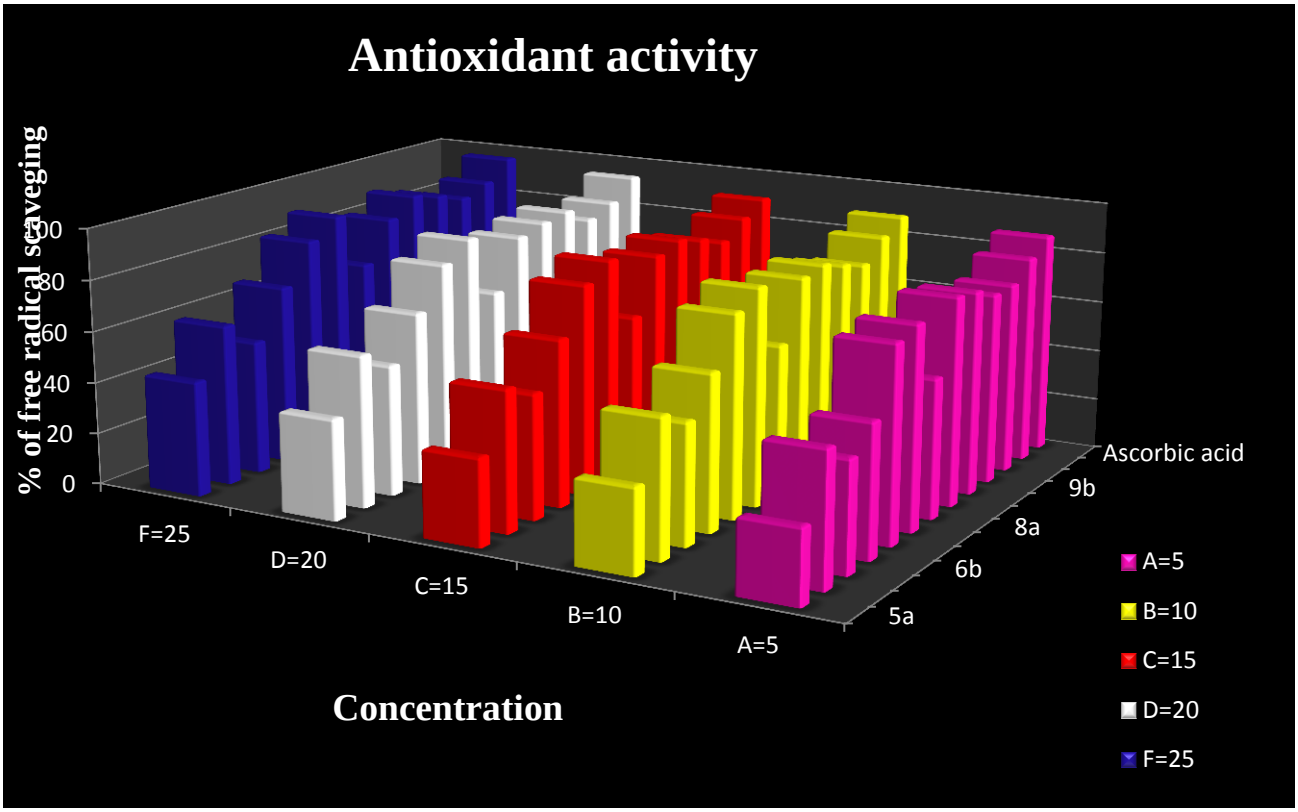

Fig. 3: antioxidant activity of the compound 5(a-b) - 10(a-b)

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