IJPAC : 13(3-4), (July-December 2018)

Synthesis and characterization of (2*E*)-1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl]propan-2-one *N*-[(1*Z*)-(4-chlorophenyl) methylidene] thiosemicarbazone Benzoxazole Derivatives and their biological Activities

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ABSTRACT: In this study a new series of (2*E*)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one *N*-[(1*Z*)-(4-chloroyphenyl)methylidene]thiosemicarbazone derivatives have been synthesized by standard physicochemical methods. The structures of newly synthesized benzoxazole derivatives ($\mathbf{5}_{a-f}$) were characterized by elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. The synthesized compounds ($\mathbf{5}_{a-f}$) has been screened for their antimicrobial activity using the disc diffusion and minimum inhibitory concentration (MIC) method against the selected bacterias (*Staphylococcus aureus, Staphylococcus epidemidis*, *Bacillus subtilis*, *P.aeruginosa, Vibrio cholerae* and *E. coli*) and fungal strain (*Aspergillus aureus* and *Aspergillus fumigates*). Among all synthesized benzoxazole derivatives $\mathbf{5}_{b}$, $\mathbf{5}_{c}$, $\mathbf{5}_{d}$ and $\mathbf{5}_{f}$ were found to be more potent antibacterial active against all tested strains. The *In vitro* antimicrobial activity at MIC level showed that the compound $\mathbf{5}_{a}$ also exhibit potent antimicrobial activity as similar to compound $\mathbf{5}_{c}$. The antioxidant properties were evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method. Compounds $\mathbf{5}_{a}$ and $\mathbf{5}_{b}$ showed predominant antioxidant activities among the synthesized analogues.

Keywords: (2*E*)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one *N*-[(1*Z*)-(4-chloroyphenyl) methylidene]thiosemicarbazone, benzoxazole derivatives, antimicrobial activity, MIC, antioxidant activities.

1. INTRODUCTION

The rising prevalence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. In fact every day more common and uncommon bacteria previously susceptible to common antimicrobials are reported to have developed resistance to different antibiotics. To prevent the emergence and dissemination of resistant bacteria, as a consequence of this feature it is necessary to develop a new effective antibacterial agents.

The benzoxazole have been the aim of many researchers for many years because they constitute an important class of heterocyclic compounds exhibiting various types of biological properties such as anticancer[1], antibacterial[2], anti HIV-1[3], antioxidant[4], cyclooxygenase inhibitory[5], antifungal[6], antibacterial[7], melatonin receptor agonist[8], antibiotic[9], antimycobacterial activities[10].

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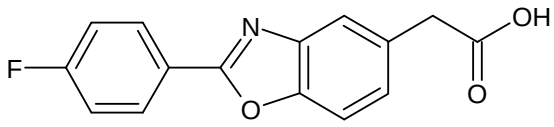
Considering the above observations and in connection to previous publications involving the synthesis of new biologically active benzoxazole. Being a heterocyclic compound, benzoxazole finds use in research as a starting material for the synthesis of bioactive structures. It is found within the chemical structures of pharmaceutical drugs such as Flunoxaprofen.



Flunoxaprofen

The literature survey is raveled that thiosemicarbazone derivatives are of huge importance because of their versatile biological and pharmacological activities. On the other hand Thiosemicarbazides are potent intermediates for the synthesis of pharmaceutical and bioactive materials and the derivatives of thiosemicarbazone

have received considerable attention because of their anti-amoebic[11], trypanocidal[12], Anticancer[13], anti HIV[14], anti-tumor[15], anti-inflammatory[16] CNS stimulant properties[17], they also find applications as dyes, lubricants and analytical reagents[18], antiviral agents[19].

In the present work we have synthesized a series of benzoxazole derivatives including thiosemicarbazone moiety. The desired compound has been synthesized from (2*E*)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone(4) on treatment with different aromatic aldehydes and the Compounds $5_{(a-f)}$ has been screened for their antimicrobial activity using the disc diffusion and minimum inhibitory concentration (MIC) method against the selected bacterial and fungal strain, and the antioxidant properties were evaluated.

2. MATERIALS AND METHODS

2.1. General Experiments

2-Amino-5-nitrophenol, AR grade chloroacetone, absolute ethanol were procured from Sigma-Aldrich (INDIA), Himedia (INDIA), Labo Chemicals (INDIA). Freshly distilled solvents were used for all synthetic purposes. All other chemicals were of AR grade. The progress of reaction was monitored by TLC. Yields refer to isolated yields after column chromatographic purification of compounds that have a purity of ≥ 97%.

The products of these reactions were confirmed by spectral data. ^{1}H and ^{13}C NMR spectra recorded on Bruker 400 MHz spectrometer at Sophisticated Analytical Instruments Facility, Cochin University, Cochin, India. The chemical shifts have been proven in δ values (ppm) with tetramethylsilane (TMS) as an internal standard. The signals were designated as follows: s, singlet; d, doublet; t, triplet and m, multiplet. Molecular weights of unknown compounds were characterized by LCMS. Melting points were determined in an electrically heated apparatus. The Fourier transform infrared (FT-IR) spectra of the compounds were taken as KBr pellet (100 mg) the usage of a Shimadzu Fourier Transform Infrared (FT-IR) spectrometer.

2.2. Synthesis of benzoxazole derivatives (5_{a-f})

The preparation of (2*E*)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one *N*-[(1*Z*)-(4-chloroyphenyl) methylidene]thiosemicarbazone the benzoxazole derivative involving three intermediates (2, 3 and 4).

2.2.1. Synthesis of 6-nitro-1, 3-benzoxazole- 2-tiole (2)

The mixture of 2-amino-5-nitro phenol **(1)** (0.01 mol) and carbon disulfide (0.01 mol) in presence of potassium hydroxide were refluxed up to 6 h in 30 mL of methanol. The product was confirmed by TLC [Ethyl acetate: Pet ether (3:7)]. The reaction mass was poured onto crushed ice and acidify with glacial acetic acid (pH 6), yellow colored solid separated out, filtered and recrystallized from ethanol. Color: Yellow; Yield: (92%), m.p. 196-198 C^0; IR (KBr, cm^{-1}): 3386 cm^{-1} (ν -SH); ^{1}H NMR (DMSO-d$_6$, δ ppm): 14.2(s, H -SH), 8.407~8.412 (d, H Ar-H), 8.206~8283 (dd, H Ar-H), 7.388~7.410 (d H Ar-H); MS: m/z =196.

2.2.2. Synthesis of 1-[(6-nitro-1, 3-benzoxazol-2-yl) sulfanyl] propan-2-one (3)

The compound 6-nitro-1,3-benzoxazole-2- tiole (0.01 mol) and chloroacetone (0.01 mol) in presence of potassium hydroxide (0.01 mol) in (30) mL of ethyl acetate ware refluxed for 5 h. The product was confirmed by TLC [Ethyl acetate: Pet ether (7:3)]. The reaction mixture was poured on to the crushed ice, acidifies with glacial acetic acid thus pale brown solid separates and filtered. The obtained product was recrystallized using ethanol. Color: Pale brown: Yield: (94 %), m.p. 210-212 C^0; IR (KBr, cm^{-1}): 1714 cm^{-1} (ν C=O); ^{1}H NMR (DMSO-d$_6$, δ ppm): 8.605~8.609(d, H Ar-H); 8.250~8.605 (dd, H Ar-H); 7.803~7.825 (d H Ar-H); 4.246 (s, 2H -CH$_2$); 2.084 (s, 3H -CH$_3$); ^{13}C NMR (DMSO-d$_6$, δ ppm): 205.09, 170.02, 151.02, 147.06, 121.45, 121.33, 105.99, 43.33, 15.02; MS: m/z = 253.24.

2.2.3. Synthesis of (2E)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-onethiosemicarbazone (4) Mixture of 1-[(5-nitro-1, 3-benzoxazol-2-yl)sulfanyl]propan-2-one (0.01mol) and thiosemicarbazide (0.01 mol) was heated under reflux in ethanol in presence of hydrochloric acid, the product was confirmed by TLC [Ethyl acetate : Pet ether (4: 6)]. The resulting mixture was further allowed for 6 h. The mixture was poured to crushed ice, the orange colored solid separated out and it was filtered and recrystallized by methanol. Yield: (95 %), m.p. 220-222 C^0 ; IR (KBr, cm^{-1}): 3300 cm^{-1} (ν N-H), 1700 cm-1 (ν C=S),

1650 cm^{-1} (ν C=N); ^1H NMR (DMSO-d_6, δ ppm): 10.368 (s, 2H -NH_2), 8.629 (s, H Ar-H), 8.294~8.304 (d, H Ar-H), 7.835~7.857 (s, H Ar-H), 4.246 (s, 2H -CH_2), 2.084 (s, 3H -CH_3), 1.284 (s, -SH); ^{13}C NMR (DMSO-d_6, δ ppm): 179.43, 170.31, 155.08,150.83, 147.68, 144.99, 121.47, 121.32, 107.29, 40.54, 16.39; MS: m/z =326.26.

2.2.4. General procedure for the synthesis of (2E)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone derivatives (5_{a-f})

Mixture of (2*E*)-1-[(5-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone (0.01 m) and substituted aromatic aldehyde (0.01 m) in 50 mL of ethanol was refluxed for 4 h in presence of catalytic amount of glacial acetic acid , the resulting mixture was poured onto crushed ice. The pale yellow colored solid separated out, filtered and washed with cold water. The product was recrystallized from dry ethanol. The compounds (**5_{a-f}**) can be prepared by following similar procedure.

2.2.4.1 Synthesis of (2E)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[(1Z)-(4-chlorophenyl)methylidene]thiosemicarbazone (5a)

Color: pale brown; Yield: (84 %); m.p. 216-218 C^0 ; IR (KBr, cm-1): 3321 cm^{-1} (ν -NH), 1712 cm^{-1} (ν C=S), 1656 cm^{-1} (ν C=N); ^1H NMR (DMSO-d_6, δ ppm): 11.847 (s, H –NH), 8.469 (s, H -CN), 8.197~8.177 (d, H Ar-H), 8.161~8.155 (d, H Ar-H), 7.598~7.538 (m, 4H Ar-H), 6.886 (s, H Ar-H), 4.208 (s, 2H –CH_2), 2.064 (s, 3H –CH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 190.42, 165.27, 163.31, 155.83, 150.82, 147.74, 144.27, 138.27, 132.38, 130.72, 130.62, 129.72, 129.72, 120.44, 120.42, 107.25, 42.75, 16.27; Elemental analysis (%) found (Calculated) for $C_{18}H_{14}ClN_5O_3S_2$ C - 48.27 (48.52), H - 3.15 (3.17), N -15.64 (15.62); MS: m/z = 447.98 (M+), 449.23 (M+2)

2.2.4.2. Synthesis of (2E)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[(1Z)-(4-methoxyphenyl)methylidene]thiosemicarbazone 5(b) Color: pale brown; Yield: (75 %); m.p. 242-244 C^0 IR (KBr, cm-1): 3322 cm^{-1} (ν N-H), 1713 cm^1 (ν C=S), 1655 cm^{-1} (ν C=N); ^1H NMR (DMSO-d_6, δ ppm): 10.536 (s, H -NH), 8.519 (s, H -CN), 8.193~8.085 (d, H Ar-H), 7.669~7.639 (d, H Ar-H), 7.635~7.201 (m, 4H Ar-H), 6.879 (s, H Ar-H), 4.225 (s, 2H –CH_2), 3.068 (s, 3H –CH_3), 2.268 (s, 3H –CH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 192.55, 167.62, 165.02, 162.36, 156.32, 152.62, 146.36, 144.60, 131.07, 130.62, 126.30, 120.04, 120.02, 114.03, 114.02, 106.34, 55.40, 43.43, 15.38 ; Elemental analysis (%) found (Calculated) for $C_{19}H_{17}N_5O_4S_2$: C - 51.46 (51.47), H - 3.86(3.84), N -15.79 (15.80); MS: m/z = 443.88.

2.2.4.3. Synthesis of (2E)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-oneN-[(1Z)-(4-bromophenyl)methylidene]thiosemicarbazone 5(c) Color: dark brown; Yield: (82 %); m.p. 232-236 C^0 IR (KBr, cm-1): 3324 cm^{-1} (ν N-H), 1714 cm^{-1} (ν C=S), 1656 cm^{-1} (ν C=N); ^1H NMR (DMSO-d_6, δ ppm): 10.503 (s, H -NH), 8.919 (s, H -CN), 8.187~8.861 (d, H Ar-H), 7.861~7.855 (d, H Ar-H), 7.834~7.401 (m, 4H Ar-H), 7.159 (s, H Ar-H), 4.205 (s, 2H –CH_2), 2.258 (s, 3H -CH_3); ^{13}C NMR (DMSO-d_6, δ ppm); 192.23, 170.28, 163.28, 155.78, 150.74, 147.24, 144.12, 132.28, 131.62, 131.41, 131.40, 125.21, 120.24,120.22, 106.28, 40.28, 15.58 ; Elemental analysis (%) found (Calculated) for $C_{18}H_{14}BrN_5O_3S_2$: C - 43.91 (43.43), H- 2.87 (2.85), N -14.22 (14.24); MS: m/z = 492.42 (M+), 494.12 (M+ 2).

2.2.4.4. Synthesis of (2E)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[(1Z)-(4-nitrophenyl)methylidene]thiosemicarbazone 5(d) Color: yellow; Yield: (76%); m.p. 214-216 C^0; IR (KBr, cm-1): 3324 cm^{-1} (ν N-H), 1711 cm^{-1} (ν C=S), 1656 cm^{-1} (ν C=N), ^1H NMR (DMSO-d_6, δ ppm): 10.353 (s, H -NH), 8.139 (s, H -CN), 8.593~8.587 (d, H Ar-H), 8.261~8.255 (d, H Ar-H), 8.239~8.801 (m, 4H Ar-H), 7.459 (s, H Ar-H), 4.305 (s, 2H –CH_2), 2.058 (s, 3H -CH_3); ^{13}C NMR (DMSO-d_6, δ ppm); 190.55, 167.36, 163.05, 160.79, 155.36, 151.04, 150.02, 147.76, 144.40, 139.40, 129.07, 129.02, 120.06, 120.02, 116.03, 116.02, 105.30, 42.53, 16.32; Elemental analysis (%) found (Calculated) for $C_{18}H_{14}N_6O_5S_2$:C - 47.16 (47.25), H - 3.08 (3.10), N -18.33 (18.30); MS: m/z = 458.56

2.2.4.5. Synthesis of (2E)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[(1Z)-(4-hydroxyphenyl)methylidene]thiosemicarbazone 5(e) Color: orange ; Yield: (70%); m.p. 218-220 C^0; IR (KBr, cm-1): 3450 cm^{-1} (ν O-H), 3334 cm^{-1} (ν N-H), 1721 cm^{-1} (ν C=S), 1646 cm^{-1} (ν C=N); ^1H NMR (DMSO-d_6, δ ppm): 11.907 (s, H -NH), 10.556 (s, H -OH), 8.569 (s, H -CN), 8.297~8.277 (d, H Ar-H), 8.261~8.255 (d, H Ar-H), 7.698~7.638 (m, 4H Ar-H), 6.986 (s, H Ar-H), 4.258 (s, 2H –CH_2), 2.094 (s, 3H -CH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 190.25,

167.32, 163.02, 160.76, 155.32, 151.02, 147.76, 144.40, 130.07, 130.02, 125.30, 120.04, 120.02, 116.03, 116.02, 105.30, 42.33, 16.38. Elemental analysis (%) found (Calculated) for $C_{18}H_{15}N_5O_4S_2$: C – 50.34 (50.36), H - 3.52 (3.51), N -16.31 (16.34); MS: m/z = 429.08

2.2.4.6. Synthesis of (2E)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[(1Z)-(3-flurophenyl)methylidene]thiosemicarbazone 5(f) Color: orange; Yield: (74%); m.p. 210-212 C⁰; IR (KBr, cm-1): 3328 cm^{-1} (ν N-H), 1714 cm^{-1} (ν C=S), 1654 cm^{-1} (ν C=N), ^{1}H NMR (DMSO-d_6, δ ppm): 10.506 (s, H -NH), 8.619 (s, H -CN), 8.093~8.087 (d, H Ar-H), 7.661~7.655 (d, H Ar-H), 7.639~7.201 (m, 4H Ar-H), 6.859 (s, H Ar-H), 4.258 (s, 2H – CH_2), 2.094 (s, 3H -CH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 190.55, 167.92, 165.02, 162.76, 156.32, 152.02, 147.36, 144.60, 130.97, 130.72, 125.30, 120.04, 120.02, 116.03, 116.02, 106.30, 43.43, 15.38; Elemental analysis (%) found (Calculated) for $C_{18}H_{14}FN_5O_3S_2$:C - 50.11 (50.14), H - 3.27 (3.25), N -16.23(16.22); MS: m/z = 431.48

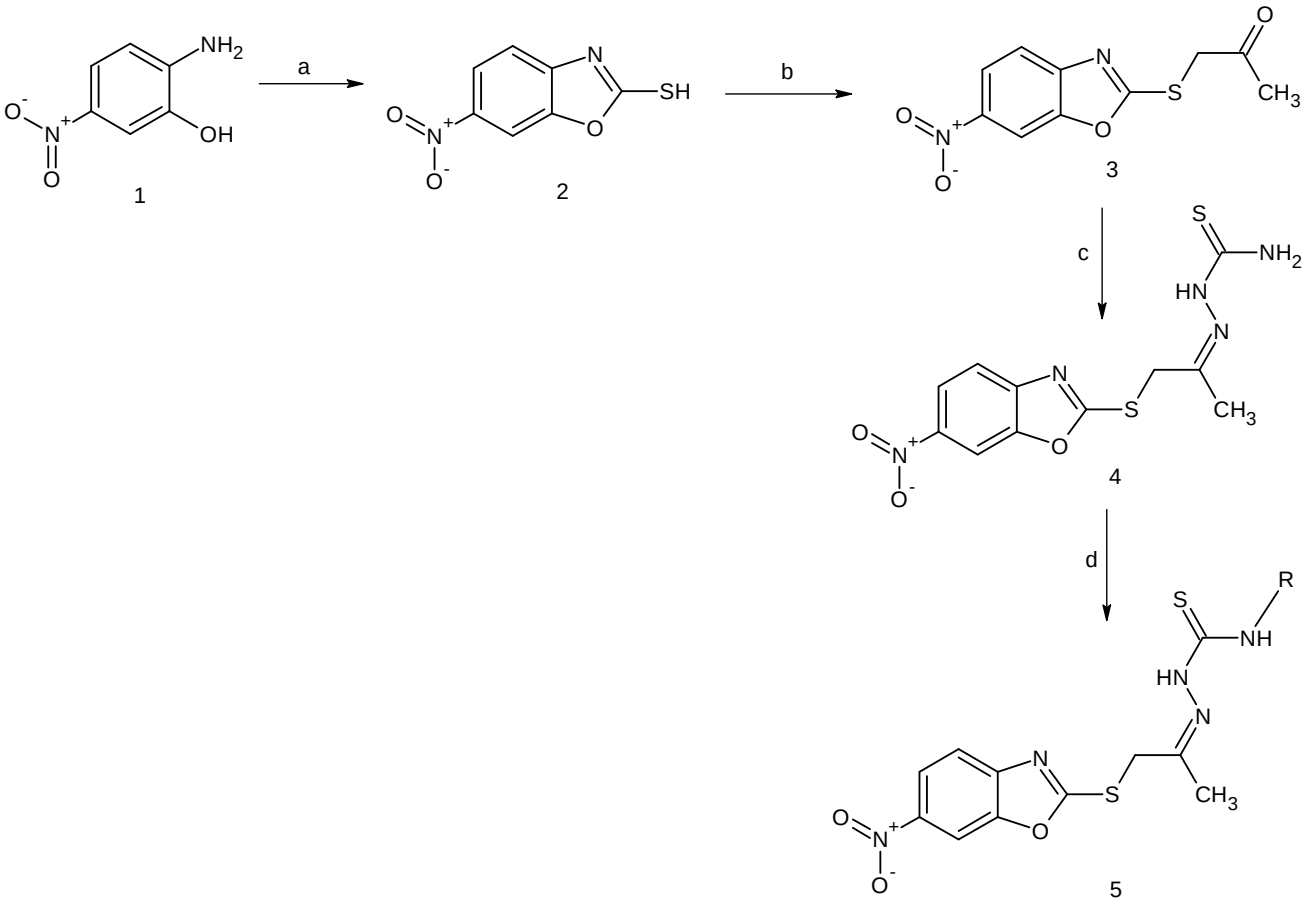


Figure 1: Scheme

General synthetic route to access A-ring variants, Reagents and conditions (a). CS_2, KOH, MeOH. (b) $ClCH_2COCH_3$, KOH, Ethyl acetate. (c) $NH_2NHCSNH_2$. AcOH, EtOH, (d) RCHO, DMF AcOH.

R

5a: 4-Cl-C_6H_4

5b: 4-OCH_3-C_6H_4

5c: 4-Br-C_6H_4

5d: 4-NO_2-C_6H_4

5e: 4-OH-C_6H_4

5f: 4-F-C_6H_4

2.3. Evaluations of biological activities

2.3.1. Antibacterial screening

The newly synthesized compounds were evaluated for *in vitro* growth inhibitory activities against a panel of standard strains of pathogenic microorganisms including three Gram-positive bacteria, three Gram-negative bacteria namely *staphylococcus aureus, staphylococcus epidemidis* and *bacillus cereus* and gram negative bacteria namely *pseudomonas aeruginosa, vibrio cholerae* and *escherichia coli* by agar well diffusion method. The standard drug as Tetracycline, 100 µg/mL of sterile distilled water, three different concentrations (100, 50 and 25 µg /mL in 10% DMSO) and control (10% DMSO) were introduced to respective labeled wells. The plates were allowed to stand for 30 min. and were incubated at 37 ºC for 24 h in upright position and the zone of inhibition was recorded. During this period, the test solution diffused and zone of inhibition were recorded using vernier calipers.

2.3.2. Antifungal screening

The newly synthesized compounds were evaluated against *Aspergillus aureus* and *Aspergillus fumigates* fungus, using the sabouraud dextrose agar diffusion method. Wells were made (6mm diameter) with a sterile cork borer. The standard drug namely Fluconazole used 100 µg/mL of sterile distilled water) and control was added to respectively labeled wells. To these wells 140 µL from each (100, 50 and 25 vg/mL in 10% DMSO) of the test stock solution compounds were introduced and the plates were allowed to cool for an hour to facilitate the diffusion. The plates were incubated at 37 ºC for 48 h. At the end of the incubation period, the diameter of the zone of inhibition around the wells was measured using vernier calipers.

2.3.3. Free-radical-scavenging activity using the DPPH method

The synthesized compounds were performed for scavenging activity by DPPH method as per literature [30]. The compounds of different concentrations were dissolved in menthaol and were added to each vial of 5mL. To this test vials 3 mL of 0.004% DPPH in methanol was added and the mixtures have been incubated in dark condition at ambient temperature for 30 min. Ascorbic acid was used as the standard. The absorbance reduced while the DPPH is scavenged by way of an antioxidant, through contribution of hydrogen to shape a strong DPPH molecule. DPPH scavenging activity calculated by the use of the following equation and absorbance measured at 517 nm.

Scavenging ratio (%) = [(Ai Ao) D (Ac Ao)] × 100%

Where

Ai is the absorbance within the presence of the check compound.

Ao is absorbance of the clean inside the absence of the check compound.

Ac is the absorbance within the absence of the test compound.

3. RESULTS AND DISCUSSION

3.1. Chemistry

The starting material 1, 3 Benzoxazole- 2- tiole (2) was synthesized by the reaction of 2-amino 5-nitro phenol with carbon disulfide in presence of potassium hydroxide in methanol and the product was condensed with chloroacetone in ethyl acetate to get 1-[(6-nitro-1, 3-benzoxazol-2-yl) sulfanyl] propan-2-one (3) and the compound 3 on treated thiosemicarbazide afforded intermediate benzoxazole compound (2*E*)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone (4).

The compound 4 was Characterized by IR, the spectrum showed absorption at 3300 cm^{-1} which was due to the N-H stretching. The band appeared at 1700 cm-1 is due to C=S stretching and absorption at 1650 cm^{-1} which was due to the C=N stretching which was confirmed the structure of the compound.

The ^1H NMR spectrum of compound 4 displayed a singlet at δ 2.084 which was due to three C-H_3 protons. Two singlets at δ 1.284 and δ 4.246 were due to S-H proton and C-H_2 protons, a multiplet at δ 7.835-8.629 were due to aromatic protons. A singlet at δ 10.36 appeared due to N-H_2 protons confirmed the formation of compound 4. The mass spectrum of 4 showed a molecular ion peak at m/z = 326.26, which was in agreement with the molecular formula $C_{11}H_{11}N_5O_3S_2$. The intermediate benzoxazole compound(2*E*)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone (4) was condensed with 4-

chlorobenzaldehyde to produce target compound (2*E*)-1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one *N*-[(1*Z*)-(chlorophenyl) methylidene]thiosemicarbazone 5a.

The compound 5a was characterized by IR the spectrum exhibited strong absorbance band at 3321 cm^{-1} which was due to the N-H stretching. The band appeared at 1712 cm^{-1} was due to C=S stretching and absorption at 1656 cm^{-1} which was due to the C=N stretching confirmed the structure of the compound.

The ^1H NMR spectrum of compound 5a displayed a singlet at δ 2.064 which was due to C-H_3 protons. A singlet at δ 4.208 was due to C-H_2 protons, a multiplet at δ 6.886-8.469 were due to sexate aromatic protons, azomethane proton displayed at δ 8.469 and a singlet near δ 11.84 appeared due to N-H proton confirmed the formation of desired product. The mass spectrum of 5a showed a molecular ion peak at m/z = 448.08 which was concurrence with molecular weights of targeted molecules $C_{18}H_{15}ClN_5O_3S_2$.

Table 1
Physical data of compounds 5 (a-f)

Compound	*R*	*Molecular formula*	*Molecular weight*	*M.P. (^{0}c)*	*% of Yield*	*Found (Calculated) %* *C*	*H*	*N*
5a	4-Cl-C_6H_4	$C_{18}H_{14}ClN_5O_3S_2$	447.91	218	84	48.27 (48.52)	3.15 (3.17)	15.64 (15.62)
5b	4-OCH_3-C_6H_4	$C_{19}H_{17}N_5O_4S_2$	443.49	244	75	51.46 (51.47)	3.86 (3.84)	15.79 (15.80)
5c	4-Br-C_6H_4	$C_{18}H_{14}BrN_5O_3S_2$	492.36	236	82	43.91 (43.93)	2.87 (2.85)	14.22 (14.24)
5d	4-NO_2-C_6H_4	$C_{18}H_{14}N_6O_5S_2$	458.47	216	76	47.16 (47.25)	3.08 (3.10)	18.33 (18.30)
5e	4-OH-C_6H_4	$C_{18}H_{15}N_5O_4S_2$	429.47	220	70	50.34 (50.36)	3.52 (3.51)	16.31 (16.34)
5f	4-F-C_6H_4	$C_{18}H_{14}FN_5O_3S_2$	431.46	212	74	50.11 (50.14)	3.27 (3.25)	16.23 (16.22)

3.2. Biological activity studies

The searching for newer antibacterial and antifungal agents was still in continuation; due to the rapid development of the resistance among bacteria and fungi. Benzoxazole derivatives including thiosemicarbazone moiety may comprise a new class of antimicrobial agents with diminished resistance.

3.2.1. In vitro antibacterial and antifungal activity

The data showed that synthesized compound and its derivatives have the capacity of inhibiting the metabolic growth of the investigated bacterial and fungal strains. Antimicrobial activity in different concentrations of benzoxazole derivatives were showed in Table 2 and Table 3. As a result of this, the primary screening against the bacterial strains in different concentrations showed good zone of inhibition as shown in fig 2. It was noticed that the presence of electron withdrawing halogen such as F or Br attached to the benzene ring displayed strong effect on the antimicrobial activity for example the presence of 4-fluorophenyl, 4-bromophenyl and 4-chlorophenyl moiety in the structure of (**5f. 5c and 5a**) showed the potent antimicrobial activity among all the tested compounds of this series. While electron-donating group such as methoxy group had a detrimental effect on antimicrobial activity. The compound **5a, 5c** and **5d** exhibited the highest antibacterial activity against gram negative bacteria *P aeruginosa*, *V cholerae* and *E coli*. The compounds **5b, 5d** and **5f** displayed good antibacterial activity towards gram positive bacteria, *S aureus*, *staphylococcus epidemidis* and *Bacillus subtilis* respectively. The compounds **5a, 5c** and **5d** performs considerable antifungal activity against *A aureus* and *A fumigates*, the primary screening against the fungal strains in different concentrations showed good zone of inhibition as shown in fig 3. The minimum inhibitory (MIC), minimum bactericidal (MBC) and minimum fungicidal concentration (MFC) values of the compounds were tested. MIC was the lowest

concentration of an antimicrobial that will inhibit the visible growth of a microorganism. The MBC/MFC was the lowest concentration of antibiotic required to kill a particular bacterium/fungi. The MIC study of both synthesized compounds against bacterial and fungal strains at different concentrations i.e., 1, 10, 25, 50, and 100µg/mL was evaluated. The MIC data of antimicrobial activity[20] of the compounds ($\mathbf{5_{a\text{-}f}}$) were reported in table 4 and table 5. The fig 4 and 5 represents the MIC activity against bacterial and fungal strains. The **5a** and **5c** compounds showed potential MIC values against bacterial and fungal strains respectively.

Table 2
Antibacterial activity data of synthesized compounds $5_{(a\text{-}f)}$

Compound	*Concentration in µg/ml*	*Growth inhibition against bacteria in mm*					
		P.aeruginosa	*S.aureus*	*V.cholerae*	*S.epidermidis*	*B.subtilis*	*E.coli*
5a	25	17.52±0.12	18.23±0.24	16.25±0.26	16.03±0.25	17.26±0.20	15.27±0.45
	50	20.12±0.12	22.56±0.07	18.42±0.31	18.10±0.26	20.28±0.05	21.54±0.03
	100	23.12±0.12	25.52±0.11	26.45±0.31	23450±0.20	27.27±0.00	24.56±0.25
5b	25	13.03±0.21	14.25±0.05	13.32±0.20	15.25±0.15	12.11±0.17	14.26±0.17
	50	17.10±0.20	17.54±0.07	18.29±0.03	17.35±0.30	14.00±0.00	16.27±0.16
	100	19.07±0.21	19.85±0.32	21.45±0.21	21.47±0.33	20.14±0..00	20.78±0.07
5c	25	16.42±0.20	15.18±0.13	16.26±0.06	16.82±0.34	18.64±0.24	12.14±0.12
	50	18.54±0.13	19.45±0.18	18.24±0.16	18.34±0.14	21.45±0.17	15.24±0.23
	100	23.12±0.10	23.51±0.57	25.89±0.16	23.45±0.23	26.84±0.14	23.81±0.18
5d	25	10.56±0.15	12.63±0.15	12.92±0.06	13.52±0.02	12.56±0.04	13.42±0.11
	50	13.20±0.15	14.22±0.56	13.06±0.04	14.96±0.04	15.90±0.06	14.23±0.20
	100	16.20±0.09	18.12±0.15	16.23±0.27	18.22±0.07	22.11±0.19	18.21±0.02
5e	25	12.08±0.20	16.70±0.20	15.28±0.05	13.30±0.19	13.28±0.05	15.21±0.33
	50	16.06±0.15	19.43±0.21	17.56±0.02	15.41±0.06	15.28±0.21	18.21±0.04
	100	20.57±0.12	21.45±0.09	20.49±0.08	19.45±0.04	21.32±0.02	20.17±0.03
5f	25	12.20±0.01	18.47±0.21	16.13±0.02	17.54±0.11	14.23±0.12	13.89±0.01
	50	15.30±0.22	22.15±0.26	18.14±0.01	19.35±0.21	18.65±0.09	19.02±0.31
	100	20.10±0.02	26.45±0.21	22.14±0.02	24.26±0.27	23.34±0.05	22.50±0.25
Control	100	0	0	0	0	0	0
Std	**100**	**24.00±0.01**	**27.12±0.01**	**28.25±0.14**	**25.03±0.31**	**30.05±0.45**	**26.11±0.20**

* Each value is expressed as mean ± SD of three replicates for the zone of inhibition

* Stnd: Tetracyclin

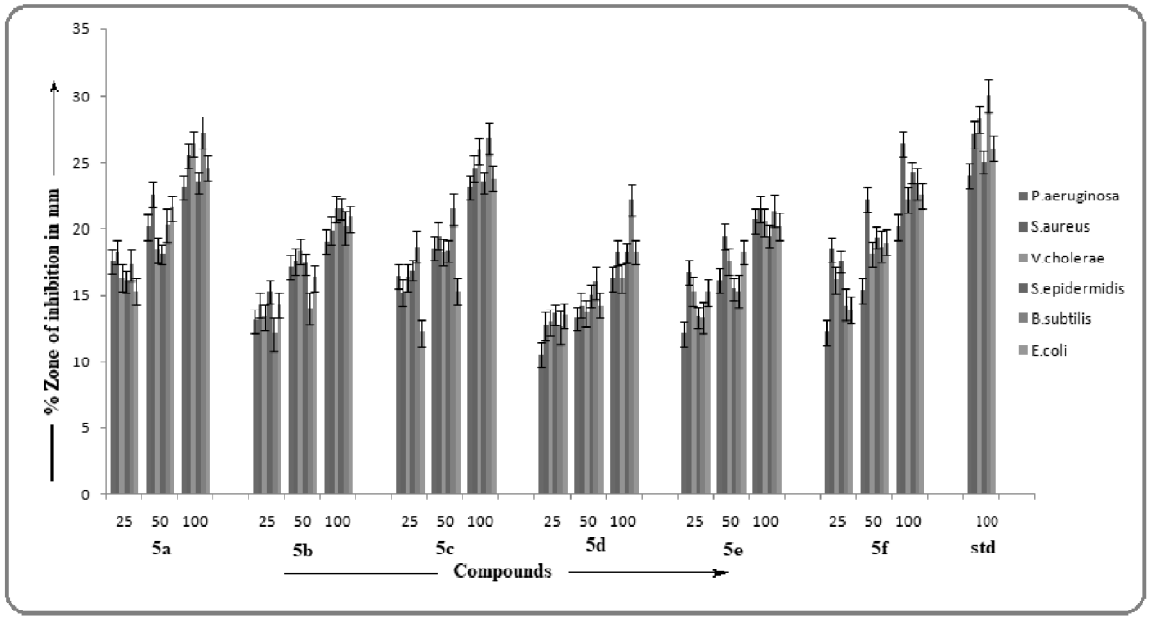


Figure 2: Antibacterial activity of compound $5_{(a\text{-}f)}$

Table 3
Antifungal activity data of synthesized compounds $5_{(a-f)}$

Compound	*Concentration in µg/ml*	*Growth inhibition against fungicides in mm*	
		A. aureus	*A.fumigatus*
5a	25	18.23±0.25	17.56±0.20
	50	22.50±0.31	21.45±0.20
	100	24.89±0.21	23.12±0.23
5b	25	14.11±0.17	15.14±0.15
	50	16.44±0.20	18.36±0.15
	100	19.59±0.33	20.35±0.10
5c	25	18.17±0.16	16.25±0.10
	50	22.13±0.14	20.20±0.07
	100	25.65±0.02	23.42±0.09
5d	25	19.42±0.17	19.21±0.14
	50	23.56±0.20	22.36±0.25
	100	26.22±0.23	23.15±0.15
5e	25	16.58±0.19	14.25±0.09
	50	18.62±0.25	17.28±0.05
	100	21.36±0.10	21.06±0.01
5f	25	14.23±0.01	16.23±0.18
	50	15.92±0.22	18.65±0.10
	100	19.23±0.11	20.23±0.01
Control	100	0	0
Std	100	28.05±0.11	24.52±0.15

* Each value is expressed as mean ± SD of three replicates for the zone of inhibition

* Stnd: Fluconazole

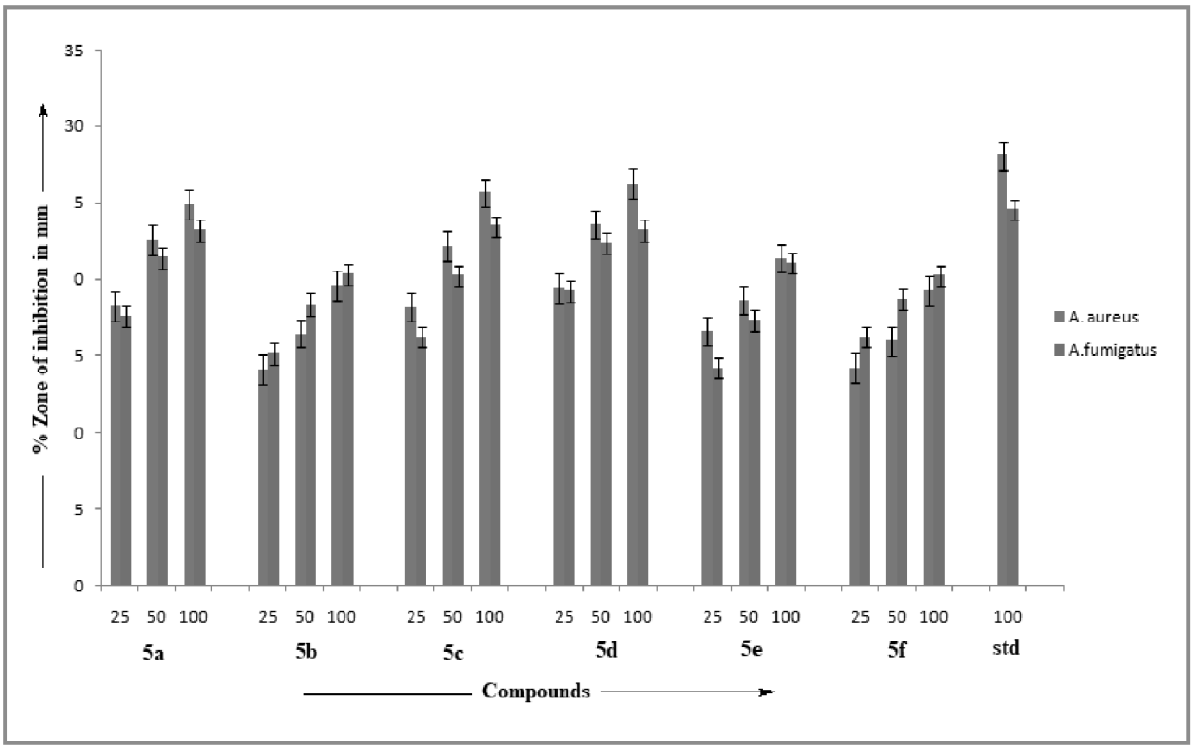


Figure 3: Antifungal activity of compound $5_{(a-f)}$

Table 4
MIC data of antibacterial activity of the synthesized compounds $5_{(a-f)}$

Compound	*Growth inhibition against bacteria in mm*					
	P.aeruginosa	*S.aureus*	*V.cholerae*	*S.epidermidis*	*B.subtilis*	*E.coli*
5a	700	700	700	500	700	500
5b	250	250	500	250	NT	250
5c	500	500	500	500	700	250
5d	250	250	250	250	250	250
5e	250	500	500	250	250	500
5f	250	700	500	700	250	250
Stnd	250	250	250	NT	NT	250

* Stnd: Tetracycline

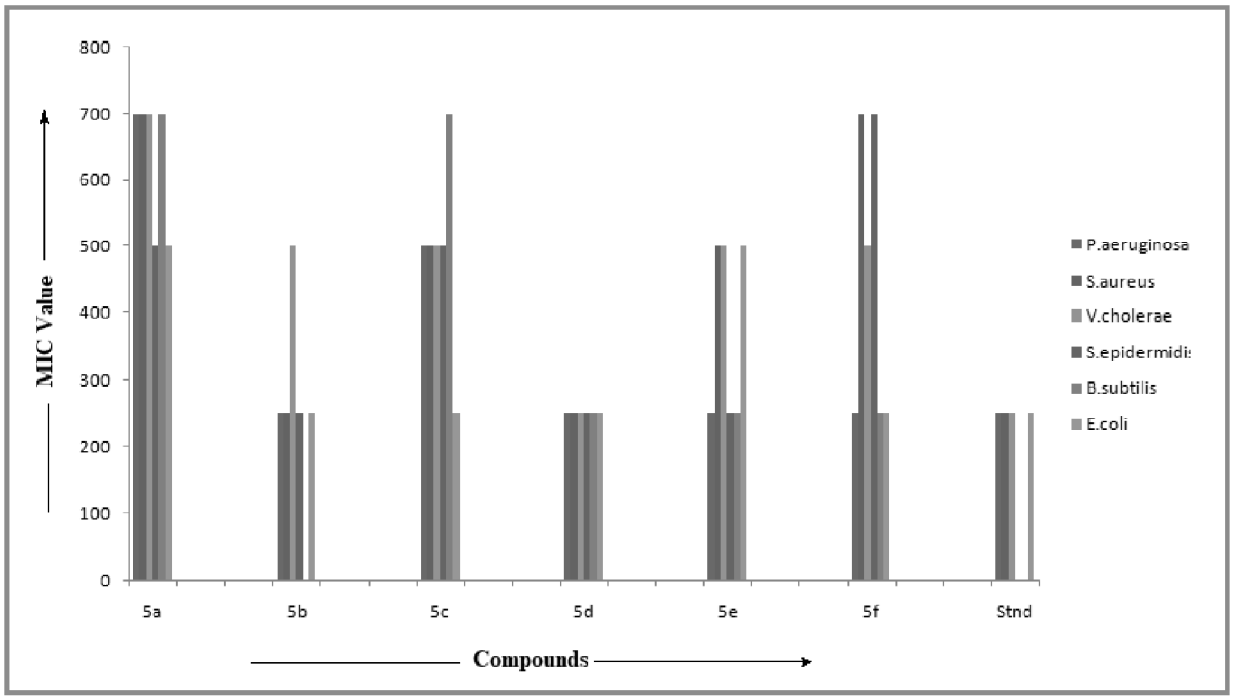


Figure 4: MIC data of Antibacterial activity of compound $5_{(a-f)}$

Table 5
MIC data of antifungal activity of the synthesized compounds $5_{(a-f)}$

Compound	*Growth inhibition against fungicides in mm*	
	A. aureus	*A.fumigatus*
5a	700	700
5b	250	500
5c	700	500
5d	700	700
5e	700	500
5f	250	500
Stnd	250	NT

* Stnd: Fluconazole

3.2.2. *Free-radical-scavenging activity using the DPPH method*

The DPPH radical scavenging activity data represented in table 6 and fig 5. DPPH solution in methanol showed strong absorbance at 517 nm. If DPPH abstracts a hydrogen radical from an external source, the absorption decreases stoichiometrically depending on the number of electrons or hydrogen atoms. The newly synthesized compounds showed significantly higher activity but lower when compared to ascorbic acid (vitamin C) as standard. The compound **5a** and **5b** exhibited potent scavenging activity almost close to the standard Vitamin-C and **5e** compound showed better inhibitions activity against free radical and other synthesized compounds showed moderate activity.

Table 6
Scavenging activity

Concentration in mL	*5a*	*5b*	*5c*	*5d*	*5e*	*5f*
0	-	-	-	-	-	-
5	22±0.01	21±0.21	19±0.05	16±0.04	20±0.01	15±0.01
10	25±0.05	24±0.24	20±0.21	19±0.02	24±0.04	16±0.08
15	30±0.07	28±0.20	24±0.21	21±0.04	27±0.03	18±0.04
20	32±0.06	31±0.09	28±0.22	22±0.07	32±0.07	21±0.07
25	36±0.01	35±0.01	29±0.07	26±0.21	33±0.04	22±0.01
Ascarbic acid	40±0.08	38±0.21	34±0.04	30±0.25	36±0.08	24±0.02

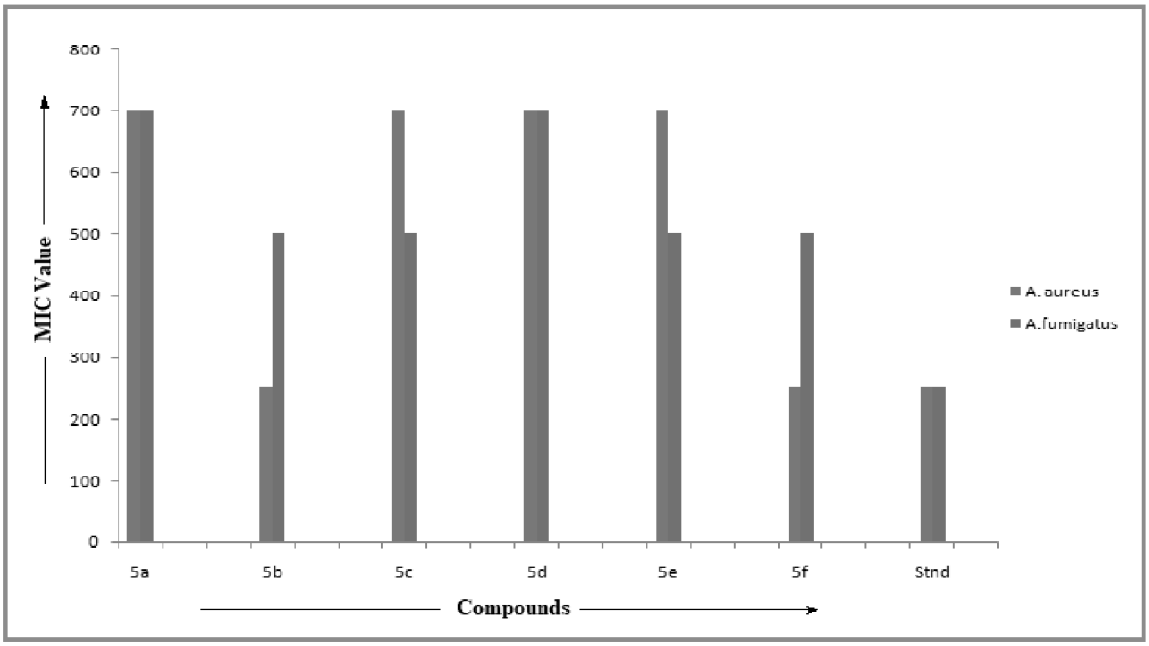


Figure 5: MIC data of Antifungal activity of compound $5_{(a\text{-}f)}$

4. CONCLUSION

New series of benzoxazole derivatives were synthesized through reaction (2*E*)-1-[(5-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone with different aromatic aldehyde. All the newly synthesized molecules were characterized by IR, ^{1}H NMR ^{13}C NMR and mass spectral analysis. For compounds, the antibacterial, MIC and antioxidant activity were evaluated. Nitro substituted benzoxazole derivatives exhibited promising Compound **5a, 5b, 5c, 5d, 5e,** and **5f** were exhibited effective *in vitro* antibacterial, MIC with antioxidant activity with effective result. By considering effective biological activity, we can conclude that benzoxazole was 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

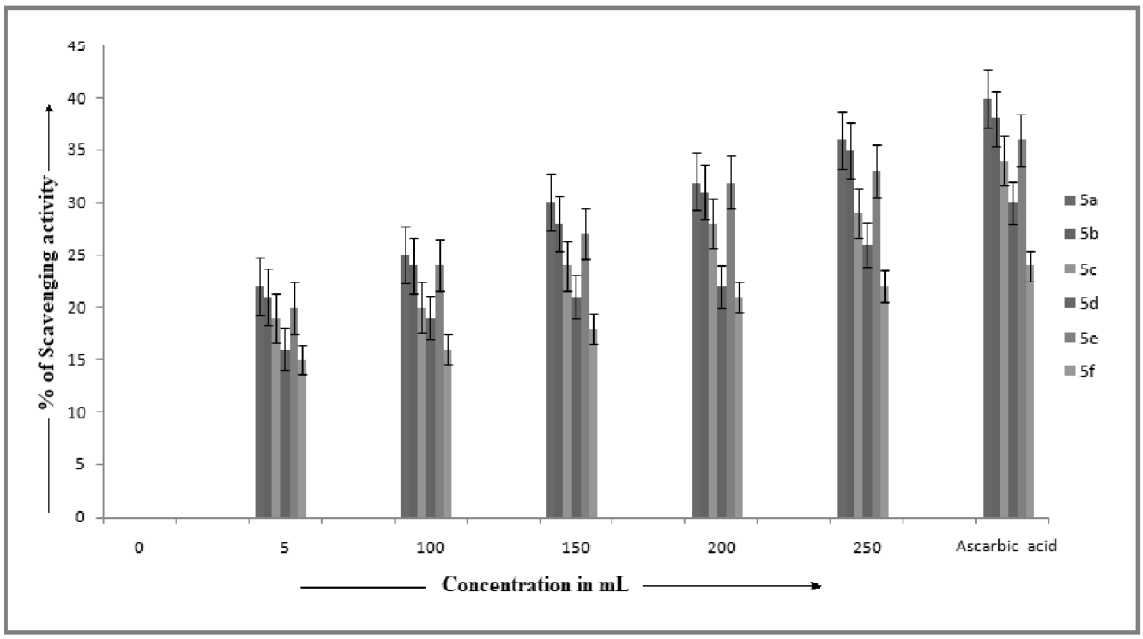


Figure 6: Free-radical-scavenging activity

the modification of different functional group in the target compounds.

Acknowledgements

The authors are thankful to the Principal and the Department of Chemistry and bio chemistry, Sahyadri Science College, Shimoga, Karnataka, India, for providing the necessary research facilities. We are also grateful to Sophisticated Analytical Instruments Facility, Cochin University, Cochin, Kerala, India, and MIT Manipal.

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