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Research Article

Design, Synthesis and Evaluation of *In Vitro*, Anti-Microbial Activity and Antioxidant Activities of Novel 2-{[(1-Acetyl-1*H*-Benzimidazol-2-Yl)Methyl]Sulfanyl}-6-Nitro-1,3-Benzoxazole Derivatives

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ABSTRACT
The large numbers of benzoxazole derivatives have been found to exhibit a wide variety of pharmacological activities. In the current research work, the title compound was synthesized by simple, mild and efficient synthetic protocol. The identification and characterization of all the synthesized compounds were confirmed by melting point, thin layer chromatography, FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the compounds were screened for *in vitro* antimicrobial activity using the disc diffusion and minimum inhibitory concentration (MIC) method against the selected microorganisms such as (*Staphylococcus aureus, Staphylococcus epidemidis*, *Bacillus subtilis*, *P.aeruginosa, Vibrio cholerae* and *E. coli*) and two fungal strain (*Aspergillus aureus* and *Aspergillus fumigates*) and antioxidant activity by DPPH method. Compounds 6a, 6c and 6e exhibited potent activity and other compounds showed moderate activity. Compounds 6b and 6c showed good Antioxidant property.

KEYWORDS: Benzoxazole; benzimidozole; 2-{[(1-acetyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole *in vitro* antimicrobial activity; MIC; Antioxidant property

INTRODUCTION

The practice of medicinal chemistry was devoted to the discovery and development of new agents for treating disease [1]. An important aspect of medicinal chemistry has been to establish a relationship between chemical structure and pharmacological activity. The chemistry of heterocyclic compounds was the most important in the discovery of new drugs. The study of these compounds was of great interest both in theoretical as well as practical aspects [2]. Benzoxazole finds used in research as a starting material for the synthesis of larger, usually bioactive molecules. It was found within the chemical structures of pharmaceutical drugs such as Flunoxaprofen. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites which allow for functionalization. The main objective of the synthetic chemistry and medicinal chemistry was to synthesize the compounds that give more yields with purity and show promising activity as therapeutic agents with lower toxicity. Notable among these are antihistaminic [3], antifungal [4], cyclooxygenase inhibiting [5].

Benzoxazole connected to benzimidazole have been found to be of great interest due to benzimidozole also had a broad spectrum of biological activities, Literature survey showed that benzimidazole frontier in pharmaceutical research for synthesis of new derivatives play a

vital role in biological activities such as drugs. Antimicrobial [6, 7], antifungal [8], antiviral. [9, 10], anticancer [11, 12], anti-tumor [13], anti-hepatitis-C-virus [14], kinase inhibitor [15, 16], analgesic [17], antihypertensive [18], antiulcer [19] anti-inflammatory [20]. On the other hand Schiff bases have an efficient antimicrobial [21] and antifungal activities [22]. The benzimidazole nucleus has resulted in many drugs like albendazole, mebendazole, thiabendazole as antihelmintics; omeprazole, lansoprazole, pantoprazole as proton pump inhibitors and many lead compounds in a wide range of other therapeutic areas.

The search for new antimicrobial and antioxidant agents devoid of side effects continues to be an active area of research in medicinal chemistry. Although new and highly expensive drugs have been developed, their cost was beyond the common man's reach. As a consequence, these trends have emphasized the pressing need for new, more effective, cheaper and safe antimicrobial agents. In view of these facts the present work was intended for the synthesis of a novel series of benzoxazole derivatives bearing a substituted benzimidazole nucleus. All of the synthesized derivatives 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-5-nitro-1,3-benzoxazole derivatives **6**$_{(a\text{-}f)}$ were screened for antioxidant activity by DPPH assay method and antimicrobial activity against some selected bacteria and fungi species.

MATERIALS AND METHODS

General Experiments

2-Amino-5-nitrophenol, o-Phenylenediamine, potassium hydroxide, chloro acetic acid, sodium metal, carbon disulphide, LR grade hydrochloric acid and LR grade methanol were procured from Sigma-Aldrich (INDIA), Himedia (INDIA), Labo Chemicals (INDIA) (Commercially available from local sources) were used as received without further purification. Freshly distilled solvents were used for all synthetic purposes. All other chemicals were of AR grade. The progress of reaction was monitored by TLC.

The products of these reactions were confirmed by matching spectroscopic data of the products obtained with those of the reported in the literature. ^{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 are designated as follows: s, singlet; d, doublet; t, triplet and m, multiplet. Elemental analyses were carried out with a Perkin-Elmer 2400 Series II C, H and N analyzer. Molecular weights of unknown compounds were characterized by LC-MS spectroscopy, Centralized instrumentation facility, Mysore University, Karnataka, India.. Melting points were determined in an electrically heated apparatus by taking the sample in a glass capillary sealed at one end. The FT-IR spectra of the compounds were determined by using of a Shimadzu Fourier Transform Infrared (FT-IR) spectrometer.

Synthesis of 5-Nitro-1, 3 Benzoxazole- 2-tiole (2)

2 Amino 5-nitro phenol was used as starting material for synthesis of 5-Nitro-1, 3 Benzoxazole- 2- tiole. Mixture of 2-Amino 5-nitro phenol **1** (0.01 mol) and carbon disulfide (0.01 mol) in presence of potassium hydroxide, the mixture was refluxed up to 6 hrs in 30 ml by using methanol as a solvent. The mixture poured onto crushed ice and acidify with acetic acid the solid separated out was filtered and recrystallized from ethanol. It was monitored by TLC to identify the completion of reaction. Yellow; Yield: (92 %), m.p. 196-198 C$^{0;}$ IR (KBr, cm^{-1}): 3386 cm^{-1} (v -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 =196.21.

Synthesis of 2-(chloromethyl)-1*H*-benzimidazole (4)

Chloro acetic acid (0.01 mol) was dissolved in 40 mL of 4N hydrochloric acid and stirred the mixture for nearly 20 min. o-Phenalinediamine **3** (0.01 mol) was add to above reaction mixture with constant stirring and continued stirring with reflex for nearly 4 hrs. The product was confirmed by TLC, Poured the above hot solution to ice cold water with stirring and ammonia solution was added drop wise. The yellow precipitate obtained was filtered. The precipitate was then recrystallized from the methanol, Yellow solid: Yield (84%); m.p. 194 C^{0} colour: pale brown; IR (KBr, cm^{-1}): 3350 cm^{-1} (v N-H) 2862 cm^{-1} (v –CH$_2$); ^{1}H NMR (DMSO-d$_6$, δ ppm):

11.668 (s, H -NH) 7.658-7.208 (m, 4H-Ar-H), 4.565 (s, H-CH_2); MS: m/z = 167.21

Synthesis of 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-5-nitro-1,3-benzoxazole (5)

A mixture of 5-Nitro-1, 3 Benzoxazole- 2- tiole (**2**) and 2-(chloromethyl)-1*H*-benzimidazole (**4**) in dry ethanol by adding catalytic amount of potassium hydroxide was refluxed on water bath for about 8 hrs, the reaction mixture was poured on ice we get solid product and filtered off and recrystallized from ethanol to get compound 5. Yield: (92 %), m.p. 196-198 C^0 ; IR (KBr, cm^{-1}): 3300 cm^{-1} (v N-H) ^{1}H NMR (DMSO-d_6, δ ppm): 11.508 (s, H -NH), 8.649-7.852 (m, 7H-Ar-H), 4.955 (s, H-CH_2) ^{13}C NMR (DMSO-d_6, δ ppm): 165.12, 150.92, 147.62, 144.32, 141.53, 138.15, 138.13, 123.42, 123.38, 120.12, 120.10, 115.20, 115.18, 107.25, 40.25; MS: m/z =326.92

Synthesis of benzoxazole derivatives 6 (a-f)

The compound **5** was treated with various reagents in presence of suitable solvent to get target 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-5-nitro-1,3-benzoxazole derivatives **6 (a-f)**

Synthesis of 2-{[(1-acetyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole (6a)

The mixture of compound 5 (0.01 mol) and acetyl chloride (0.01 mol) was stirred with 40 ml of Acetone in the presence of potassium hydroxide (0.01 mol) as a catalyst and reaction mass was refluxed up to 8 hrs. Then the reaction mixture was poured onto crushed ice. Solid precipitation of product thus obtained was filtered, dried and recrystallized from ethanol to get compound 6a.

Colour: pale yellow; Yield: (82 %) ; colour : yellow; m.p. 198-200 C^0; IR (KBr, cm^{-1}): 1652 cm^{-1} (v C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 8.449-7.652 (m, 7H-Ar-H), 4.655 (s, H-CH_2), 2.252 (s, H-CH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 166.12, 150.62, 147.22, 144.42, 141.43, 138.05, 131.25 123.22, 123.20, 120.20, 120.18, 115.30, 115.28, 107.65, 40.351, 15.25; Elemental analysis (%) found (Calculated) for $C_{18}H_{14}ClN_5O_3S_2$ C – 55.43 (55.48), H - 3.28 (3.25), N -15.21 (15.25); MS: m/z =368.36.

Synthesis of 2-{[(1-ethyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole (6b)

The compound 5 (0.01 mol) in 40 ml of DMSO with iodoethane (1.2 eq) in presence of sodium hydroxide (0.01 mol) as a base and reaction mixture was refluxed for 6 hrs and cooled. The solid product separated was filtered, washed with DM water, dried and recrystallized from ethanol to get the compound 6b**.**

Colour: orange ; Yield: (80 %) ; colour : pale brown ; m.p. 166-168 C^0; IR (KBr, cm^{-1}): 2912 cm^{-1} (v -CH_3); ^{1}H NMR (DMSO-d_6, δ ppm): 7.852-7.305 (m, 7H-Ar-H), 4.265~4.215 (q, H-CH_2), 1.372~1.356 (t, H-CH_3); ^{13}C NMR (DMSO-d_6, δ ppm): 165.02, 152.62, 146.36, 144.60, 141.56, 138.07, 134.07 124.62, 124.30, 120.04, 120.02, 114.03, 114.02, 106.34, 55.40, 43.43, 15.38; Elemental analysis (%) found (Calculated) for $C_{17}H_{14}N_4O_3S$ C – 57.62 (57.58), H – 3.98 (3.94), N -15.81 (15.78); MS: m/z =354.38.

Synthesis of 2-({[1-(chloroacetyl)-1*H*-benzimidazol-2-yl]methyl}sulfanyl)-6-nitro-1,3-benzoxazole (6c)

The compound 5 (0.01 mol) was refluxed for 6 hrs in 30 ml of acetone with chloro acetyl chloride (1.2 eq) and pinch of base potassium carbonate as a catalyst. Then the reaction mass was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol to get final compound 6c Yield: (84 %) ; colour : pale yellow; m.p. 202-204 C^0; IR (KBr, cm^{-1}): 1752 cm^{-1} (v C=O) 2862 cm^{-1} (v –CH_2); ^{1}H NMR (DMSO-d_6, δ ppm): 7.958-7.308 (m, 7H-Ar-H), 4.565 (s, H-CH_2), 4.265 (s, H-CH_2); ^{13}C NMR (DMSO-d_6, δ ppm): 190.36 165.22, 152.32, 146.76, 144.56, 141.63, 138.27, 132.27, 123.22, 123.20, 120.07, 120.01, 115.04, 115.02, 105.24, 54.30, 42.24 ; Elemental analysis (%) found (Calculated) for $C_{17}H_{11}ClN_4O_4S$ C – 50.69 (50.70), H – 2.75 (2.78), N -13.91 (13.86); MS: m/z = 403.08. (M+), 405.23 (M+2)

Synthesis of (2-{[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]methyl}-1*H*-benzimidazol-1-yl)acetic acid (6d)

The mixture of chloroacetic acid (1.1eq) and compound 5 (0.01 mol) was stirred with 20 ml of DMF in the presence of potassium carbonate (1 eq) as a catalyst for fifteen minute and refluxed for 8 hrs. Then the reaction mixture was poured onto crushed ice. Solid product thus afforded was

filtered, dried and recrystallized from ethanol to get compound 6d.

Yield: (78 %); colour : pale brown; m.p. 192-194 C^0; IR (KBr, cm^{-1}): 1720 cm^{-1} (C=O), 3522 cm^{-1} (C-OH); ^1H NMR (DMSO-d_6, δ ppm): 11.25 (s, H-COOH) 12.865 (s, H-COOH), 8.156-7.307 (m, 7H-Ar-H), 4.865 (s, H-CH_2), 4.265 (s, H-CH_2); ^{13}C NMR (DMSO-d_6, δ ppm): 175.36, 165.22, 152.32, 146.46, 144.66, 141.53, 138.27, 134.37, 124.42, 124.34, 120.07, 120.04, 114.04, 114.02, 106.24, 55.30, 43.53; Elemental analysis (%) found (Calculated) for $C_{18}H_{14}ClN_5O_3S_2$ C – 53.12 (53.00), H – 3.15 (3.10), N -14.58 (14.60); MS: m/z =384.36

Synthesis of 1-(2-{[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]methyl}-1*H*-benzimidazol-1-yl)propan-2-one (6e)

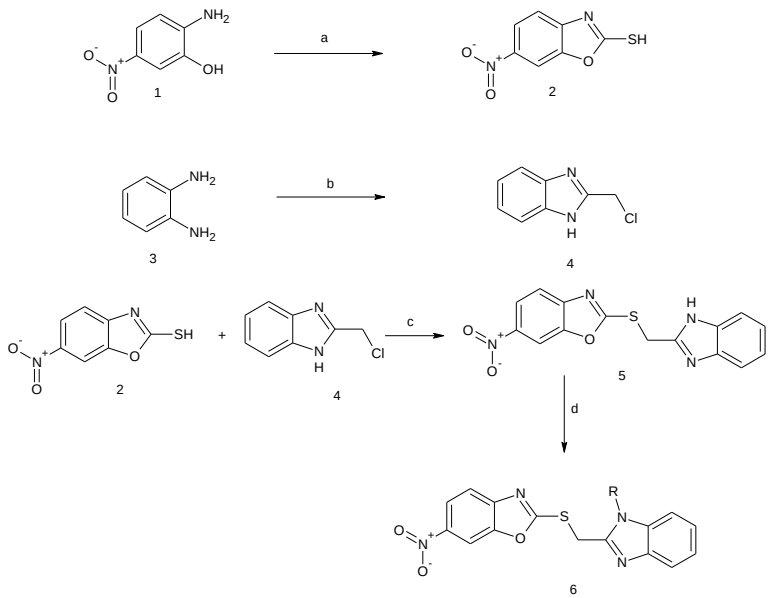
The mixture of compound 5 (0.01 mol) and chloroacetone (0.01 mol) was stirred with 30 ml of Acetone in the presence of potassium carbonate (0.01 mol) as a catalyst and reaction mixture was refluxed up to 6 hrs. Then the reaction mass was poured onto crushed ice. Solid product separates out thus obtained was filtered, dried and recrystallized from ethanol to get compound 6e.

Yield: (80 %); colour : yellow; m.p. 210-212 C^0; IR (KBr, cm^{-1}): 1702 cm^{-1} (ν C=O) 2912 cm^{-1} (ν – CH_3); ^1H NMR (DMSO-d_6, δ ppm): 7.938-7.310 (m, 7H-Ar-H), 4.765 (s, H-CH_2), 4.255 (s, H-CH_2) 1.372 (s, H-CH_3);^{13}C NMR (DMSO-d_6, δ ppm): 206.35 164.12, 150.62, 147.22, 144.42, 141.43, 138.05, 134.25 123.22, 123.20, 120.20, 120.18, 115.30, 115.28, 107.65, , 52.24, 40.31 15.23; Elemental analysis (%) found (Calculated) for $C_{18}H_{14}ClN_5O_3S_2$ C – 56.54 (56.50), H – 3.69 (3.70), N -14.65 (14.62); MS: m/z =382.39.

Synthesis of 2-({[1-(2-bromoethyl)-1*H*-benzimidazol-2-yl]methyl}sulfanyl)-6-nitro-1,3-benzoxazole (6f)

The compound 5 (0.01 mol) in 30 ml of DMSO with dibromoethane (1.2 eq) in presence of sodium hydroxide (0.01 mol) as a base and reaction mixture was refluxed for 6 hrs and cooled. The solid separated was filtered, washed with water, dried and recrystallized from methanol to get the desired compound 6f**.**

Yield: (80 %); colour : pale orange; m.p. 196-198 C^0; IR (KBr, cm^{-1}): 787 cm^{-1} (C-Br); ^1H NMR (DMSO-d_6, δ ppm): 8.449-7.652 (m, 7H-Ar-H), 4.355 (s, 2H-CH_2), 4.255 (t, 2H-CH_2) 3.553 (t, 2H-CH_2); ^{13}C NMR (DMSO-d_6, δ ppm): 165.12, 150.42, 147.02, 144.45, 141.63, 138.95, 134.35 123.08, 123.04, 120.10, 120.52, 115.32, 115.26, 106.65, , 55.24, 40.21, 32.56; Elemental analysis (%) found (Calculated) for $C_{18}H_{14}ClN_5O_3S_2$ C – 47.12 (47.08), H – 3.02 (3.00), N -12.93 (12.90); MS: m/z =434.40 (M+), 436.40 (M+2).



Scheme 1 : Synthetic route for the synthesis of compounds $6_{(a-f)}$. (a). CS_2, KOH, MeOH. (b) $ClCH_2COOH$ 4N HCl. (c) KOH, EtOH, (d) R, Acetone/ DMF, K_2CO_3.

R: 6a: $ClCOCH_3$; 6b: C_2H_5I; 6c: $ClCH_2COCl$; 6d: $CH_2ClCOOH$; 6e: $ClCH_2COCH_3$; 6f: $C_2H_4Br_2$

Evaluations of biological activities

Antibacterial screening

The synthesized compounds were screened for *in vitro* growth inhibitory activities against a panel of standard strains of pathogenic microorganisms by agar well diffusion method including two types of bacterias three Gram-positive bacteria, and other three Gram-negative bacteria namely *staphylococcus aureus, staphylococcus epidemidis* and *bacillus cereus* and gram negative bacteria namely *pseudomonas aeruginosa, vibrio cholerae* and *escherichia coli*. The test compounds **6(a-f)** 20 µg /mL in 10% DMSO, antibacterial activity were evaluated by comparing with the standard drug (Tetracycline, 100 µg/mL of sterile distilled water) and control (10% DMSO) were suspended to respective labeled wells. The plates are 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 [24]. During this period, the test solution diffused and zone of inhibition were recorded using vernier calipers.

Antifungal screening.

The newly synthesized compounds were evaluated against two fungal strains, *Aspergillus aureus* and *Aspergillus fumigates*, using the sabouraud dextrose agar diffusion method [25]. Wells were made (6mm diameter) with a sterile cork borer. The antifungal activity were evaluated by comparing with standard drug used (Fluconazole, 100 µg/mL of sterile distilled water) and control (10% DMSO) was added to respectively labeled wells. The test solution compounds of (100 µL (20 mg/mL in 10% DMSO) 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 hours. At the end of the incubation period, the diameter of the zone of inhibition around the wells was measured using vernier caliper.

Minimum Inhibitory Concentration (MIC)

The MIC of all the synthesized compounds **6**$_{(a-f)}$ was determined by micro dilution method [26]. The respective clinical antimicrobial strain was spread separately on the medium. The Wells were made (6mm diameter) with a sterile cork borer under safe aseptic conditions. The synthesized compounds at different concentrations (25, 50, and 100 μg/mL), were loaded into respective labeled wells. The drugs *Tetracycline* and *Fluconazole* were used as standard for the comparison of antibacterial activities, respectively. The results were measured in mm and presented in **Table-4 and Table-5**.

Free-radical-scavenging activity using the DPPH method

The synthesized compounds were evaluated for antioxidant scavenging activity by DPPH assay method as per literature [23]. The compounds of different concentrations were dissolved in methanol and were added to each vial of 5mL. To this vials 3 mL of 0.004% DPPH in methanol was added and the mixtures have been incubated in dark condition at room temperature for 30 min. Ascorbic acid was used as the standard. The absorbance reduced while the DPPH is scavenged by way of an antioxidant. DPPH scavenging activity calculated by the use of the following equation and absorbance measured at 517 nm.

$$\textbf{Scavenging ratio (\%)} = [(Ai - Ao) / (Ac - Ao)] \times 100\%$$

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.

RESULTS AND DISCUSSION

Chemistry

The general synthetic strategy employed to afford the title compounds in good yield was depicted in Scheme 1. The compounds were synthesized by starting material nitro substituted 2-aminophenol (**1**), it was treated with carbon disulphide and potassium hydroxide in the presence of methanol solvent to produce substituted 1,3-benzoxazole-2-thiol (**2**) compounds. And further reaction was carried with 2-(chloromethyl)-1*H*-benzimidazole **(4)** in ethanol and potassium hydroxide added in catalytic amount afforded the desired product 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-5-nitro-1,3-benzoxazole.(**5)** The compound **5** was Characterized by IR, the spectrum showed absorption at 3300 cm^{-1} which was due to the N-H stretching and the absorption band appeared at 2865 cm^{-1} which was due to the -CH_2

stretching which confirmed the structure of the compound. The ^{1}H NMR spectrum of compound **5** displayed a singlet at δ 2.955 which was due to three -CH_2 protons, a multiplet at δ 7.235-7.829 were due to aromatic protons. A singlet at δ 11.508 appeared due to -NH protons confirmed the formation of compound **5**. The mass spectrum of **5** showed a molecular ion peak at m/z = 326.925 which was in agreement with the molecular formula $C_{15}H_{10}N_4O_3S$. The intermediate benzoxazole compound 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-6-nitro-1,3-benzoxazole **5** was condensed with acetyl chloride to produce target compound 2-{[(1-acetyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole **6a**.

The compound **6a** 2-{[(1-acetyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole was characterized by IR the spectrum exhibited strong absorbance band at 2821 cm^{-1} which was due to the -CH_2 stretching. The band appeared at 1712 cm^{-1} was due to -C=O stretching and absorption at 1656 cm^{-1} which was due to the -CH_3 stretching confirmed the structure of the compound. The ^{1}H NMR spectrum of compound **6a** 2-{[(1-acetyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole displayed a singlet at δ 1.372 which was due to -CH_3 protons. A singlet at δ 4.265 was due to C-H_2 protons, a multiplet at δ 7.309-7.856 were due to sexate aromatic protons; it confirmed the formation of desired product. The mass spectrum of 6a showed a molecular ion peak at m/z = 368.36 which was concurrence with molecular weights of targeted molecules $C_{17}H_{12}N_4O_4S$.

Table 1: Physical data of compounds 6 (a-f)

Compound	R	Molecular formula	Molecular weight	M.P. (^{0} c)	% of Yield	Found (Calculated) %		
						C	H	N
6a	$ClCOCH_3$	$C_{17}H_{12}N_4O_4S$	368.36	200	82	55.43 (50.44)	3.28 (3.25)	15.12 (15.14)
6b	C_2H_5I	$C_{17}H_{14}N_4O_3S$	354.38	168	80	57.62 (57.56)	3.98 (3.94)	15.81 (15.84)
6c	$ClCH_2COCl$	$C_{17}H_{11}ClN_4O_4S$	402.81	204	84	50.69 (50.64)	2.75 (2.72)	13.91 (13.90)
6d	$CH_2ClCOOH$	$C_{17}H_{12}N_4O_5S$	384.36	194	78	53.12 (53.10)	3.15 (3.16)	14.12 (14.08)
6e	$ClCH_2COCH_3$	$C_{18}H_{14}N_4O_4S$	382.39	212	80	56.54 (56.50)	3.69 (3.70)	14.65 (14.63)
6f	$C_2H_4Br_2$	$C_{17}H_{13}BrN_5O_3S$	433.27	198	80	47.12 (47.10)	3.02 (3.01)	12.93 (12.94)

Pharmacology studies

Some microorganisms have become resistant to conventional antibiotics were often Abused/overused against microbial pathogens. So, currently various classes of antibiotic compounds were being synthesized and used in the treatment and prevention of microbial infections. The benzoxazole moiety was a versatile lead molecule in the pharmaceutical drug development and has a broad range of biological activities such as antibacterial and antifungal activity.

In vitro antibacterial and antifungal activity

The antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the used standard reference. The observed inhibition zones are presented in **Table 2** and **Table 3**. As a result of this, the primary screening against the bacterial strains showed good zone of inhibition as shown in **fig 2** and **fig 3.** It was noticed that the presence of electron withdrawing halogens attached to the benzimidazole ring displayed strong effect on the antimicrobial activity for example the presence of bromo and chloro substituent respectively in the structure, which was responsible for the enhanced activity of the compounds of (**6c and 6e**) showed the potent antimicrobial activity among all the tested compounds of this series. While electron-donating group such as methoxy group has a moderate effect on antimicrobial

activity. The compound **6b, 6c** and **6e** exhibited highly significant antibacterial activity against gram negative bacteria *P aeruginosa*, *V cholerae* and *E coli*. The compounds **6a** and **6c** displayed good antibacterial activity towards gram positive bacteria, *S aureus*, *staphylococcus epidemidis* and *Bacillus subtilis* respectively. All the tested compounds inhibited the spore germination against tested fungi. In general, most of the compounds showed slightly higher antifungal activity towards *A aureus* and *A fumigates*.

The compounds **6c** and **6e** performs highest antifungal activity against *A aureus* and *A fumigates*, the primary screening against the fungal strains showed good zone of inhibition as shown in **fig 3**. The Minimum inhibitory concentration (MIC) of the most active synthesized compounds **6**$_{(a-f)}$ were evaluated *in vitro* using the serial dilution technique. The results of minimum inhibitory concentration were depicted in **table 4** and **table 5**. The MIC study of both synthesized compounds against bacterial and fungal strains at different concentrations i.e., 25, 50 and 100µg/mL was evaluated. The MIC zones of inhibition for antimicrobial activity of the compounds **6**$_{(a-f)}$ were reported in **fig 4 and fig 5** which was evident for the inhibition of bacterial and fungal strains. The **6a** and **6c** compounds showed potential MIC values against bacterial and fungal strains respectively.

Table 2: *In vitro* antibacterial activities of the target compounds of series 6 $_{(a-f)}$

	Zone of inhibition in mm					
Compounds	***S. aureus***	***S. epidemidis***	***Bacillus cereus***	***P.aeruginosa***	***Vibrio cholerae***	***E. coli***
6a	16±0.01	21±0.02	14±0.14	22±0.10	18±0.14	16±0.10
6b	18±0.12	20±0.06	18±0.02	14±0.08	20±0.04	18±0.04
6c	23±0.05	27±0.04	22±0.06	23±0.05	25±0.02	22±0.02
6d	16±0.03	18±0.10	16±0.01	17±0.03	14±0.08	16±0.08
6e	23±0.11	27±0.04	22±0.04	23±0.09	24±0.06	22±0.04
6f	16±0.02	18±0.14	14±0.06	15±0.02	18±0.10	16±0.05
DMSO	-	-	-	-	-	-
Std	25	30	24	25	27	25

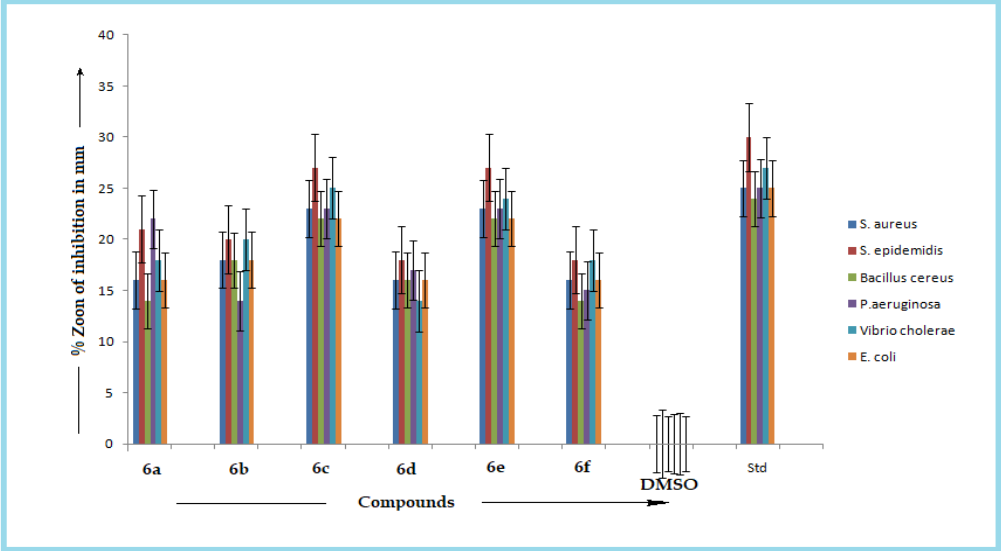


Fig. 2: Antibacterial activity of compound 6$_{(a-f)}$

Table 3: *In vitro* Antifungal Activity of compounds 6(a-f)

Compounds	Zone of inhibition in mm	
	A. aureus	*A.fumigatus*
6a	17±0.02	20±0.02
6b	16±0.04	19±0.04
6c	23±0.10	26±0.06
6d	16±0.08	19±0.09
6e	23±0.03	26±0.10
6f	18±0.05	19±0.02
DMSO	-	-
Std* *(Fluconazole)*	25	30

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

Table 4: MIC data of Antibacterial activity of synthesized compounds 6(a-f)

Compound	Concentration in *µg/ml*	Growth inhibition against *bacteria in mm*					
		P.aeruginosa	***S.aureus***	***V.cholerae***	***S.epidermidis***	***B.subtilis***	***E.coli***
6a	25	14.25±0.12	15.25±0.24	16.47±0.26	15.22±0.25	13.47±0.20	14.89±0.45
	50	18.25±0.12	18.20±0.07	19.24±0.31	17.28±0.26	16.56±0.05	17.24±0.03
	100	21.25±0.12	23.25±0.11	22.56±0.31	19.25±0.20	21.25±0.00	19.51±0.25
6b	25	14.10±0.12	13.45±0.24	14.41±0.26	13.15±0.25	14.48±0.20	13.78±0.45
	50	16.25±0.12	15.98±0.07	17.45±0.31	16.24±0.26	15.85±0.05	14.88±0.03
	100	20.21±0.12	18.55±0.11	19.23±0.31	20.15±0.20	19.56±0.00	19.47±0.25
6c	25	16.56±0.21	15.42±0.05	17.01±0.20	16.85±0.15	17.45±0.17	15.66±0.17
	50	19.25±0.20	19.45±0.07	18.24±0.03	18.34±0.30	21.45±0.00	15.24±0.16
	100	23.14±0.21	25.45±0.32	26.28±0.21	23.98±0.33	27.45±0.00	24.56±0.07
6d	25	11.25±0.20	10.25±0.13	13.24±0.06	10.89±0.34	11.89±0.24	12.36±0.12
	50	14.25±0.13	15.24±0.18	14.85±0.16	13.49±0.14	14.85±0.17	13.44±0.23
	100	18.47±0.10	20.12±0.57	21.56±0.16	20.78±0.23	23.47±0.14	21.85±0.18
6e	25	14.35±0.15	15.89±0.15	16.24±0.06	15.89±0.02	14.76±0.04	16.42±0.11
	50	18.25±0.15	19.56±0.56	18.44±0.04	19.23±0.04	18.55±0.06	18.36±0.20
	100	22.26±0.09	26.23±0.15	26.87±0.27	23.14±0.07	28.26±0.19	24.89±0.02
6f	25	13.25±0.20	16.70±0.20	15.78±0.05	14.99±0.19	13.28±0.05	12.98±0.33
	50	16.21±0.15	19.43±0.21	17.52±0.02	18.56±0.06	16.23±0.21	18.24±0.04
	100	19.23±0.12	21.56±0.09	22.12±0.08	20.56±0.04	22.89±0.02	20.65±0.03
Control	100	0	0	0	0	0	0
Std *(Tetracycline)*	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*

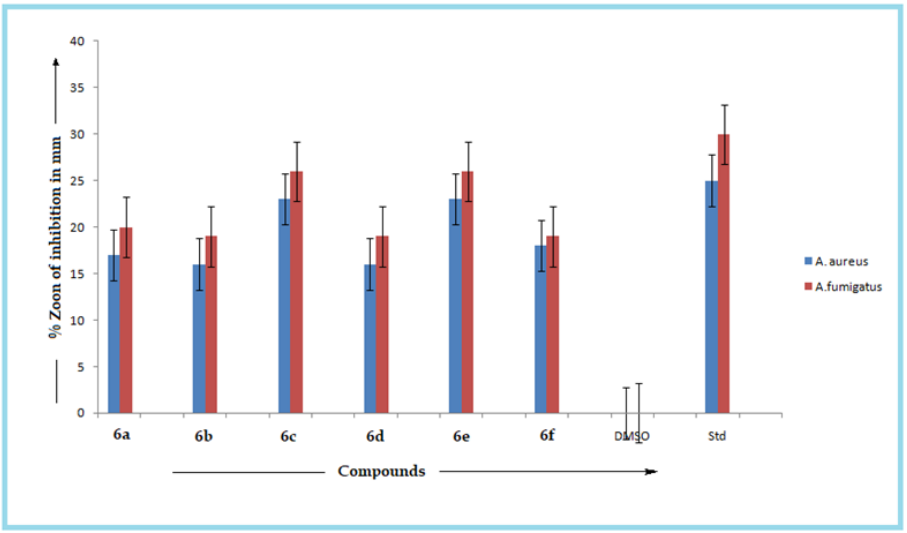


Fig. 3: Antifungal activity of compound 6(a-f)

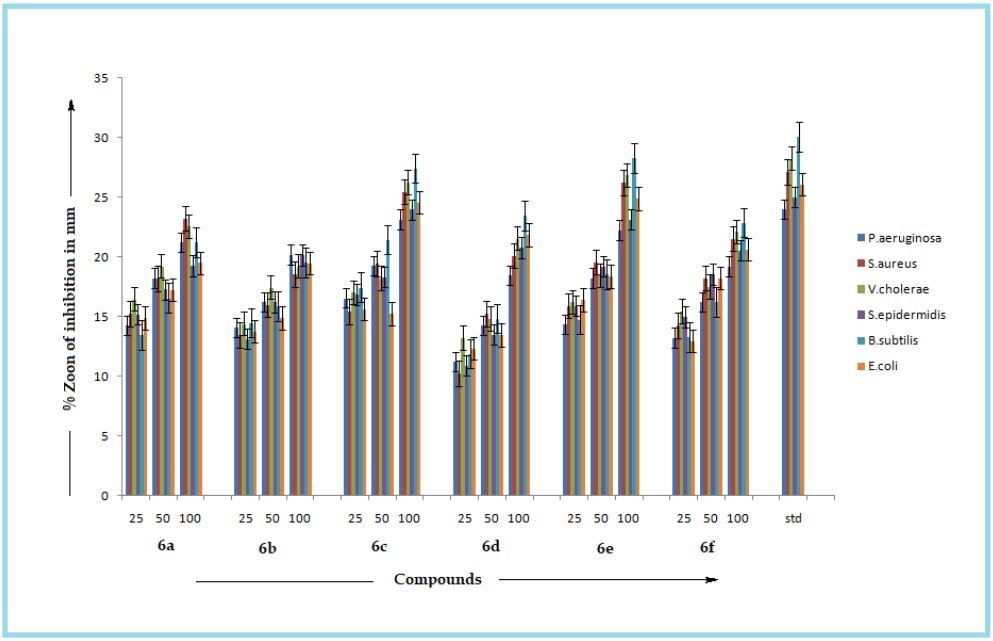


Fig 4: MIC data of Antibacterial activity of compound 6(a-f)

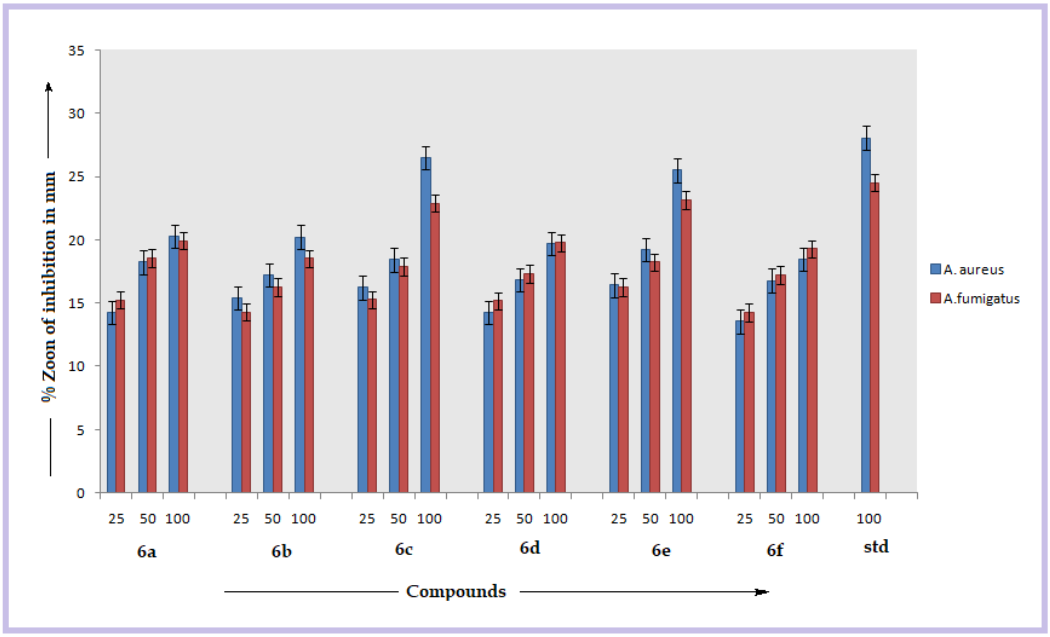


Fig. 5: MIC data of Antifungal activity of compound 6(a-f)

Table 5: MIC data of Antifungal activity of synthesized compounds 6(a-f)

Compound	Concentration in *µg/ml*	Growth inhibition against *fungicides in mm*	
		A. aureus	***A.fumigatus***
6a	25	14.25±0.25	15.24±0.20
	50	18.23±0.31	18.56±0.20
	100	20.27±0.21	29.92±0.23
6b	25	15.41±0.17	14.29±0.15
	50	17.24±0.20	16.27±0.15
	100	20.22±0.33	18.51±0.10
6c	25	16.22±0.16	15.29±0.10
	50	18.44±0.14	17.89±0.07
	100	26.49±0.02	22.89±0.09
6d	25	14.25±0.17	15.19±0.14
	50	16.82±0.20	17.33±0.25
	100	19.72±0.23	19.77±0.15
6e	25	16.42±0.19	16.27±0.09
	50	19.24±0.25	18.24±0.05
	100	25.49±0.10	23.14±0.01
6f	25	13.54±0.01	14.25±0.18
	50	16.78±0.22	17.22±0.10
	100	18.45±0.11	19.27±0.01
Control	100	0	0
Std *(Fluconazole)*	100	28.05±0.11	24.52±0.15

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

Free-radical-scavenging activity using the DPPH method.

The DPPH radical scavenging activity data was dipicted in **table 6** and **fig 6**. 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 displayed potent activity but lower when compared to standard ascorbic acid (vitamin C) as standard. The compound **6a** and **6b** showed potent scavenging activity almost close to the standard Vitamin-C and **6e** compound showed better inhibitions activity against free radical and other synthesized compounds showed moderate activity.

Table 6: Scavenging activity of Benzoxazole derivatives 6 (a-f)

Concentration in mL	6a	6b	6c	6d	6e	6f
0	-	-	-	-	-	-
5	20±0.01	22±0.21	21±0.05	14±0.04	18±0.01	16±0.01
10	23±0.05	26±0.24	22±0.21	16±0.02	22±0.04	18±0.08
15	28±0.07	30±0.20	26±0.21	20±0.04	24±0.03	20±0.04
20	31±0.06	33±0.09	32±0.22	24±0.07	28±0.07	23±0.07
25	34±0.01	36±0.01	34±0.07	26±0.21	32±0.04	26±0.01
Ascarbic acid	38±0.08	40±0.21	39±0.04	30±0.25	34±0.08	28±0.02

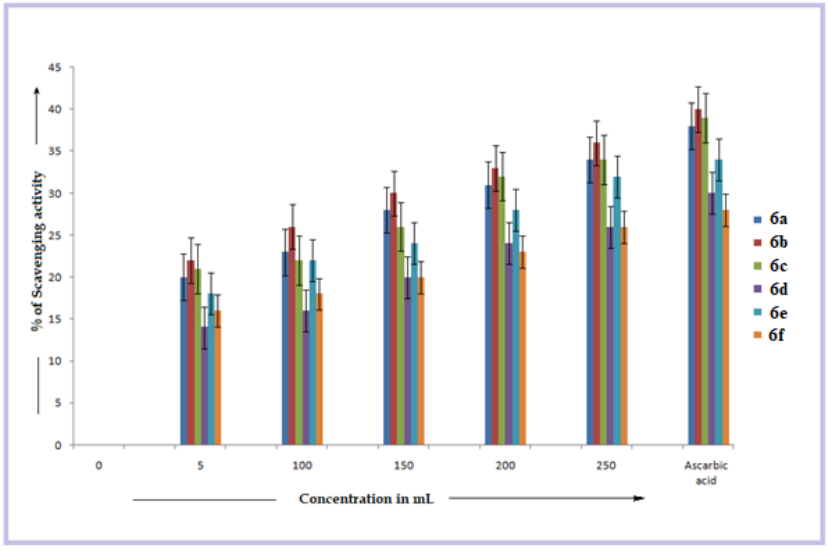


Fig.6: **Free-radical-scavenging activity.**

CONCLUSION

In this present paper we have described simple and efficient protocol for the preparation of 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-5-nitro-1,3-benzoxazole derivatives with good yields. All the newly synthesized molecules were characterized by IR, ^{1}H NMR ^{13}C NMR and mass spectral analysis. The synthesized compounds have been screened for their *in-vitro* antibacterial, MIC and antioxidant activity were evaluated. Nitro substituted benzoxazole derivatives exhibited promising Compound **6a, 6b, 6c, 6d, 6e,** and **6f** were exhibited effective *in vitro* antibacterial, MIC with antioxidant activity with effective results were observed. By considering effective biological activity, benzoxazole moiety was a potent medicinal value molecule in-vitro studies of these compounds evidenced that, the chloro and bromo group in the compound that enhances the antimicrobial as well as antioxidant activities, which might serve as new templates in the synthesis and development of significant therapeutics. Therefore, it can be concluded that such

compounds exert their pharmacological effects. This has resulted good impact on chemists for further investigations in the field of medicinal chemistry.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

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