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Research Article

SYNTHESIS OF 1,3,4-OXADIAZOLE INCORPORATED AZO DYE DERIVATIVES AS A POTENT BIOLOGICAL ACTIVITY MOLECULES

SHRIDHAR A.H[1], J.KESHAVAYYA[1,*] AND JOY H. HOSKERI[2]

[1]Department of Studies and Research in Chemistry, School of Chemical Sciences, Kuvempu University, Jnana Sahyadri Shankaraghatta 577451, India, [2]Department of Biochemistry, Kuvempu University, Jnana Sahyadri, Shankaraghatta, 577451 Karnataka, India. Email: jkeshavyya@gmail.com.

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ABSTRACT

In present study 5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol)azo dye were synthesized by multistep reaction sequences, Structure of newly synthesized compounds characterized and confirmed by IR, NMR and Mass spectral studies. The synthesized compounds were screened for their antimicrobial and *in vitro* antioxidant properties. Synthesized compounds were found to be potent antibacterial and antioxidant agents. All the synthesized compounds exhibit significant biological activity and are certainly hold greater promise for discovering safer biologically active molecules.

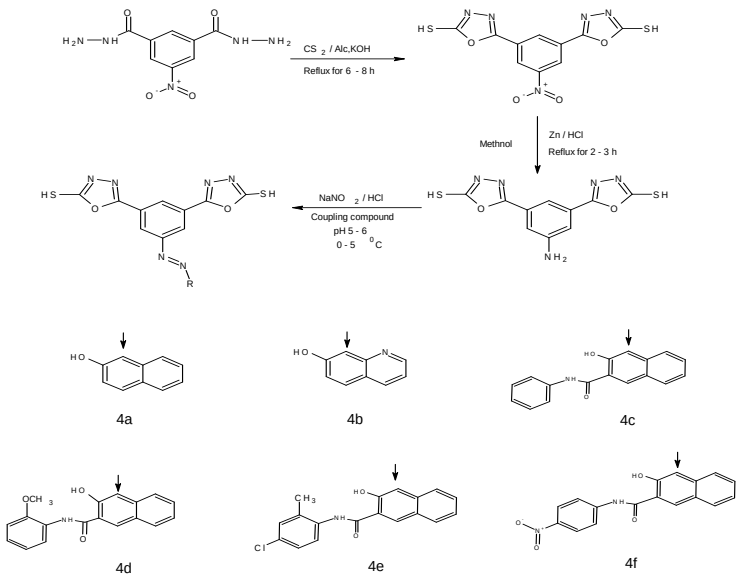
Keywords: Bis (1,3,4-oxadiazole)azo dye, 8-hydroxy quinoline, Anti-microbial activity, *In-vit*ro antioxidant activity.

INTRODUCTION

In recent fungal infections have become an important complication and a major cause of morbidity and mortality in immune compromised individuals suffering from tuberculosis, cancer, or AIDS. In organ transplant cases, free radicals generates due stress leads to the cell damage in living beings. The growing incident of bacterial resistant to existingantibiotic pocess a serious medical problem in treating pathogenic infection [**1**]. It has been known that the activity of azo linkage increases with the incorporation of suitable heterocyclic moiety and are well known for their medicinal importance, for their usage as antineoplastics [**2**], antidiabetics [**3**], antiseptics [**4**], antiseptics, anti-inflammatory, and other useful chemotherapeutic agents [**5-6**], antibacterial activities [**7-9**]. Azo dyes are used as hypnotic drugs for the nervous system, in detecting cancer as chemotherapeutic agents and are involved in the structure of nucleic acids in living cells [**10**]. Azo dyes are also known to be involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and nitrogen fixation [**11-12**]. Evans blue and congo red are being studied as HIV inhibitors of viral replications. This effect is believed to be caused by binding of azo dyes to both protease and reverse transcriptase of virus [**13**].

1,3,4-oxadiazoles are of significant interest in synthetic and medicinal chemistry due to its wide range of biological activities such as anti fungal [**14**] antimicrobial [**15- 16**], anti-inflammatory and analgesic [**17-18**], hypolipidemic [**19**], anti tubercular [**20-21**], anti-convulsant [**22-23**], cytotoxicity [**24**]. Some oxadiazole were used in high technological biological studies, [**25-30**] like antimicrobial, anti-tubercular, anti-inflammatory, anticonvulsant [**31**], hypnoti, anesthetic activity[**32**].1,3,4-oxadiazoles showed antibacterial properties similar to those of well known sulfonamide drugs. The oxadiazole nucleus with N=C-S linkage exhibits a large number of pharmacological activities [**33**].

As less reports on biological activity of azo dye moiety, in view of the above mentioned findings and our previous reports, [**34 - 37**] in the present study we made an efficient attempt to synthesize azo dyes derivative containing 1,3,4-oxadiazole, possessing a potent biological activity. Structures of the synthesized compounds were confirmed by ^{1}H NMR, IR and Mass spectral studies and compounds were screened for their antimicrobial and *in vitro*- antioxidant property.



Scheme 1: synthesis of 5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dyes

MATERIALS AND METHODS

All analytical grade chemicals were used directly as purchased. Melting point of the synthesized compounds was determined in scientific melting point apparatus and uncorrected. The progress of reaction was monitored by TLC using silica gel coated plates (0.5 mm thickness, Merck) and spots were visualized under UV radiation. Synthesized compounds were recrystalized using suitable solvent system. Infra-Red spectra were recorded on Perkin Elmer-spectrum RX-1model spectrophotometer using KBr pellets. NMR spectra were recorded by Bruker DRX 400MHz spectrometer and acquired on a Bruker Avance-2 model spectrophotometer using DMSO as a solvent and TMS as an internal reference. The crude compounds were purified by recrystalization method.

Synthesis of aryl 3-nitroiso-phthalic acid dihydrazide (1)

The 3-nitroiso-phthalic acid dihydrazide (**1**) was synthesized by adopting reported method. 0.2 mmol of Hydrazine hydrate was added drop-wise to the solution of iso-phthalic ester (0.1 mmol) in dried ethanol (30 mL) with vigorous stirring. The resulting mixture was refluxed for 4-6 h. The progress of the reaction was monitored by TLC with the solvent system petroleum ether: ethyl acetate (1:1) as the mobile phase and visualized in under UV light. Filtered the yellow solid formed after the distillation of excess ethanol followed by cooling to room temperature. Washed with brine and dried.

Synthesis of 5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) (2):

5-nitroiso-phthalic dihydrazide (1 mmol) was added to a mixture of alcoholic KOH (3 mmol, 60mL) in a RB flask and Carbon disulphide (6 mmol) was added drop wise. Reflux the contents for about 10 - 12hrs. The progress of the reaction was monitored by TLC using silica gel plates, petroleum ether: ethyl acetate (7:3) as the eluting system and visualized in UV light. The reaction mixture was cooled to room temperature. Poured to ice-cold water and neutralized by using dilute HCl. The crude product obtained was filtered, washed with water and dried and recrystalized from the ethanol to get a pure product.

General procedure for the synthesis of 5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) aniline (3)

A suspension of nitro compound (1 mmol), $SnCl_2 2H_2O$ (5 mmol) were dissolved in 0.02 M methanolic HCl solution and refluxed for 3-4 hrs at 70-80 ^{0}C under nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate, and washed with aq. $NaHCO_3$ and water. The organic layer was dried over anhydrous sodium sulfate, concentrated, and the recrystalized from methanol.

General procedure for the synthesis of 5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dye (4a-f)

The newly synthesized amine (1 mmol) was taken in a HCl and cooled to 0-5 °C, $NaNO_2$ dissolved in a suitable solvent (1.25 mmol) was added drop wise with constant stirring without allowing the temperature to rise above 10 °C to get a diazonium salt. After complete addition the reaction mixture was adjusted to pH 5-6, coupling compound (1 mmol) was dissolved in a suitable solvent and cooled to 0-5 °C and this solution was added to the above mixture gradually without allowing the temperature rise above 0-5 °C. After complete addition, the reaction mixture was stirred for 1- 2 h for the completion of reaction. The dye obtained was filtered and thoroughly washed with water, dried and recrystalized using methanol that afforded a red coloured dye.

Spectral Data of Synthesized Compound

5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dye (4a)

This dye was isolated as red colour solid with 62% yield, m.p. 218-219 ^{o}C; IR (V max, cm$^{-1)}$ = 3472(br,-OH), 3100 (C-H stretching), 2977 (H-C=O:C-H stretching), 2587 (SH),1567(C=N, stretching), 1434 (C=C ring stretching), 1544 (-N=N-). ^{1}H NMR (DMSO) δ ppm = 14.7(s,1H, -OH), 13.8 (s,2H, SH), 8.63(d, 2H, Ar-H), 8.13 (s, 1H, Ar-H), 7.93(d,1H, Ar-H), 7.62((d,1H.Ar-H), 7.58(d, 1H, Ar-H), 7.32 (t, 1H, Ar-H), 7.23(t, 2H, Ar-H), 7.25(d, 1H, Ar-H). MS *m/z* =449 (M$^+$).Anal. Calcd. For $C_{20}H_{12}N_6O_3$; C,53.56; H,2.70; N,18.74. Found; C,53.14; H,3.16; N,18.42.

5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dye (4b)

This dye was isolated as red colour solid with 54% yield, m.p. 232-233 ^{o}C; IR (V max, cm$^{-1)}$ = 3463(br,-OH), 3100 (C-H stretching), 2897 (H-C=O:C-H stretching), 2546 (SH),1567(C=N, stretching), 1578 (-N=N-). ^{1}H NMR (DMSO) δ ppm = 14.8(s,1H, -OH), 12.9 (s,2H, SH), 8.76 (s, 1H, Ar-H), 8.63(d,1H, Ar-H), 8.24(d, 2H, Ar-H), 7.93((d,1H.Ar-H), 7.86(d, 1H, Ar-H), 7.29 (d, 1H, Ar-H), 7.18(t, 1H, Ar-H). MS *m/z* = 450.46 (M$^+$) Anal. Calcd. For $C_{19}H_{11}N_7O_3$; C,50.77; H,2.47; N,21.81. Found; C,50.43; H,2.86; N,20.96.

5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dye (4c)

This dye was isolated as red colour solid with 67% yield, m.p. 256-257 ^{o}C; IR (V max, cm$^{-1)}$ = 3416(br,-OH), 3100 (C-H stretching), 3198(-NH-), 2977 (H-C=O:C-H stretching), 2587 (SH),1567(C=N, stretching), 1544 (-N=N-),1434 (C=C ring stretching). ^{1}H NMR (DMSO) δ ppm = 14.9(s,1H, -OH), 13.2 (s,2H, SH), 10.8 (s,1H, NH), 8.54 (s, 1H, Ar-H), 8.18(d,2H, Ar-H), 7.96(s, 1H, Ar-H), 7.68((d,2H.Ar-H), 7.62(d, 1H, Ar-H), 7.54 (d, 1H, Ar-H), 7.38 (t, 1H, Ar-H), 7.24 (d, 2H, Ar-H), 7.21(t, 1H, Ar-H),6.94 (t, 1H, Ar-H). MS *m/z* = 568.17(M$^+$). Anal. Calcd. For $C_{27}H_{17}N_7$; C,57.13; H,3.02; N,17.27. Found; C,57.46; H,3.47; N,17.53.

5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dye (4d)

This dye was isolated as red colour solid with 67% yield, m.p. 256-257 ^{o}C; IR (V max, cm$^{-1)}$ = 3472(br,-OH), 3100 (C-H stretching), 3198(-NH-), 2977 (H-C=O:C-H stretching), 2587 (SH),1567(C=N, stretching), 1544 (-N=N-),1434 (C=C ring stretching), ^{1}H NMR (DMSO)δ ppm = 14.5(s,1H, -OH), 13.6 (s,2H, SH), 11.3 (s,1H, NH), 8.59 (s, 1H, Ar-H), 8.23(d,2H, Ar-H), 7.84(s, 1H, Ar-H), 7.62((d,1H.Ar-H), 7.58(d, 1H, Ar-H), 7.38(t,1H, Ar-H), 6.84 (m, 4H, Ar-H), 7.28 (t, 1H, Ar-H),3.83(s,1H,-OCH_3). MS *m/z* = 598.23 (M$^+$) Anal. Calcd. For $C_{28}H_{19}N_7$; C,56.27; H,3.20; N,16.41. Found; C,56.83; H,3.67; N,16.21.

5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dye (4e)

This dye was isolated as red colour solid with 62 % yield, m.p. 247-248 ^{o}C; IR (V max, cm$^{-1)}$ = 3472(br,-OH), 3100 (C-H stretching), 3198(-NH-), 2977 (H-C=O:C-H stretching), 2587 (SH),1567(C=N, stretching), 1544 (-N=N-),1434 (C=C ring stretching). ^{1}H NMR (DMSO) δ ppm = 14.7(s,1H, -OH), 13.7 (s,2H, SH), 11.8 (s,1H, NH),8.51 (s, 1H, Ar-H), 8.14(d,2H, Ar-H), 7.92(s, 1H, Ar-H), 7.68(d, 1H, Ar-H), 7.59 (d, 1H, Ar-H), 7.48 (d, 1H, Ar-H), 7.38 (t, 1H, Ar-H), 7.24 (t, 1H, Ar-H) 7.08 (d, 2H, Ar-H), 0.96 (s, 3H, CH_3). MS *m/z* = 617.62 (M$^+$) Anal. Calcd. For $C_{28}H_{18}N_7$; C,54.59; H,2.94; N,15.91. Found; C,54.87; H,2.36; N,16.13.

5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dye (4f)

This dye was isolated as red colour solid with 57 % yield, m.p. 283-284 ^{o}C; IR (V max, cm$^{-1)}$ = 3472(br,-OH), 3100 (C-H stretching), 3198(-NH-), 2977 (H-C=O:C-H stretching), 2587 (SH),1567(C=N, stretching), 1544 (-N=N-),1434 (C=C ring stretching). ^{1}H NMR (DMSO) δ ppm = 14.2(s,1H, -OH), 13.9 (s,2H, SH), 11.3 (s,1H, NH),8.58 (s, 1H, Ar-H), 8.19(d,2H, Ar-H), 8.13(d,2H, Ar-H), 7.90(d, 3H, Ar-H), 7.62(d, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 7.32 (t, 1H, Ar-H), 7.27 (t, 1H, Ar-H). MS *m/z* = 613.14 (M$^+$) Anal. Calcd. For $C_{27}H_{16}N_8$; C, 54.94; H,2.63; N,18.29. Found; C, 54.51; H, 3.16; N, 18.62.

Determination of Minimal Inhibitory Concentrations (MIC)

The agar dilution susceptibility test was performed based on modified method of NCCLS, 2003 and CLSI, 2009 to determine the MIC of the synthesized compounds. The test compounds **4a-f** was dissolved in sterilized 5% DMSO (400 mg/mL concentration) was

taken as standard stock. A series of two fold dilutions of each compound in the final concentration of 40, 20, 10, 5 and 2.5 mg/mL were prepared in nutrient agar for bacteria and potato dextrose agar for fungi. After solidification, the plates were spotted with 100 µL of overnight grown bacterial cultures approximately containing 1×10^4 CFU/mL. The test was carried out in triplicates. The plates of bacterial culture were incubated at 37°C for 18 – 24 h and fungal cultures were incubated at 24°C for 24-48 h. After incubation, the MIC was determined.

Antimicrobial activity

The antimicrobial activity of newly synthesized compounds **4a-f** was determined by well plate method in nutrient agar (antibacterial activity) [**38**] and Sabouraud dextrose agar (antifungal activity). The in vitro antibacterial activity was carried out against 24 h old cultures of bacterial strains and 72 h old cultures of fungal strains. In this work, *E. coli, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi,* were used to investigate the antibacterial activities and *Pseudomonas Aeruginosa,* Candida albicans, *Candida parapsilosis*, were used to investigate the antifungal activities.

The test compounds were dissolved in dimethyl sulfoxide (DMSO) at concentration of 100 and 50 mg/mL. Approximately 1 cm3 of a 24 h broth culture was placed in sterile Petri dishes. Molten nutrient agar kept at 45 ^{0}C was then poured into the Petri dishes and allowed to solidify. Six millimeter diameter holes were then punched carefully using a sterile cork borer and completely filled with the test solutions. The plates were incubated for 24 h at 37 ^{0}C. The inhibition zone that appeared after 24 h, around the holes in each plate were measured as zone of inhibition in mm. Experiments were duplicated and standard deviation was calculated. The zone of inhibition of antifungal activity was determined using 72 h old broth culture. The results were compared with Fluconazole**.**

In-vitro antioxidant screening

The free radical scavenging activity of synthesized **4a – 4f** were measured by DPPH (2,2-diphenyl 1-picrylhydrazyl) using the method proposed by Brand-williams et al [**39**]. The reaction mixture of the synthesized compounds viz. **4a – 4f** at different concentrations ranging from 25 to 100 µg aliquots were taken an volume was made up to 3 mL by using methanol, to this 1 mL of 0.1 mM solution of DPPH in methanol was added and kept in the dark for 30 min at room temp, O.D was measured at 517 nm and the inhibition concentration was calculated using formula given below. 3mL of methanol and 1mL of DPPH was used as control.

$$\% \text{ of inhibition} = [(A^0 - A^1)/A^0] \times 100.$$

A^0 = the absorbance of the control at 517nm,

A^1 = the absorbance of the compound 4a – 4g at 517 nm.

RESULTS AND DISCUSSION

Chemistry

As depicted in the **scheme 1.** 1, 3, 4-oxadiazole azo dye derivatives were synthesized by a multi-step reaction sequence. 1,3,4-oxadiazole were prepared by reacting 5-nitro bis iso-phthalic dihydrazide with CS_2 and alcoholic KOH gives bis1,3,4-oxadiazoles in a good yield. 5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) were reacted with Zn / HCl using ethanol as a solvent to convert nitro group to amine. The amine group were diazotized and coupled with 8- hydroxy quinoline to obtain 1,3,4- oxadiazole substituted azo dye **4 (a-f)**. The compounds were recrystalized from Methanol. The purity of the compounds was checked by TLC. Spectral data ^{1}H NMR, IR and Mass spectra of all synthesized compounds were recorded and found in full agreement with the proposed structures. The elemental analysis results were within ± 0.4% of the theoretical values.

The IR spectra of **1** showed absorption peak at 3336.4 cm^{-1} due to hydrazide peak at 1507.1 cm^{-1} due to NO_2 and the peak at 1633.3 cm^{-1} due to C=O absorption. These spectral data of synthesized 5-nitro, iso-phthalic dihydrazide stand in good agreement with those reported in the literature [**40**]. The IR spectrum of the compound **2** showed absorption peak at 1561.4 cm^{-1} due to C=N stretching vibration. The absence of C=O peak at 1633.3 cm^{-1} and absence of $NHNH_2$ at 3336.4 cm^{-1} confirms the formation of oxadiazoles. The ^{1}H NMR spectrum revealed a singlet at δ 13-16 due to -OH protons and δ 6-8 due to aromatic proton. The IR spectrum of compound (**4 a-f)** showed absorption at 3300-3400 cm^{-1}, attributed to –OH, 1600-1700 cm^{-1}, due to N=N, absorption at 670 cm-1 assigned to stretching absorptions of C=N, C-O-C and C-S groups. The IR, ^{1}H NMR and Mass spectral data was found in good agreement with the newly synthesized compounds.

Pharmacology

Azoles exert antifungal activity through inhibition Based on the structure of the active site of oxadiazoles and extensive investigation of the structure-activity relationships (SAR) of azole, it was found that oxadiazole ring, having oxygen, nitrogen and the hydroxyl group were the pharmacophores of antifungal agents [**41**].

Evaluation of minimal inhibitory concentrations (MIC)

The MIC values of all the compounds (**4a-f)** were carried out using concentrations ranging from 2.5 to 20 mg/ mL. Compound **4c** showed significant inhibition at 2.5 mg/mL against *Pseudomonas aureginosa, E. coli and Candida parapsilosis.* While, compound 4a and 4d showed maximum inhibitory activity against *Pseudomonas aureginosa* and *Candida parapsilosis* at MIC 2.5 mg/mL. Lowest MIC was shown by compound **4c.** Compounds **4b, 4c** and **4d** demonstrated efficient MIC when compared to other test compounds. Results of MIC are depicted in **Table 1.**

Table 1: In vitro minimum inhibition concentrations evaluation of test compounds against Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, Escherchia coli, Candida albicans and Candida parapsilosis

Test pathogenic microorganisms	Test Compounds (mg/ml)						
	4a	4b	4c	4d	4e	4f	4g
Staphylococcus aureus	09	08	20	10	05	*	20
Pseudomonas aureginosa	07	25	*	25	10	20	*
Bacillus subtilis	12	20	05	05	05	10	10
Escherchia coli	*	26	10	13	10	20	10
Candida albicans	24	10	10	05	25	05	20
Candida parapsilosis	13	23	*	16	05	05	*

* indicates values more than 40 mg/ml. The value of each constituents consisted of ± S.E.M. of 03 replicates. ND – Not Defined.

Antimicrobial screening

All synthesized compound having heterocyclic system containing bridgehead nitrogen and oxygen atoms possess enhanced antimicrobial activity. Compound **4b** showed significant results in inhibiting *S. aureus and B. subtilis* growth with 14.67 ± 1.86mm and 15.33 ± 2.03mm zone of inhibition when compared to other compounds. Compounds **4b** and **4e** against *P. aureginosa* produced 20 ± 0.58 mm and 19.5 ± 0.96mm zone of inhibition this was comparable to the effect of the standard used. Compound **4d** was significant and showed 22.01 ± 0.98 mm zone of inhibition against *P. aureginosa*.

Test compounds other than **4d** showed similar effect than the standard drug ampicillin against *E.coli.* Compound **4d** showed significant inhibition against *Candida albicans and Candida*

parapsilosis with 19.5± 1.13mm and 17.33 ± 1.01 mm zone of inhibition when compared to other compounds but less efficient than the standard drug fluconazole. Evaluation of antimicrobial activity revealed that the all the synthesized compounds were effective in inhibiting the bacterial and fungal growth but with some exceptions. Among all test compounds, compound **4b, 4d** and **4e** showed significant antimicrobial activity when compared to other compounds. Specifically, compound **4b** and **4c** having methoxy, halogen and electron donating atom was more efficient than other compounds but less potent than standard drug ampicillin. Result of *in-vitro* antimicrobial activity is depicted in **Table 2**.

***In vitro* antioxidant screening**

The free radical scavenging activity of test samples **4a-f** was measured by DPPH method according to Brand-williams et al., All the compounds having a electron donating a exhibited free radical scavenging capacity by comparison with the standard Butylated Hdroxytoulene (BHT). DPPH assay were carried out for compounds **4a-f** for different concentration from 50 and 100 µM concentration. All the compounds have an electron donating atoms and conjugated pi bond are having ability to scavenge free radicals.

Among the test compounds, **4b** and **4d** having electron donating atoms showed significant amount of DPPH activity (>75 %) for concentration 100 µM. Remaining compounds were no significant compared to the standard BHT. The variation exhibited in DPPH scavenging capacity could be attributed to the effect of different substitution and results were graphically presented in **fig. 1.**

Table 2: Antimicrobial activity of the synthesized compounds (4a- f) against Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, Escherchia coli, Candida albicans and Candida parapsilosis

Compounds	Zone of inhibition (mm)					
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Candida parapsilosis*
4a	16.33 ± 0.56	14.17 ± 0.95	19.01 ± 0.33	11.1 ± 1.2	15.33 ± 1.2	14.67 ± 0.33
4b	14.67 ± 1.86	15.33 ± 2.03	19.17 ± 1.01	20 ± 0.58	17.33 ± 0.88	17 ± 0.8
4c	15 ± 1.15	11 ± 0.58	14.67 ± 0.95	19 ± 1.06	17 ± 0.58	16.27 ± 1.01
4d	25 ± 0.86	23.67 ± 1.45	26.83 ± 0.88	22.01 ± 0.98	19.5± 1.13	17.33 ± 1.01
4e	17.33 ± 0.88	16.83 ±1.4	17.67 ± 0.5	19.5 ± 0.96	14.67 ± 1.45	16.17 ± 0.95
4f	13.33 ± 1.2	9 ± 1.13	20± 0.58	17.33 ± 0.58	14.67 ± 1.86	14.01 ± 0.33
4g	18.67 ± 0.88	17.33 ± 1.01	16.67 ± 1.45	16 ± 0.33	16.33 ± 0.56	14 ± 2.52
Ampicillin	17 ± 1.53	20.67 ± 0.33	14.33 ± 1.45	19.67 ± 0.88	-	-
Fluconazole	-	-	-	-	19.3 ± 0.33	18.83 ± 1.13

The value of each constituents consisted of ± S.E.M. of 03 replicates. ND – Not Defined.

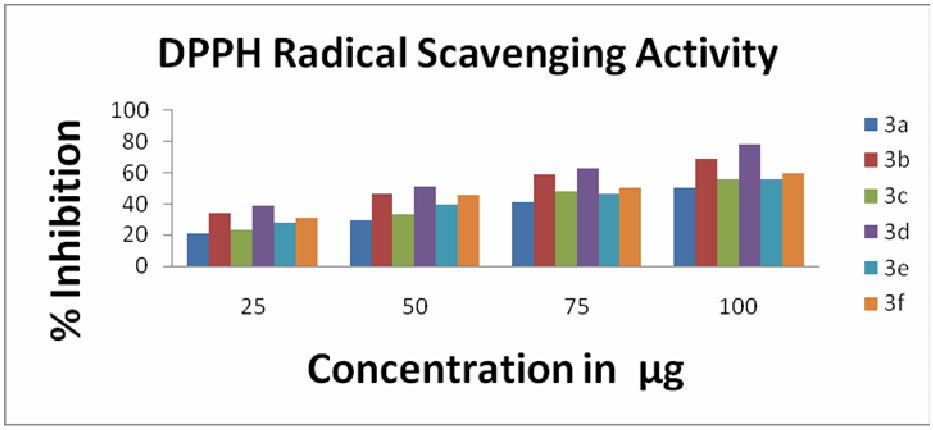


Fig. 1: DPPH radical scavenging activity.

CONCLUSION

This investigation proposes a convenient, economical and useful method for the synthesis of 5,5'-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiol) azo dye, coupled with quinoline, and napthols which are biologically active molecules possessing safer antimicrobial and *in vitro* antioxidant property. The new class of heterocycles, 1,3,4-oxodiazole azo dyes derivatives proved to be a safer up to upper most dosage and exhibit a significant antimicrobial and antioxidant activity. 1,3,4-oxodiazole azo dye having electron donating atoms, shows active antioxidant capacity. The preliminary antimicrobial activity studies revealed that the azo dye having 1,3,4-oxodiazole moiety exhibited a potential antimicrobial activity. Hence, it can be concluded that, this class of compounds certainly holds a greater promise in discovering a safer antimicrobial and antioxidant agent.

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