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ORIGINAL ARTICLE

Synthesis and biological activities of Bis alkyl 1,3,4-oxadiazole incorporated azo dye derivatives

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Abstract 3,5-Bis(alkyl-1,3,4-oxadiazole-2-yl azo dyes were synthesized by a multi-step reaction sequence. Structures of newly synthesized compounds were characterized and confirmed by IR, NMR, and Mass spectral studies. The synthesized compounds were screened for their antimicrobial and *in vitro* antioxidant properties. The results of this investigation revealed that the newly synthesized compounds are potent antibacterial and antioxidant agents. All the synthesized compounds exhibit significant biological activity and certainly hold a greater promise for discovering potent biologically active molecules.

1. Introduction

In recent years, fungal and bacterial infections have become an important complication and a major cause of morbidity and mortality. The growing incidence of fungal and bacterial resistance to existing antibiotics poses a serious medical problem in treating pathogenic infections. Several five-membered hetero cyclic drugs possess diverse biological effects. Nitrogen and oxygen containing five membered azoles are important bioactive agents, due to their vast pharmacological and industrial applications. Synthesis of such heterocyclic compounds is of pharmaceutical importance and a foremost task for chemists. 1,3,4-Oxadiazole derivatives are heterocyclic compounds which exhibit remarkable pharmacological activities. It has been known that the activity of azo linkage increases with the incorporation of a suitable heterocyclic moiety. Heterocyclic azo compounds are well known for their medicinal importance and are recognized for their use as antineoplastics (Child et al., 1977), antidiabetics (Garg and Praksh, 1972), antiseptics (Browing et al., 1926), anti-inflammatory, antibacterial (Khedr et al., 2011; Nikhil et al., 2011) and other useful chemotherapeutic agents (Bae et al., 2003; Sanjay et al., 2007). 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 (Zeynel et al., 2008).

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Azo dyes are known to be involved in a number of biological reactions such as inhibition of DNA, RNA, protein synthesis, carcinogenesis and nitrogen fixation (Browing et al., 1926; Rajendra et al., 1998). Evans blue and Congo red are being studied as HIV inhibitors. This effect is believed to be caused by binding of azo dyes to both protease and reverse transcriptase of this dreadful virus (Fatma and Eser, 2007). 1,2,3-Oxadiazoles can also act as HIV integrase inhibitors (Sicardi et al., 1980).

1,3,4-Oxadiazoles are five membered heterocyclic compounds having significant position in synthetic and medicinal chemistries due to their wide array of biological activities such as anti fungal (Parkash et al., 2010) antimicrobial (Sridhara et al., 2010), anti-inflammatory, analgesic (Akhter et al., 2009; Idrees et al., 2009; Tozcoparan et al., 2000), hypolipidemic (Jayashankar et al., 2009) anti tubercular (Kumar et al., 2010; Kucukguzel et al., 2002), anti-convulsant (Singh and Pankaj, 2010; Bhat et al., 2010), and cytotoxic agents (Padmavathi et al., 2009), also as prostaglandin receptor antagonists (George et al., 2000) and anti oxidant agent (Abdu Musad et al., 2011; Bondock et al., 2009).

In view of the above mentioned findings and our previous findings on synthesis and pharmacological activities of heterocyclic compounds (Keshavayya et al., 2011a,b, 2006, 2007), in the present study we made an efficient attempt to synthesize alkyl bis 1,3,4-oxadiazole substituted azo dyes coupled with quinoline, possessing potent biological activities. Structures of the synthesized compounds were confirmed by ^{1}H NMR, IR and Mass spectral studies. Synthesized compounds were screened for their antimicrobial and *in vitro* antioxidant properties.

2. Results and discussion

As depicted in Scheme 1, 1,3,4-oxadiazole azo dye derivatives were synthesized by a multi-step reaction sequence. 1,3,4-Oxadiazoles were prepared by reacting 5-nitro bis iso-phthalic dihydrazide with appropriate long chain fatty acids in the presence of phosphorous oxychloride gives 5-nitro alkyl bis 1,3,4-oxadiazoles in good yields. 5-Nitro alkyl bis 1,3,4-oxadiazoles were allowed to react with Zn/HCl using ethanol as a solvent to convert the nitro group to amine. The newly synthesized amine group was diazotized and coupled with 8-hydroxy quinoline to obtain bis alkyl 1,3,4-oxadiazole substituted azo dye **4 (a–f)**. The synthesized compounds were recrystallized using methanol. The purity of the compounds was checked by TLC. The structures of the newly synthesized compounds were characterized by ^{1}H NMR, IR and Mass spectra studies. The synthesized compounds were found in good agreement with the spectral data. The elemental analysis results were matched within $\pm 0.4\%$ of the theoretical values.

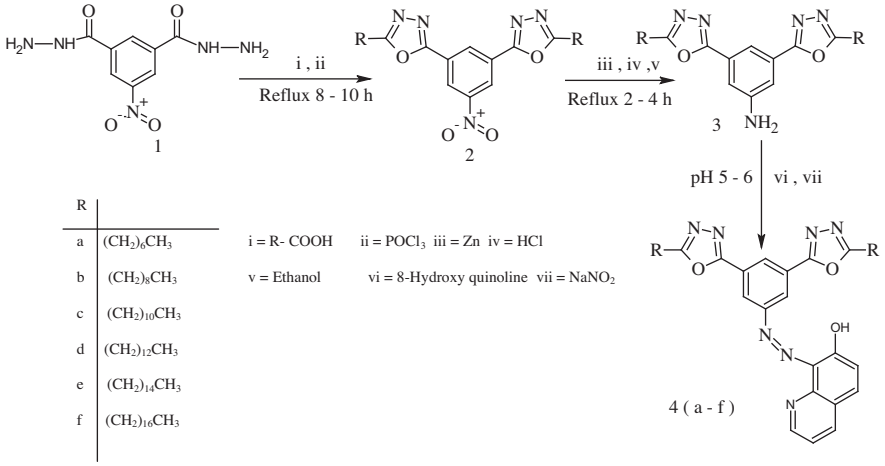
The IR spectra of **1** showed an absorption peak at 3336.4 cm^{-1} due to hydrazide, a peak at 1507.1 cm^{-1} due to NO_2 and a peak at 1633.3 cm^{-1} due to C═O absorption. These spectral data of synthesized 5-nitro, iso-phthalic dihydrazide stand in good agreement with those reported in the literature (Palekar et al., 2009). 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 $NHNH_2$ 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, δ 2–3 due to long chain aliphatic protons and δ 6–8 due to aromatic proton. The IR spectrum of compounds **4 a–f** showing an absorption peak at 3300–3400 cm^{-1}, was attributed to ─OH, at 1600–1700 cm^{-1}, due to N═N, absorption at 1500–1600 and C═N at 2921 (aliphatic chain). The IR, ^{1}H NMR and mass spectral data were found in good agreement with the newly synthesized compounds.

3. 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 has revealed that the oxadiazole ring, having oxygen, nitrogen and the hydroxyl group was the pharmacophore of antifungal agents [**41**].

3.1. Evaluation of minimal inhibitory concentrations (MICs)

Evaluation of MIC values of all the compounds **4a–f** was 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, Escherichia coli,* and *Candida parapsilosis*. While, compounds 4a and **4d** showed maximum inhibitory activity against *P. aureginosa* and *C. parapsilosis* at MIC 2.5 mg/mL, lowest MIC was shown by compound



Scheme 1

Table 1 *In vitro* minimum inhibition concentration evaluation of test compounds against *Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, Escherichia coli, Candida albicans and Candida parapsilosis*.

	5a4	5b2	5c1	5d3	5e5	5f6
Staphylococcus aureus	20	10	05	10	20	20
Pseudomonas aureginosa	10	05	05	10	10	10
Bacillus subtilis	10	10	2.5	05	20	20
Escherichia coli	10	05	2.5	05	10	–
Candida albicans	05	2.5	2.5	05	10	20
Candida parapsilosis	–	05	05	10	10	–

4c. Compounds **4b**, **4c** and **4d** demonstrated efficient MIC when compared to other test compounds. Results of MIC are depicted in Table 1.

3.2. Antimicrobial screening

All synthesized compounds having heterocyclic system containing bridgehead nitrogen and oxygen atoms possess enhanced antimicrobial activity. Among the synthesized compounds **4c** having moderate aliphatic chain length showed significant results in inhibiting *Staphylococcus aureus and Bacillus subtilis* growth with 21.83 ± 0.88 mm and 24.33 ± 1.01 mm zones of inhibition respectively when compared to other compounds. Compounds **4b** and **4a** against *P. aureginosa* produced 21.01 ± 0.56 mm and 17.69 ± 0.33 mm zones of inhibition respectively; this was comparable to the effect of the standard used. Whereas compound **4d** was significant and showed 17.5 ± 1.2 mm. Test compounds other than **4c** showed moderate effect when compared to the standard drug ampicillin against *E. coli*. Compound **4c** showed significant inhibition against *Candida albicans and C. parapsilosis* with 25.67 ± 0.58 mm and 17.33 ± 1.01 mm zones of inhibition respectively 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 the compounds tested, compounds **4b**, 4d, and **4a** showed significant antimicrobial activity when compared to other compounds. Specifically, compound **4c** was more efficient than other compounds but less potent than standard drug ampicillin. Results of *in vitro* antimicrobial activity are depicted in Table 2.

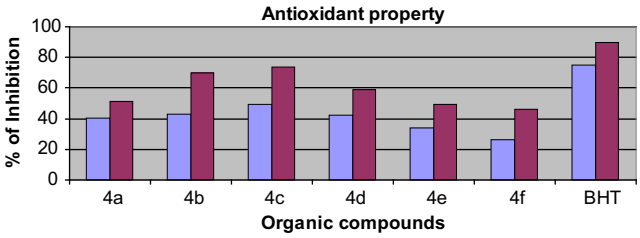


Figure 1 DPPH radical scavenging activity.

3.3. 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 synthesized compounds having electron donating groups like oxygen and nitrogen, exhibited free radical scavenging capacity in comparison with the standard Butylated Hydroxytoulene (BHT). DPPH assay was carried out for compounds **4a–f** at different concentrations from 50 to 100 μM concentration. Among the tested compounds, 4b and **4c** showed significant DPPH scavenging activity (> 70%) at concentration 100 μM whereas other test compounds were less potent than the standard BHT (Fig. 1). The variation exhibited in DPPH scavenging capacity could be attributed to the effect of different substitutions and results are presented in Table 3.

4. Experimental

All analytical grade chemicals were used directly. Melting point of the synthesized compounds was determined in a 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 recrystallized using suitable solvent system. Infra-red spectra were recorded on a Perkin Elmer-spectrum RX-1model spectrophotometer using KBr pellets. NMR spectra were recorded by a Bruker DRX 400 MHz spectrometer and acquired on a Bruker Avance-2 model spectrophotometer using DMSO as a solvent

Table 2 Antimicrobial activity of the synthesized compounds (**4a–f**) against *Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis, Escherichia coli, Candida albicans and Candida parapsilosis*.

Compounds	Zone of inhibition (mm)					
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Candida parapsilosis*
	ATCC 25923	ATCC 6633	ATCC 25922	ATCC 27853	ATCC 10231	ATCC 90018
4a	10.33 ± 1.12	16.67 ± 0.95	17.88 ± 1.53	17.69 ± 0.33	19.13 ± 0.33	ND
4b	17 ± 1.53	17.67 ± 0.88	18.83 ± 1.4	21.01 ± 0.56	22.33 ± 0.67	18.01 ± 0.33
4c	21.83 ± 0.88	24.33 ± 1.01	23 ± 0.33	20 ± 0.33	25.67 ± 0.58	17.33 ± 1.01
4d	17.33 ± 0.67	18.83 ± 0.33	18.33 ± 0.88	17.5 ± 1.2	18.98 ± 1.17	16.98 ± 1.13
4e	12.28 ± 0.88	11.67 ± 0.33	17.17 ± 1.45	15.11 ± 0.33	16.33 ± 1.86	15.17 ± 1.52
4f	10.33 ± 0.67	9.67 ± 1.01	13.67 ± 0.95	ND	11.33 ± 1.2	ND
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.

Table 3 DPPH radical scavenging activity of compound **4a–f**.

Concentration in μM	4a	4b	4c	4d	4e	4f	BHT
50	40.17	43.11	49.16	42.13	34.24	26.33	75.13
100	51.19	69.89	73.67	59.13	49.33	46.03	90.02

and TMS as an internal reference. The crude compounds were purified by recrystallization method.

*4.1. Synthesis of aryl 3-nitro iso-phthalic acid dihydrazide (**1**)*

The 3-nitro iso-phthalic acid dihydrazide (**1**) was synthesized by adopting reported method. Hydrazine hydrate (0.2 mmol) was added drop-wise to the solution of iso-phthalic ester (0.1 mmol) in 30 mL of dried ethanol 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 under UV light. The excess ethanol was distilled out and the contents were cooled at room temperature. The yellow solid mass formed was filtered and washed thoroughly using brine solution and the resultant was dried.

*4.2. General procedure for the synthesis of 2, 2′-(5-nitrobenzene-1, 3-diyl) bis (5-alkyl-1,3,4-oxadiazole) (**2**)*

A suspension of 5-nitrobenzene-1,3-dicarbohydrazide (1 mmol) and the appropriate long chain fatty acids (2 mmol) in $POCl_3$ (10 ml) were refluxed for 6–8 h. The progress of the reaction was monitored on TLC by using silica gel plates using petroleum ether and ethyl acetate (7:3) as the eluting system and visualized in UV light. The reaction mixture was allowed to cool to room temperature and slowly poured over crushed ice kept overnight. The solid thus, separated out was neutralized with anhydrous sodium bicarbonate, filtered, washed with water and recrystallized using ethanol.

4.3. General procedure for the synthesis of 3,5-bis(alkyl-1,3,4-oxadiazol-2-yl)aniline

A suspension of nitro compound (1 mmol) and $SnCl_2 2H_2O$ (5 mmol) was dissolved in 0.02 M methanolic HCl solution and refluxed for 3–4 h at 70–80 °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 sulphate, concentrated, and then recrystallized from methanol.

*4.4. General procedure for the synthesis of 3,5-bis(alkyl-1,3,4-oxadiazol-2-yl azo dye (**4a–f**)*

The newly synthesized amine (1 mmol) was taken in HCl and cooled to 0–5 °C, $NaNO_2$ dissolved in a suitable solvent (1.25 mmol) was added dropwise 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, the 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 recrystallized using methanol that afforded a red coloured dye, a red colour solid with 83% yield, m.p. 168–169 °C.

4.5. Spectral data of synthesized compound

*4.5.1. 3,5-Bis (alkyl-1,3,4-oxadiazol-2-yl azo dye (**4a**)*

This dye was isolated as red colour solid with 83% yield, m.p. 168–169 °C; IR (Vmax, cm^{-1}) = 2849 (C—H), 1630 (C═N), 1507 (NO_2), 2921 (aliphatic chain), ^{1}H NMR ($CDCl_3$) (δ ppm) = 0.92 (*t*, 6H, aliphatic CH_3), 1.27 (Br, S, 12H, CH_2), 1.33 (*m*, 4H, Ar–H), 1.6 (*t*, 4H, CH_2) 2.54 (*m*, 4H, CH_2), 8.76 (*d*, 1H, Ar–H), 8.07 (*d*, 2H, Ar–H), 7.95 (*d*, 1H, Ar–H), 7.92 (*s*, 1H, Ar–H), 7.83 (*d*, 1H, Ar–H), 7.32 (*d*, 1H, Ar–H), 7.18 (*t, 1H, Ar–H), 14.63 (s, 1H, OH) .MS m/z* 582.27 (M^+) ^{13}C NMR ($CDCl_3$) (δ ppm) = 14.6, 23.1, 26.0–32.4 (aliphatic-C), 118.6, 118.3, 121.3, 123.8, 127.4, 131.4, 134.6, 134.5, 137.8, 143.1, 150.6, 150.8, 153.2. (Ar–C), 162.6 (C5 of oxadiazole), 168.8 (C2 of oxadiazole). Anal. Calcd. For $C_{33}H_{39}N_7O_3$; C, 68.14; H, 6.76; N, 16.86. Found; C, 68.23; H, 6.59; N, 16.91.

4.5.2. 3,5-Bis (alkyl-1,3,4-oxadiazol-2-yl azo dye (4b)

This dye was isolated as red colour solid with 78% yield, m.p. 175–176 °C; IR (Vmax, cm^{-1}) = 2849 (C—H), 1630 (C═N), 1507 (NO_2), 2921 (aliphatic chain), ^{1}H NMR ($CDCl_3$) (δ ppm) = 0.94 (*t*, 6H, aliphatic CH_3), 1.29 (*m*, 20H, CH_2) 1.34 (*m*, 4H, CH_2) 1.63 (*m*, 4H, CH_2), 2.59 (*t*, 4H, CH_2) 8.76 (*d*, 1H, Ar–H), 8.12 (*d*, 2H, Ar–H), 7.97 (*d*, 1H, Ar–H), 7.93 (*s*, 1H, Ar–H), 7.82 (*d*, 1H, ArH), 7.31 (*d*, 1H, Ar–H), 7.15 (*t*, 1H, Ar–H). 14.7 (*s*, 1H, OH) .MS *m/z* = 638.46 (M^+) ^{13}C NMR ($CDCl_3$) (δ ppm) = 14.8, 23.3, 26.0–32.6 (aliphatic-C), 118.4, 118.7, 121.7, 123.5, 127.1, 131.8, 133.2, 134.3, 138.2, 143.7, 150.2, 151.3, 153.7. (Ar–C), 162.2 (C5 of oxadiazole), 169.2 (C2 of oxadiazole). Anal. Calcd. For $C_{37}H_{47}N_7O_3$; C, 69.67; H, 7.43; N, 15.37. Found; C, 68.92; H, 7.53; N, 15.58.

4.5.3. 3,5-Bis (alkyl-1,3,4-oxadiazol-2-yl azo dye (4c)

IR (Vmax, cm^{-1}) = 2849 (C—H), 1630 (C═N), 1507 (NO_2), 2921 (aliphatic chain) ^{1}H NMR ($CDCl_3$) (δ ppm) = 15.2 (*s*, 1H), 0.95 (*t*, 6H, aliphatic CH_3), 1.26 (*m*, 28H, CH_2) 1.31 (*m*, 4H, CH_2) 1.64 (*m*, 4H, CH_2), 2.58 (*t*, 4H, CH_2) 8.79 (*d*, 1H, Ar–H), 8.11 (*d*, 2H, Ar–H), 7.95 (*d*, 1H, Ar–H), 7.90 (*s*, 1H, Ar–H), 7.85 (*d*, 1H, Ar–H), 7.29 (*d*, 1H, Ar–H), 7.18 (*t*, 1H, Ar–H). 24.7 (*s*, 1H, OH). ^{13}C NMR ($CDCl_3$) (δ ppm) = 14.3, 23.2, 29.8, 30.0, 30.2, 32.2, 32.5 (aliphatic-C), 118.6, 118.4, 121.3, 123.6, 127.7, 131.4, 134.5, 135.3, 137.3, 143.4, 150.7, 150.4, 153.7. (Ar–C), 162.4 (C5 of oxadiazole), 169.1 (C2 of oxadiazole). MS *m/z* = 694.17(M^+). Anal. Calcd. For $C_{41}H_{55}N_7O_3$; C, 70.96; H, 7.99; N, 14.13. Found; C, 69.98; H, 7.74; N, 16.15.

4.5.4. 3,5-Bis (alkyl-1,3,4-oxadiazol-2-yl azo dye (4d)
This dye was isolated as red colour solid with 86% yield, m.p. 188–189 °C; IR (Vmax, cm^{-1}) = 2849 (C—H), 1630 (C═N), 1507 (NO_2), 2921 (aliphatic chain) ^1H NMR ($CDCl_3$) (δ ppm) = 15.4 (*s*, 1H), 0.97 (*t*, 6H, aliphatic CH_3), 1.28 (*m*, 36H, CH_2) 1.33 (*m*, 4H, CH_2) 1.624 (*m*, 4H, CH_2), 2.56 (*t*, 4H, CH_2) 8.72 (*d*, 1H, Ar–H), 8.32 (*d*, 2H, Ar–H), 7.98 (*d*, 1H, Ar–H), 7.91 (*s*, 1H, Ar–H), 7.82 (*d*, 1H, Ar–H), 7.27 (*d*, 1H, Ar–H), 7.14 (*t*, 1H, Ar–H). MS *m*/*z* = 751.23 (M^+) Anal. Calcd. For $C_{45}H_{63}N_7O_3$; C, 72.06; H, 8.47; N, 13.307. Found; C, 71.52; H, 7.98; N, 14.16.

4.5.5. 3,5-Bis (alkyl-1,3,4-oxadiazol-2-yl azo dye (4e)
This dye was isolated as red colour solid with 77% yield, m.p. 192–193 °C; IR (Vmax, cm^{-1}) = 2849 (C—H), 1630 (C═N), 1507 (NO_2), 2921 (aliphatic chain) ^1H NMR ($CDCl_3$) (δ ppm) = 15.8 (*s*, 1H), 0.93 (*t*, 6H, aliphatic CH_3), 1.24 (*m*, 44H, CH_2) 1.36 (*m*, 4H, CH_2) 1.63 (*m*, 4H, CH_2), 2.58 (*t*, 4H, CH_2) 8.76 (*d*, 1H, Ar–H), 8.34 (*d*, 2H, Ar–H), 7.93 (*d*, 1H, Ar–H), 7.96 (*s*, 1H, Ar–H), 7.84 (*d*, 1H, Ar–H), 7.29 (*d*, 1H, Ar–H), 7.16 (*t*, 1H, Ar–H). MS *m*/*z* = 806.35 (M^+) Anal. Calcd. For $C_{49}H_{71}N_7O_3$; C, 73.01; H, 8.88; N, 12.16. Found; C, 74.19; H, 8.62; N, 15.89.

4.5.6. 3,5-Bis (alkyl-1,3,4-oxadiazol-2-yl azo dye (4f)
This dye was isolated as red colour solid with 84% yield, m.p. 198–199 °C; IR (Vmax, cm^{-1}) = 2849 (C—H), 1630 (C═N), 1507 (NO_2), 2921 (aliphatic chain) ^1H NMR ($CDCl_3$) (δ ppm) = 14.3 (*s*, 1H), 0.98 (*t*, 6H, aliphatic CH_3), 1.25 (*m*, 52H, CH_2) 1.36 (*m*, 4H, CH_2) 1.68 (*m*, 4H, CH_2), 2.52 (*t*, 4H, CH_2) 8.79 (*d*, 1H, Ar–H), 8.38 (*d*, 2H, Ar–H), 7.96 (*d*, 1H, Ar–H), 7.92 (*s*, 1H, Ar–H), 7.87 (*d*, 1H, Ar–H), 7.31 (*d*, 1H, Ar–H), 7.12 (*t*, 1H, Ar–H).\ MS *m*/*z* = 862.97 (M^+) Anal. Calcd. For $C_{53}H_{79}N_7O_3$; C, 73.83; H, 9.32; N, 11.37. Found; C, 73.62; H, 9.52; N, 11.85.

4.6. Determination of minimal inhibitory concentrations (MIC)

The agar dilution susceptibility test was performed based on the modified method of NCCLS, 2003 and CLSI, 2009 to determine the MIC of the synthesized compounds. The test compounds (**4a–g**) dissolved in sterilized 5% DMSO (400 mg/mL concentration) were taken as standard stock. A series of two fold dilutions of each compound in the final concentrations 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.

4.7. Antimicrobial activity

The antimicrobial activity of newly synthesized compounds **4a–f** was determined by well plate method in nutrient agar (antibacterial activity) (Lehrer et al., 1991) 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, S. aureus, B. subtilis, Salmonella typhi,* were used to investigate the antibacterial activities and *Pseudomonas Aeruginosa, C. albicans*, *C. parapsilosis*, were used to investigate the antifungal activities.

The test compounds were dissolved in dimethyl sulphoxide (DMSO) at concentration of 100 and 50 mg/mL. Approximately 1 cm^3 of a 24 h broth culture was placed in sterile petri dishes. Molten nutrient agar kept at 45 °C was then poured into the Petri dishes and allowed to solidify. Six millimetre 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 °C. The inhibition zone was observed and documented after 24 h for bacterial culture and 72 h for fungal cultures. The clear zone around the holes in each plate was measured as zone of inhibition in mm. Experiments were triplicates and standard deviation was calculated. The zone of inhibition of antifungal activity was determined after 72 h culture. The results were compared with Fluconazole.

4.8. In vitro antioxidant screening

The free radical scavenging activity of synthesized **4a–g** was measured by DPPH (2,2-diphenyl 1-picrylhydrazyl) using the method proposed by Brand-Williams et al. (1995). The reaction mixture of the synthesized compounds *viz*. **4a–g** at different concentrations ranging from 25 to 100 µg aliquots was taken and the 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 temperature, O.D was measured at 517 nm and the inhibition concentration was calculated using the formula given below. 3 mL of methanol and 1 mL of DPPH were used as controls.

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

$A°$ = the absorbance of the control at 517 nm,
A^1 = the absorbance of the compound **4a–g** at 517 nm.

5. Conclusion

This investigation proposes a convenient, economical, cheaper and useful method for the synthesis of 3,5-bis(alkyl-1,3,4-oxadiazol-2-yl) azo dyes, coupled with quinoline, which are biologically active molecules possessing antimicrobial and *in vitro* antioxidant properties. These new classes of heterocycles, exhibit a significant antimicrobial and antioxidant activities. 1,3,4-Oxodiazole azo dye coupled with 8-hydroxy quinoline showed active antioxidant capacity than 1,3,4-oxodiazole azo dye coupled with naphthols. 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 new class of compounds certainly holds a greater promise in discovering a potent antimicrobial and antioxidant agent.

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