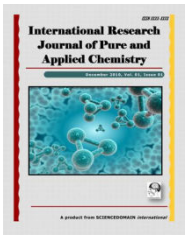

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Synthesis of Some Novel Bis 1,3,4-Oxadiazole Fused Azo Dye Derivatives as Potent Antimicrobial Agents

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ABSTRACT

3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dyes were synthesized by multistep reaction sequences, which is diazotized and coupled with different napthols and quinoline. Structure of newly synthesized compounds were characterized and confirmed by IR, NMR and Mass spectral studies. The newly synthesized azo dye fused with (5-(furan-2-yl)-1,3,4-oxadiazole) were screened for their acute toxicity and gross behavioral studies and in-vitro anti-microbial activity. Synthesized compounds exhibit significant biological activity and can certainly hold greater promise in discovering safer biologically active molecules.

Keywords: 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl) azo dyes; Anti-microbial activity; Acute toxicity and gross behavioral studies;

1. INTRODUCTION

Heterocycles by far are the largest classical division of organic chemistry and are of immense importance. Synthesis of such heterocyclic compounds is of pharmaceutical importance and a foremost task of chemists due to its vast pharmacological and industrial applications. Nitrogen and oxygen containing five member heterocyclic compounds have

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been used as a scaffold to synthesize numerous therapeutic molecules. Heterocyclic azo compounds are known for their medicinal importance, recognized for their use as antineoplastics (Child et al., 1977) antidiabetics (Garg et al., 1972) antiseptics (Browing et al., 1926) antibacterial (Swati et al., 2011; Kalpana et al., 2011) and are known to be involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and nitrogen fixation (Rajendra et al., 1998; Browing et al., 1926). 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 retrovirus. It has been found that the activity of azo compound increases on the incorporation of suitable heterocyclic moiety. 1,3,4-oxadiazoles are a class of heterocycles they are of significant interest in medicine and pesticide chemistry in a number of biological targets including anti-inflammatory agents (Chena et al., 2010, Omar et al., 1996), antibacterial (Singh et al., 2010; Iqbal et al., 2006), tuberculostatic (Shahar Yar et al., 2007), anti-convulsant (Bhat et al., 2010; Shahar yar et al.,2009) and antimicrobial activity (Kristin et al., 2009; Gulay Ahin et al., 2002). 1,2,3-oxadiazoles can also be used as HIV integrase inhibitors (Zhao et al., 1997) or as prostaglandin receptor antagonists (Ann et al., 2000), antinociceptive activity (Santagati et al., 1994). 1,3,4-oxadiazoles exhibits potent biological activity, it is probably by the virtue of -N=C-O- grouping). Recently we have reported synthesis, characterization and pharmacological studies of novel bis 1,3,4-oxadiazole and 1,2,4-triazole derivatives (Shridhar et al., 2011).

New antibiotics that are active against resistant bacteria are required to combat with the present scenario of bacterial pathogenesis. Bacteria have lived on earth since several billion years. During this time, they encountered in nature a wide range of naturally occurring antibiotics. To survive bacteria developed antibiotic resistance mechanism (Raja et al., 2010). In spite of decades of effort it has been difficult to obtain food free of pathogenic bacteria (Singh et al., 2011). Hence there is a great demand for new compounds that can combat with their resistant bacteria. With this knowledge we made an efficient attempt in synthesizing new antimicrobial compounds. In the present endeavor we synthesized furan 1,3,4-oxadiazole azo dyes coupled with quinoline and napthols. Structures of newly synthesized compounds were characterized and confirmed by IR, NMR and Mass spectral studies and the synthesized compounds were tested for their utility as possible anti-microbial agents and compounds were tested for their acute toxicity and grass behavioral studies. All newly synthesized compounds exhibited potent antimicrobial property.

2. MATERIALS AND METHODS

All analytical grade chemicals of were used directly. Melting points were 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. Infra-Red spectra were recorded on Perkin Elmer-spectrum RX-1model spectrophotometer using KBr pellets. NMR spectra was recorded by Bruker DRX400 MHz spectrometer and acquired on a Bruker Avance-2 model spectrophotometer using $CDCl_3$/DMSO as a solvent and TMS as an internal reference.

2.1 Synthesis of Bis 1,3,4-oxadiazole Substituted Azo Dye

2.1.1 Synthesis of aryl 3- nitro iso-phthalic acid dihydrazide (1)

The hydrazine hydrate (0.2 M) was added drop-wise to the solution of iso-phthalic ester in (0.1 M) in 30 mL of dried ethanol with vigorous stirring. The resulting mixture was refluxed for 4-6 hrs. The Excess ethanol was distilled out and the contents were cooled to a room temperature. The progress of the reaction was monitored by TLC with petroleum ether, ethyl acetate (1:1) as the eluting solvent and visualized in a UV light. The yellow solid mass formed was filtered, washed thoroughly with brine solution and the resultant dried.

2.1.2 Synthesis of 2,2'-(5-nitrobenzene-1,3-diyl)bis(5-(furan-2-yl)-1,3,4-oxadiazole)(3)

A suspension of 5-nitrobenzene-1,3-dicarbohydrazide (1 mmol) and the appropriate furan-2- carboxylic acid (2 mmol) in $POCl_3$ (10 mL) was refluxed for about 6-8 hrs. The progress of the reaction was monitored on TLC by using silica gel plates using petroleum ether and ethyl acetate (6:4) as the eluting system and visualized in UV light. The reaction mixture allowed cooled to room temperature, slowly poured over crushed ice kept overnight. The solid thus, separated out was neutralized with anhydrous sodium bicarbonate, filtered, washed with water and recrystalized with ethanol.

2.1.3 General procedure for the synthesis of 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)aniline(4)

A suspension of *2,2'*-(5-nitrobenzene-1,3-diyl)bis(5-(furan-2-yl)-1,3,4-oxadiazole) (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.

2.1.4 General procedure for the synthesis of 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye (5a-g)

The newly synthesized amine 4 (1 mmol) was taken in a HCl and cooled to 0-5^{0}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^{0}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^{0}C and this solution was added to the above mixture gradually without allowing the temperature rise above 0-5^{0}C after complete addition, the reaction mixture was stirred for 1-2 hrs for the completion of reaction. The dye obtained was filtered and washed with water, dried and recrystalized with methanol to afford red coloured dye.

2.2 In-Vitro Antimicrobial Activity

2.2.1 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. The test compounds (5a-g) dissolved in

sterilized DMSO (40 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 ℃ for 18 – 24 hrs and fungal cultures were incubated at 24℃ for 24-48 hrs. After incubation, the MIC was determined.

2.2.2 Antimicrobial screening

The antimicrobial activity of the test compounds (5a-g) were screened by agar well radial diffusion method against bacterial strains belonging to *Staphylococcus aureus*, *Pseudomonas aeruginosa, Bacillus subtilis* and *Escherchia coli* and fungal strains *Candida albicans* and *Candida parapsilosis*, respectively (Cowen et al., 1993). The microbial strains were collected from different infectious status of the patients with the help of authorized physicians, in district health center of Gulberga, Karnataka state, India. The clinical isolates were identified in Microbiology Laboratory, Gulberga University following the standard method (Islam et al., 2008). The bacterial and fungal spore suspensions were diluted in 10^{-1} to 10^{-8} phosphate buffered saline. Samples were homogenized and then loaded in six aliquots of 20 µL each onto nutrient agar plates. The working cultures of bacteria and fungi were prepared by inoculating a loopful of each test microorganism in 3 mL of nutrient broth and potato dextrose broth respectively. Broths were incubated at 37℃ and 24℃ for 24-48 hrs. The suspension was diluted with sterile distilled water to obtain approximately 10^6 CFU/mL.

The test compounds were dissolved in 10% aqueous dimethyl sulfoxide (DMSO; that enhances compound solubility) to get stock solutions. Commercial bactericide ampicillin and fungicide fluconazole were used as standard (100 µg/100 µL of sterilized distilled water) concomitantly with the test samples.

A sensitive agar well radial diffusion technique (Lehrer et al., 1991) was used for the assessment of antimicrobial activity of the test samples. Sterilized nutrient agar medium and potato dextrose agar medium were poured into sterilized petridishes seperately. Nutrient broth containing 100 µL of 24 hrs incubated bacterial cultures of clinical isolates was spread on the nutrient agar medium. Potato dextrose broth containing 100 µL of 48 hrs incubated fungal cultures of clinical isolates was spread on the potato dextrose agar medium. Wells were created using a sterilized cork borer in an aseptic condition. 20 µL of test compounds and 100 µL of standard drug ciprofloxacin were loaded on to their corresponding wells. The bacterial plates were incubated at 37℃ for 24 hrs and fungal plates were incubated at 24℃ for 48 hrs. The diameter of the zone of complete inhibition of the bacteria and fungi was measured to the nearest around each well and readings were recorded in mm. The results of these experiments are expressed as mean ± S.E.M. of three replicates in each test.

2.3 Acute Toxicity and Gross Behavioral Studies

The acute oral toxicity study for the test compounds 5a-g were evaluated according to the OECD guidelines No.420 using Swiss albino male mice weighing 25-30 g (OECD Guidelines for acute toxicity studies, 2008). Each group consisting of 6 male mice (overnight fasted) was kept in the colony cage at 25 ± 2℃ with 55% relative humidity and 12 hrs light/dark cycle. A specified fixed dose of 250, 500, 1000 and 1500 mg/kg was selected and administered orally as a single dose as fine suspension prepared in saline using gum acacia

(5%). The acute toxic symptoms and the behavioral changes produced by the test compounds were observed continuously at interval time of 4 hrs. (4^{th}, 8^{th},12^{th}, and 24^{th} hrs) onset of toxic symptoms and gross behavioral changes were also recorded (Ghosh et al., 1984; Nilanjan et al., 2010; Jaouhari et al., 1999; Turner et al., 1965; Segovia et al., 2002). The experimental studies revealed that all the categories of synthesized dyes derivatives are quite safe up to 1500 mg/kg and no mortality in animals was recorded except in compound 5e. Further, no significant gross behavioral changes were observed in experimental animals except in the compound 5e at concentration 1000 and 1500 mg/kg, which showed depression on the first day and mortality on second day.

3. RESULTS AND DISCUSSION

3.1 Chemistry

As depicted in the scheme 1, 1, 3, 4-oxadiazole azo dye derivatives were synthesized by a multi-step reaction sequence. 5-nitro bis iso-phthalic hydrazide was synthesized according to reported method. The IR spectra of compound 5-nitro bis iso-phthalic hydrazide showed absorption peak at 3336.4 cm^{-1} due to $NHNH_2$, 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 were in agreement with earlier report (Keshavayya et al., 2007). The IR spectrum of the compound 3 showed absorption peak 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 IR spectrum of the compounds 5a- g showed 3394.8 cm^{-1} due to phenolic(-OH), 2977.8 cm^{-1} due to (CH_2), 1624.8 cm^{-1} due to (N=N), 1505.1(C=C) confirms the formation of azo dyes.

3.2 Physical and Spectral Data of Synthesized Compounds

3.2.1 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye (5a)

This dye was isolated as red colour solid with 63% yield, m.p. 168-169 ^oC; IR (KBr) cm^{-1} =3472(br,-OH), 3100 (C-H stretching), 2977 (H-C=O:C-H stretching), 1567(C=N, stretching) 1544 (-N=N-). ^1H NMR (DMSO) δ ppm = 14.3(s,1H, -OH),8.76(d, 2H, Ar-H), 7.97(s, 1H, Ar-H), 7.82(s,1H, Ar-H), 7.65((d,1H.Ar-H), 7.56(d, 1H, Ar-H), 7.42(d, 2H, Ar-H), 7.34 (t, 1H, Ar-H), 7.23(t, 1H, Ar-H), 7.19(d,1H,Ar-H),6.4(m,4H,Ar-H). MS *m/z* =515 (M^-). Anal. Calcd. For $C_{28}H_{16}N_6O_5$; C,65.12; H,3.12; N,16.27. Found; C, 65.35; H, 3.18; N, 16.32.

3.2.2 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye (5b)

This dye was isolated as red colour solid with 70% yield, m.p. 173-174 ^oC; IR (KBr) cm^{-1} = 3464(br,-OH), 3114 (C-H stretching), 2956 (H-C=O: C-H stretching), 1558(C=N, stretching) 1562 (-N=N-). ^1H NMR (DMSO) δ ppm = 14.6(s,1H, -OH),8.75(d, 2H, Ar-H), 8.51(s, 1H, Ar-H), 7.98(d,1H, Ar-H), 7.94((s,1H.Ar-H), 7.87(d, 1H, Ar-H), 7.44(d, 2H, Ar-H), 7.28 (d, 1H, Ar-H), 7.18(t, 1H, Ar-H), 6.5(m, 4H, Ar-H). MS *m/z* =516 (M^-). Anal. Calcd. For $C_{27}H_{15}N_7O_5$; C,62.67; H,2.92; N,18.95. Found; C, 62.65; H, 2.89; N, 18.92.

3.2.3 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye (5c)

This dye was isolated as red colour solid with 62% yield, m.p. 173-174 ^oC; IR (KBr) cm^{-1} = 3582(br,-OH), 3463 (N-H stretching) 3117 (C-H stretching), 2952 (H-C=O: C-H stretching),

1558(C=N, stretching) 1562 (-N=N-). ^{1}H NMR (DMSO) δ ppm = 14.4(s,1H, -OH), 10,7(s,1H,NH), 8.73(d, 2H, Ar-H), 8.54(s, 1H, Ar-H), 7.94((s,1H.Ar-H), 7.64(d,2H, Ar-H), 7.62(d,1H,Ar-H), 7.55 (d, 1H, Ar-H), 7.44(d,2H,Ar-H) 7.34(t, 1H, Ar-H),7.26(t,2H,Ar-H)7.22(t,1H,Ar-H),7.06 (t,1H,Ar-H),6.5(m, 4H, Ar-H). MS *m/z* = 634 (M^-).Anal. Calcd. For $C_{35}H_{21}N_7O_6$; C, 66.14; H, 3.33; N, 15.53. Found; C, 66.21; H, 3.24; N, 15.57.

3.2.4 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye (5d)

This dye was isolated as red colour solid with 56% yield, m.p. 185-186 ^oC; IR (KBr) cm^{-1} = 3576(br,-OH), 3472 (N-H stretching), 3113 (C-H stretching), 2967 (H-C=O: C-H stretching), 1562 (C=N, stretching), 1567 (-N=N-).2815 (-OCH_3). ^{1}H NMR (DMSO) δ ppm = 14.3(s,1H, -OH), 10,9(s,1H,NH), 8.72(d, 2H, Ar-H), 8.52(s, 1H, Ar-H), 7.92(s,1H.Ar-H),7.54(d,2H,Ar-H),7.66(d,1H,Ar-H),7.44(d,2H,Ar-H),7.32(t,1H,Ar-H),7.23(t,1H,Ar-H)7.01(t,1H,Ar-H),6.82 (t,1H,Ar-H),6.81(d,1H,Ar-H),6.5(m, 4H, Ar-H),3.86(s,3H,CH_3).MS *m/z* = 664 (M^-).Anal. Calcd. For $C_{36}H_{23}N_7O_7$; C, 64.96; H, 3.48; N, 14.73. Found; C, 64.93; H, 3.45; N, 14.71.

3.2.5 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye (5e)

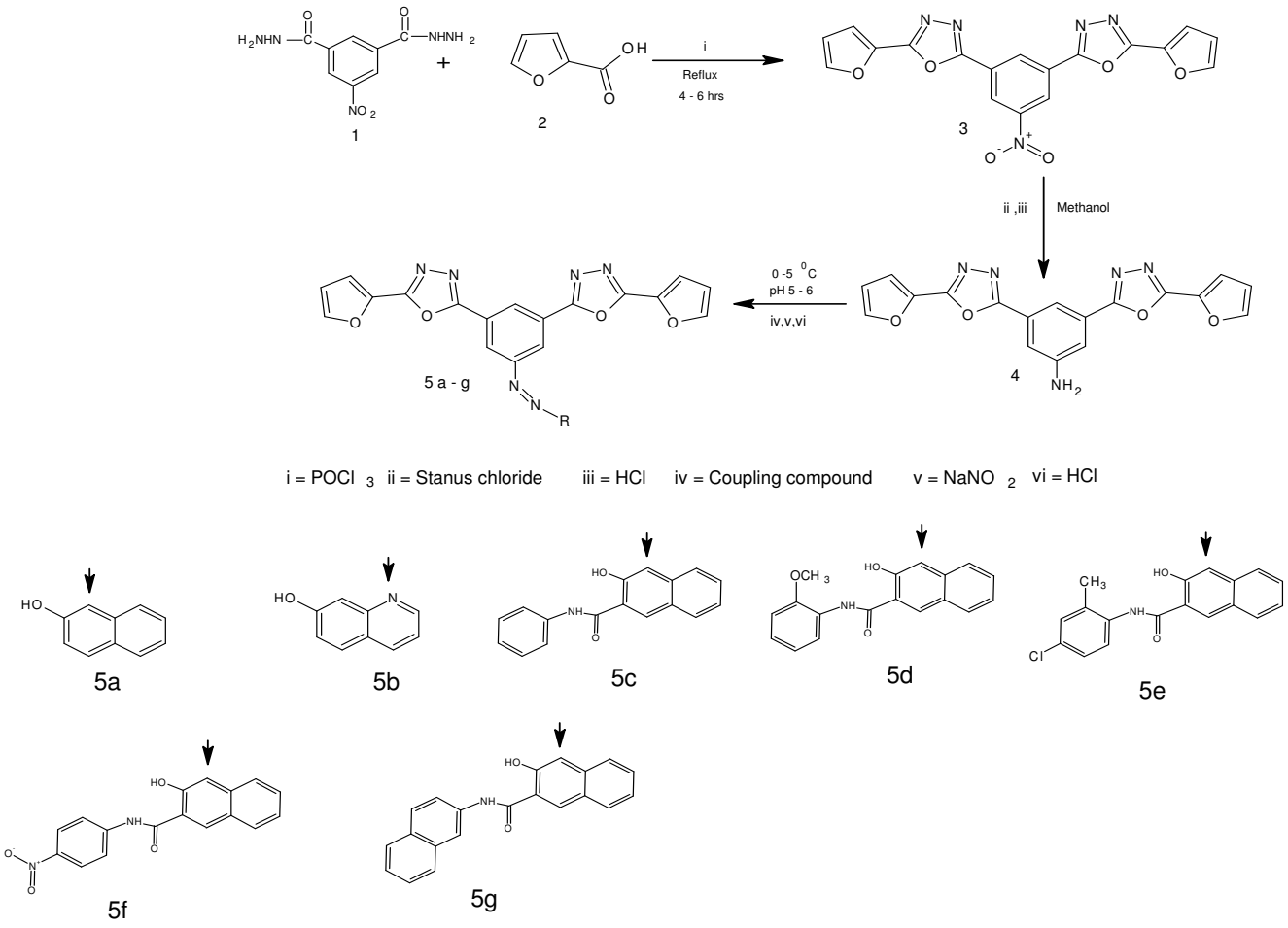
This dye was isolated as red colour solid with 56% yield, m.p. 192-1193 ^oC; IR (KBr) cm^{-1} = 3582(br,-OH), 3472(N-H stretching), 3113 (C-H stretching), 2967 (H-C=O: C-H stretching), 1562 (C=N, stretching), 1567 (-N=N-). 7869 (C-Cl). ^{1}H NMR (DMSO) δ ppm = 14.7 (s,1H, -OH), 10,4 (s,1H,NH), 8.74(d, 2H, Ar-H), 8.53(s, 1H, Ar-H), 7.95(s,1H.Ar-H),7.65(d,1H,Ar-H),7.57(d,1H,Ar-H),7.48(d,1H,Ar-H),7.44(d,2H,Ar-H),7.37(t,1H,Ar-H), 7.24 (t,1H,Ar-H)7.08(d,1H,Ar-H),7.05(d,1H,Ar-H),6.7(m,4H,Ar-H),2.83(s,3H,Ar-H) MS *m/z* = 698 (M^-).Anal. Calcd. For $C_{36}H_{23}ClN_7O_7$; C, 61.76; H, 3.17; N, 14.01. Found; C, 61.73; H, 3.15; N, 14.04.

3.2.6 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye (5f)

This dye was isolated as red colour solid with 70% yield, m.p. 187-188 ^oC; IR (KBr) cm^{-1} = 3464(br,-OH), 3472(N-H stretching), 3114 (C-H stretching), 2956 (H-C=O: C-H stretching), 1562 (-N=N-), 1558(C=N, stretching), 1535(N=O). ^{1}H NMR (DMSO) δ ppm = 13.9 (s,1H, -OH), 11.2 (s,1H,NH), 8.76 (d, 2H, Ar-H), 8.58(s, 1H, Ar-H), 8.54(s,1H,Ar-H),8.04(d,1H.Ar-H),7.98(d,1H,Ar-H),7.94(d,1H,Ar-H),7.56(d,1H,Ar-H),7.52(t,1H,Ar-H), 7.46(d,2H,Ar-H), 7.34(t,1H,Ar-H),7.67(d,1H,Ar-H),7.28(t,1H,Ar-H),6.4(m,4H,Ar-H), MS *m/z* = 680 (M^-).Anal. Calcd. For $C_{35}H_{20}N_8O_8$; C,61.77; H,2.96; N,16.46. Found; C,61.67; H,2.85; N,16.56.

3.2.7 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye (5g)

This dye was isolated as red colour solid with 52% yield, m.p. 213-214 ^oC; IR (KBr) cm^{-1} = 3464(br,-OH), 3472(N-H stretching), 3114 (C-H stretching), 2956 (H-C=O: C-H stretching), 1562 (-N=N-), 1558(C=N, stretching).^{1}H NMR (DMSO) δ ppm = 14.3(s,1H, -OH), 10.9 (s,1H,NH), 8.76 (d, 2H, Ar-H), 8.56(s, 1H, Ar-H), 7.93(d,1H,Ar-H), 7.68 (d,1H,Ar-H), 7.55 (d,1H,Ar-H),7.54 (d,1H,Ar-H),7.52(d,1H,Ar-H), 7.47(d,1H,Ar-H), 7.42(d,2H,Ar-H) , 7.38(t,1H,Ar-H),7.25(t,1H,Ar-H),7.23(t,1H,Ar-H),7.02(t,1H,Ar-H),6.78(d,1H,Ar-H),6.74(d,1H,Ar-H), 6.4 (m,4H,Ar-H). MS *m/z* = 680 (M^-).Anal. Calcd. For $C_{39}H_{23}N_7O_6$; C,68.32; H,3.38; N,14.30. Found; C,68.34; H,3.36; N,14.33.



Scheme 1. Synthesis of 1,3,4-oxadiazole azo dyes

3.3 *In-Vitro* Antimicrobial Screening

3.3.1 Evaluation of minimal inhibitory concentrations (MIC)

The MIC values of all the test compounds ranged from 2.5 to 20 mg/ mL. Compound 5a, 5c, 5f and 5g showed significant inhibition at MIC 2.5 mg/mL against *Pseudomonas aureginosa,* E. coli and Candida parapsilosis. Whereas, compound 5b, 5d and 5e showed maximum activity against *Pseudomonas aureginosa* and *Candida parapsilosis* at MIC 2.5 mg/mL. Lowest MIC was observed by compound 5d. Compounds 5b, 5d, 5e and 5f demonstrated efficient MIC when compared to other test compounds. At 20 mg/ml most of the test compounds demonstrated inhibitory activity, hence in general this concentration (20 µg/µL) was used to evaluated antimicrobial activity. Results of MIC are depicted in Table 1.

3.3.2 Antimicrobial screening

All synthesized compound having heterocyclic system containing bridgehead nitrogen and oxygen atoms possess enhanced antimicrobial activity. Compound 5b showed significant results in inhibiting *S. aureus* and *B. subtilis* with 25 ± 0.86 mm and 23.67 ± 1.45 mm zone of inhibition when compared to other compounds. Compounds 5d and 5e against *P. aureginosa* produced 19.21 ± 0.33 and 19.5 ± 0.96 mm zone of inhibition this was comparable to the effect of standard used. Whereas compound 5b having furan oxadiazole and quinoline moiety was significant and showed 22.01 ± 0.98 mm. Test compounds other than 5d having furan oxadiazole ring and OCH_3 group showed better effect than the standard drug ampicillin against *E. coli.* Compound 5d showed significant inhibition against *Candida albicans and Candida parapsilosis* with 14.67 ± 1.45 mm and 16.27 ± 0.95 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 the test compounds 5b, 5d and 5e showed significant antimicrobial activity when compared to other compounds. Specifically, compound 5b was more efficient than other compounds but less potent than standard drug ampicillin. Result of *in-vitro* antimicrobial activity is depicted in Table 2.

3.3.3 Acute toxicity and gross behavioral studies

From the preliminary toxicity studies, it was observed that, the test compounds 5b, 5d, 5e and 5f have revealed good safety profile till the uppermost dose (2500 mg/kg). No mortality and behavioral changes of animals observed even after 24 hrs in compound 5b, 5d, 5e and 5f, but mortality was seen in the compound 5a and 5g at the concentration 1000 and 1500 mg/kg and behavioral changes were also recorded for same concentration.

Table 1. *In vitro* minimum inhibition concentrations of synthesized compound (5a-g)

Test pathogenic microorganisms	Test Compounds (mg/mL)						
	5a	5b	5c	5d	5e	5f	5g
Staphylococcus aureus	20	10	20	05	05	10	20
Pseudomonas aureginosa	20	2.5	*	2.5	10	05	*
Bacillus subtilis	10	05	05	20	05	10	10
Escherchia coli	*	10	10	2.5	10	*	10
Candida albicans	05	05	10	05	2.5	20	20
Candida parapsilosis	05	10	*	2.5	05	10	*

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

Table 2. Antimicrobial activity of the synthesized compounds (5 a- g)

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**
5a	16.33 ± 0.56	14.17 ± 0.95	19.01 ± 0.33	11.1 ± 1.2	15.33 ± 1.2	14.67 ± 0.33
5b	25 ± 0.86	23.67 ± 1.45	26.83 ± 0.88	22.01 ± 0.98	19 ± 1.13	17.33 ± 1.01
5c	15 ± 1.15	11 ± 0.58	14.67 ± 0.95	19 ± 1.06	17 ± 0.58	16.27 ± 1.01
5d	17.33 ± 0.88	16.83 ±1.4	17.67 ± 0.5	19.21 ± 0.33	14.67 ± 1.45	16.17 ± 0.95
5e	22.67 ± 0.88	21.33 ± 1.01	18.67 ± 1.45	19.5 ± 0.96	17.33 ± 0.56	15.48 ± 2.52
5f	13.33 ± 1.2	9 ± 1.13	20± 0.58	17.33 ± 0.58	14.67 ± 1.86	14.01 ± 0.33
5g	14.67 ± 1.86	15.33 ± 2.03	19.17 ± 1.01	20 ± 0.58	17.33 ± 0.88	17 ± 0.8
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.

4. CONCLUSION

We report a convenient, economically cheaper and useful method for the synthesis of 3,5-bis(5-(furan-2-yl)-1,3,4-oxadiazol-2-yl)azo dye biologically active molecules possessing safer antimicrobial activity. The new class of heterocycles, furan, 1,3, 4- oxodiazole azo dyes derivatives proved to be a safer up to upper most dosage and are exhibit a significant antimicrobial activity. The preliminary antimicrobial activity studies revealed that the azo dye fused with 1,3,4-oxodiazole moiety exhibited a higher antimicrobial activity. Hence, it is concluded that, this class of compounds certainly holds a greater promise in discovering safer antimicrobial agents.

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