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

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ABSTRACT

In present study 5,5’-(5-nitrobenzene-1,3-diyl)bis(1,3,4-oxadiazole-2-thiolazo 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-vitro 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 AIDS. In organ transplant cases, free radicals gene rates due stress participates in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and nitrogen fixation [11-13]. 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-cancer [22-23], cytotoxicity [24]. Some oxadiazole were used in high technological biological studies, like antimicrobial, anti-tubercular, anti-inflammatory, anticonvulsant 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, 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 1H 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](image-url)
MATERIALS AND METHODS
All analytical grade chemicals were used directly as purchased. Melting points of the synthesized compounds 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 recrystallized using suitable solvent system. Infra-red spectra were recorded on Perkin Elmer-spectrum RX-1 model 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 recrystallization 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 dissolved in 3 mL HCl and cooled to 0-5 °C. After complete addition, the reaction mixture was stirred for 1-2 hrs. 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 TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C. After complete addition the reaction mixture was adjusted to pH 5-6, The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C, NaNO2 was added with constant stirring without allowing the temperature rise above 0-5 °C. After complete addition the reaction mixture was stirred for 1-2 hrs without allowing the temperature rise above 0-5 °C. After complete addition, the reaction mixture was stirred for 1-2 hrs. The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light.

General procedure for the synthesis of 5,5′-(5-nitrobenzene-1,3-diy1)bis(1,3,4-oxadiazole-2-thiol) aniline (3)

A suspension of nitro compound (1 mmol). SnCl2·2H2O (5 mmol) were dissolved in 0.02 M methanolic HCl solution and refluxed for 3-4 hrs at 70-80 °C under nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate, 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-diy1)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, NaNO2 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, 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-diy1)bis(1,3,4-oxadiazole-2-thiol) azo dye (4a-f)

This dye was isolated as red colour solid with 62% yield, m.p. 284-288°C; IR (ν max cm⁻¹) = 3472 (br, OH), 3100 (CH-stretching), 2940 (C-H stretching), 1603 (C=O stretching), 1514 (C=N ring stretching). The newly synthesized amine (1 mmol) was taken in a 10% HCl and cooled to 0-5 °C, NaNO2 was added with constant stirring without allowing the temperature rise above 0-5 °C. After complete addition the reaction mixture was stirred for 1-2 hrs. The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C. After complete addition the reaction mixture was adjusted to pH 5-6, The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C, NaNO2 was added with constant stirring without allowing the temperature rise above 0-5 °C. After complete addition the reaction mixture was stirred for 1-2 hrs. The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C, NaNO2 was added with constant stirring without allowing the temperature rise above 0-5 °C. After complete addition the reaction mixture was stirred for 1-2 hrs. The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C, NaNO2 was added with constant stirring without allowing the temperature rise above 0-5 °C. After complete addition the reaction mixture was stirred for 1-2 hrs. The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C, NaNO2 was added with constant stirring without allowing the temperature rise above 0-5 °C. After complete addition the reaction mixture was stirred for 1-2 hrs. The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C, NaNO2 was added with constant stirring without allowing the temperature rise above 0-5 °C. After complete addition the reaction mixture was stirred for 1-2 hrs. The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light. The reaction mixture was cooled to 0-5 °C, NaNO2 was added with constant stirring without allowing the temperature rise above 0-5 °C. After complete addition the reaction mixture was stirred for 1-2 hrs. The progress of the reaction was monitored by TLC using silica gel coated plates (0.5 mm layer) and visualized in UV light.

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 were dissolved in sterilized 5% DMSO (400 mg/ml concentration)
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 × 10⁸ 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 broth was 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°C. The inhibition zone that appeared after 24 h, around the holes in each plate were measured by DPPH (2,2-diphenyl 1-picrylhydrazyl) using the method proposed by Brand-williams et al [39].

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 C₅H₇ and alcoholic KOH gives bis 1,3,4-oxadiazoles in a good yield. 5.5’-[5-nitrobenzene-1,3-diy]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 was diazotized and coupled with 8- hydroxy quinoline to obtain 1,3,4-oxadiazole substituted azo dye 4 (a-f). The compounds were recrystallized from Methanol. The purity of the compounds was checked by TLC. Spectral data - 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 NO₂ 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 NH/NH₂ at 3336.4 cm⁻¹ confirms the formation of oxadiazoles. The H NMR spectrum revealed a singlet at 8 13-16 due to aromatic protons. 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=C and C-S groups. The IR, 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 aeruginosa; E. coli and Candida parapsilosis. While, compound 4a and 4d showed maximum inhibitory activity against Pseudomonas aeruginosa 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 aeruginosa, Bacillus subtilis, Escherchia coli, Candida albicans and Candida parapsilosis |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Test pathogens/ microorganisms | Test Compounds (mg/mL) | | | | | | |
| | 4a | 4b | 4c | 4d | 4e | 4f | 4g |
| Staphylococcus aureus | 09 | 08 | 20 | 10 | 05 | * | 20 |
| Pseudomonas aeruginosa | 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. aeruginosa 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. aeruginosa.

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.
parapsilosis with 19.5 ± 1.13 mm 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 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 an electron donating a exhibited free radical scavenging capacity by comparison with the standard Butylated Hydroxytoluene (BHT). DPPH assay was 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 aeruginosa, Bacillus subtilis, Escherichia coli, Candida albicans and Candida parapsilosis

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Staphylococcus aureus</th>
<th>Bacillus subtilis</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
<th>Candida albicans</th>
<th>Candida parapsilosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>16.33 ± 0.56</td>
<td>14.17 ± 0.95</td>
<td>19.01 ± 0.33</td>
<td>11.1 ± 1.2</td>
<td>15.33 ± 1.2</td>
<td>14.67 ± 0.33</td>
</tr>
<tr>
<td>4b</td>
<td>14.67 ± 1.86</td>
<td>15.33 ± 2.03</td>
<td>19.17 ± 1.01</td>
<td>20 ± 0.58</td>
<td>17.33 ± 0.88</td>
<td>17 ± 0.8</td>
</tr>
<tr>
<td>4c</td>
<td>15 ± 1.15</td>
<td>11 ± 0.58</td>
<td>14.67 ± 0.95</td>
<td>19 ± 1.06</td>
<td>17 ± 0.58</td>
<td>16.27 ± 1.01</td>
</tr>
<tr>
<td>4d</td>
<td>25 ± 0.86</td>
<td>23.67 ± 1.45</td>
<td>26.83 ± 0.88</td>
<td>22.01 ± 0.98</td>
<td>19.5 ± 1.13</td>
<td>17 ± 1.01</td>
</tr>
<tr>
<td>4e</td>
<td>17.33 ± 0.88</td>
<td>16.83 ± 1.14</td>
<td>17.67 ± 0.5</td>
<td>19.5 ± 0.96</td>
<td>14.67 ± 1.45</td>
<td>16.17 ± 0.95</td>
</tr>
<tr>
<td>4f</td>
<td>13.33 ± 1.2</td>
<td>9 ± 1.13</td>
<td>20 ± 0.58</td>
<td>17.33 ± 0.58</td>
<td>14.67 ± 1.86</td>
<td>14.01 ± 0.33</td>
</tr>
<tr>
<td>4g</td>
<td>18.67 ± 0.88</td>
<td>17.33 ± 1.01</td>
<td>16.67 ± 1.45</td>
<td>16 ± 0.33</td>
<td>16.33 ± 0.56</td>
<td>14 ± 2.52</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>17 ± 1.53</td>
<td>20.67 ± 0.33</td>
<td>14.33 ± 1.45</td>
<td>19.67 ± 0.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>19.3 ± 0.33</td>
<td>18.83 ± 1.13</td>
</tr>
</tbody>
</table>

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-oxidiazole-2-thiol) azo dye, coupled with quinoline, and naphthols which are biologically active molecules possessing safer antimicrobial and in vitro antioxidant property. The new class of heterocycles, 1,3,4-oxidiazoleazo dyes derivatives proved to be a safer up to uppermost dosage and exhibit a significant antimicrobial and antioxidant activity. 1,3,4-oxidiazoleazo dye having electron donating atoms, shows active antioxidant capacity. The preliminary antimicrobial activity studies revealed that the azo dye having 1,3,4-oxidiazole 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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REFERENCE


19. Kumar, S.S. Lamani and O.Kotresh. Synthesis and Biological Activity of Some Novel 4-[3-Mercapto-1,3,4-thiadiazol-2-yl]-2-phenyl-5-2-phenylvinyl]-2,4-dihydro-3H-1,2,4-triazole-3-one. E.Journal of Chemistry. 2010;7: 545.
