Synthesis, Identification and Antibacterial Potency of Azo Dyes having Quinolin-8-ol and Active Methylene Moiety

Kanchan R. Damade1,*, Jyotsana D. Shinde2, Dinesh J. Bhojsing2, Vijay S. Patil2, Bhaiya S. Chauhan2, Kiran R. Kapadane3 & Chandan I. Rajput4

1, 2 & * Department of Chemistry, Arts, Commerce and Science College Bodwad, Distt Jalgaon, Maharashtra, INDIA

* Correspondence: E-mail: Kanchan_Damade@rediffmail.com

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ABSTRACT: In the present study we have reacted the five aryl diazonium salts prepared from nitro substituted anilines and amino substituted benzoic acids and coupled with Quinolin-8-ol and Active methylene group of pent-1,4-dione respectively. Synthesized compounds are then identified by FTIR Spectroscopical method. The final product, 1 to V formed has potential to use as azo dyes and as an intermediate in other synthetic procedures or transformations along with this it exhibits good biological activities.

Keywords: Active methylene group; diazonium salt; antibacterial activity; nitroanilines and azo dye.

INTRODUCTION: Azo compounds were under study from the long time for their remarkable partaking in pharmaceuticals, cosmetics and textiles industries. They have immense important biological activities and high therapeutic values for all mammals reported by Sisley and Porsche, 19111. reductive cleavage of Azo group gives great therapeutic properties for treatment of serious disorders in human beings1,2,3. Biological outcomes from an enzymatic metabolism (occurs in vivo)4,5 for example in skin bacteria such as Staphylococcus aureus6 which involves reduction of azo group (-N=N-) to produce toxic or nontoxic resultant amines7 which may produce carcinogenic effects8,9. Despite of having negative role for environment and human health azo groups have attracted medical attentions. Later studies showed health hazards from these azo families in the form of carcinogenic and mutagenic properties, even though many of the synthetic strategies have been developed to produce these classes of compounds for their specific physico-chemical and biological activities. The azo coupling reaction with AMG results into formation of bioactive compounds like cinolines which is one of the important class of natural products having remarkable pharmaceutical and biological importance10. Due to simple process in aqueous media we can produce wide varieties of azo dyes and can determined biological actions. In our study we synthesized novel azo compound in two different schemes forming diazonium salt of o and m substituted nitro anilines and substituted amino benzoic acids followed by coupling reaction with quinolin-8-ol and active methylene group of acetyl acetone respectively, so here in we reported some novel azo compounds and testing out their antibacterial potency on selected strains of bacteria like Staphylococcus aureus and Bacillus subtilis with discussion of their infections.

Many authors have reported about the infections registered through S. aureus and B. subtilis bacteria in human body. Logan et al. 1988 stated the infections with bacilli species in impotent patients that is who are immunologically compromised. Similarly Kiss et al.1988 also reported the B. subtilis infections in ene- vate patients. Donzis et al. 1988 reported B. subtilis eye infections due to contaminated lenses. Penington et al.1976 described infections in patients having blood cancer.

Staphylococcus aureus is also one of the dangerous pathogen found in human. Rates of S.aureus infections are high among patients with type-1 diabetes,12 intravenous drug users,13 patients facing hemodialysis,14 patients having done with major surgery,15,16 and who are HIV positive17. Patients with leukaemia are also at increased risk for staphylococcal disease18. So therefore many studies have been done for preparing antibiotics for these organisms.

MATERIALS AND METHODS: All the chemicals were of standard grade and used without further puri-
The purity of co- synthesis of azo-dyes (IV & V) was determined by melting point determination and silica gel-G TLC, elemental analysis (C,H,N), FTIR spectral data. The bacterial strains, Staphylococcus aureus and Bacillus subtilis are purchased from National Centre for Cell Science (NCCS), Pune, India and maintained at Smt. G. G. Khadse College, to determine the antibacterial activity of synthesized all five azo compounds (I-V).

**Scheme 1: General procedure for synthesis of azo dyes**

![Diagram of the synthesis process](image)

**Stage 1:** Preparation of Diazonium Salt - In 100 ml Capacity beaker add 2 ml aniline (or its derivative) and to this mixture of 5ml conc. HCl and 10 ml water were added and stirred with the glass rod to get clear solution. Cool, the solution upto 0º C by keeping in freezing mixture. Dissolving (1gm) sodium nitrite in 10 ml water. Allowed to cool the solution in ice bath to 0º C, after attaining 0º C we added NaNO₂ solution into aniline hydro chloride (or derivatives) solution drop wise with constant stirring (kept maintained temperature below 50ºC during addition). After decompose of excess of nitrous acid by adding pinch of urea filtered the solution and collect the filtrate which was diazonium salt of aniline and its derivatives.

**Stage 2:** Diazonium coupling reaction with quinolin-8-ol - Prepared a mixture of solution of 1.5gm quinolin-8-ol in 10 ml 10% NaOH and allowed to cool up to 0ºC, after attaining 0ºC ,we added solution of diazonium salt drop wise in to quinolone-8-ol in NaOH solution with constant stirring, after complete addition allowed reaction mixture to stand for 10 min in ice bath, filtered the azo colourant and washed it with cold water, dry, weight and noted the yield of clear crude azo product then recrystallized by using solvent ethanol. Recorded the dried weight and the color, melting point range of compounds.

**Scheme 2: General procedure for synthesis of azo dyes (IV & V)**

![Diagram of the synthesis process](image)

**Stage 1:** preparation of diazonium salts of amino substituted benzoic acids - Same as discussed in scheme-1.
Stage 2: Diazonium coupling reaction with AMG of Acetyl acetone (pent-2,4-dione) - Add (-o/-p) carboxy aryl diazonium salt solution drop wise, to the well cooled mixture of, Pentane-2,4-dione (1.8ml) which is dissolved in 5 ml ethanol and sodium acetate, 8-10 gm in 10-15ml of water and maintained the 0°C temperature, a coloured precipitate is separated, then by checking the absence of ester, the product obtained is recrystallized by using ethanol, dried it. Record the dried weight (in gms) and then melting point range of the compounds VI & V.

Substitution on benzoic acid: 2-NH₂ (IV) & 4-NH₂ (V)

The synthesized azo compounds (I-V) were tested for their anti-bacterial activity against two strains of bacteria named Staphylococcus aureus and Bacillus subtilis by disc diffusion assay using solutions of azo compounds at two different concentrations of 500 & 1000 µg/ml.

RESULTS AND DISCUSSION: In the present study diazonium salts of aniline (and nitro derivatives) in scheme-1 is coupled with Quinolin-8-ol which results into formation of compounds I⁹ (B.E. Ezema et.al. 2014) II & III 5-[(nitro substituted phenyl) diazenyl] quinolin-8-ol. Similarly in scheme-2, carboxy diazonium salt is coupled with AMG of Pent-2, 4-dione to form products IV & V (2,4-dioxopentan-3-yl)diazenyl) substituted benzoic acids. The synthesised compounds then screened for antibacterial activity at two antibiotic concentrations of 500 and 1000 µg/ml. All the compounds were of high purity and ascertained by melting point determinations as well as by silica gel TLC. The probable structural of the compounds (I-V) was assigned by FTIR spectroscopical method. The I.R frequencies of the presents functional groups are shown in Table 1. The name of the compound, practical yield, melting point range and color are shown in Table 2. The photographic view of antibacterial action (zone of inhibition shown in Table 3) of azo compounds on two strains Staphylococcus aureus and Bacillus subtilis has been figured as a, b, c, d and e. The antibacterial activity test shown that the synthesised compounds I and II is active against both of the bacterial strains, and compound IV is active towards only S. aureus at both of the concentrations while the azo compounds III and V has no action towards any of the tested strains. Results outcomes has also confirms that the compound II is most active against both of the bacterial strains forming growth inhibition zone upto 18mm at 1000 µg/ml while at lower concentration of 500 µg/ml there is occurrence of smaller inhibition zone of 11mm and 15mm in S.aureus and B.subtilis respectively. This concludes that azo colorant II can exhibit better antibiotic properties against S.aureus as well as B. subtilis with increasing concentration.

Table 1: FTIR spectral data for the synthesized azo compounds (I-V).

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>IR cm⁻¹</th>
<th>Assigned structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>γ N=N =1311-1193</td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ Ar-H =1502-1407</td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ CH =2992-2825</td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ C-O =1193-1072</td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ Para-sub = 898-668</td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ NO₂ =1669-1598</td>
<td></td>
</tr>
</tbody>
</table>


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Table 2: Analytical and physical data of azo colourants I-V.

<table>
<thead>
<tr>
<th>Azo compounds</th>
<th>Practical Yield</th>
<th>Melting point range</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-5-(phenyldiazenyl)quinolin-8-ol (I)</td>
<td>81.73%</td>
<td>222–226°C</td>
<td>Glittering red</td>
</tr>
<tr>
<td>(E)-5-((2-nitrophenyl)diazenyl)quinolin-8-ol (II)</td>
<td>87.16%</td>
<td>259–261°C</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>(E)-5-((3-nitrophenyl)diazenyl)quinolin-8-ol (III)</td>
<td>79.49%</td>
<td>243–246°C</td>
<td>Yellowish</td>
</tr>
<tr>
<td>(E)-2-((2,4-dioxopentan-3-yl)diazenyl) benzoic acid (IV)</td>
<td>68.84%</td>
<td>259–260°C</td>
<td>Bright yellow</td>
</tr>
<tr>
<td>(E)-4-((2,4-dioxopentan-3-yl)diazenyl) benzoic acid (V)</td>
<td>81.52%</td>
<td>240–242°C</td>
<td>Greenish yellow</td>
</tr>
</tbody>
</table>

Table 3: Showing antibacterial actions of the compounds in mm of zone of inhibition.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bacterial strain</th>
<th>Concentration</th>
<th>Concentration</th>
<th>Concentration</th>
<th>Concentration</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>500 µg/ml</td>
<td>1000 µg/ml</td>
<td>500 µg/ml</td>
<td>1000 µg/ml</td>
<td>500 µg/ml</td>
</tr>
<tr>
<td>I</td>
<td>zone diameter (mm)</td>
<td>06 08 11 18</td>
<td>- - - - - -</td>
<td>B. subtilis</td>
<td>06 08 15 18</td>
<td>08 08 - - -</td>
</tr>
<tr>
<td>II</td>
<td>zone diameter (mm)</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>S. aureus.</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
</tr>
</tbody>
</table>
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Figure 1: FTIR spectrum for azo dyes I-V shown in a-e.

Figure 2: Photographical view for antibacterial action of synthesised dyes I-V on bacterial strain S. aureus (a-e) and B. subtilis (a-e).

CONCLUSION: No report has been found for the synthesis of Dye II-V whereas the Dye –I has been reported by Ezema, 2014. In present work we reported biological potency of all dyes I-V. The results out-
come has shown that Dye –II is most active toward inhibition of S.aureus as well as B.subtilis bacterial strain with increasing antibiotic concentration from 500 µg/ml to 1000 µg/ml, this concludes dye-II produces growth inhibiting activity with increasing concentrations while both of the bacterial strains shown resistance against dye-III & V, this might be due to permeability problem of the compounds to reach up to the cell organelles or degradation potency of the bacterial cells.

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REFERENCES: