Synthesis, Characterization of Azo Dyes and their Biological Studies

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Abstract: *A series of new azo dyes (**2-6**) were synthesized by diazotization of 2 - amino, 6 - methyl pyrimidine 4-ol (**1**) and followed by coupling with different coupling components such as 1-naphthol, 2-naphthol, 6 bromo 2- naphthol, 8-hydroxy quinoline and salicylic acids. The novel azo dyes are also orange-red in color and were characterized by various physico chemical methods like IR, UV-Vis, NMR spectral techniques etc.. The synthesized compounds were screened for their biological activies(antibacterial) and the results are compared with the standards.*

Key words: Pyrimidine derivatives, Azo dyes, Synthesis, Biological Studies.

Introduction

Most of the azo dyes are synthesized by diazotization of an aromatic primary amine, followed by coupling with the components which have one or more electron rich nucleophiles such as amino and hydroxyl functionalities. Azo dyes are the most extensively used class of coloring materials because of their enormous applications in various fields of science and technology. Azo dyes are having diverse applications such as in textiles industries as dyeing agents, as paints, in leather industries and in paper industries. In recent years, the applications of azo dyes was penetrated in to the field of electronics as a storage components in the DVD-R (Digital versatile Disc-Recordable) because of its merits as stable metal azo dyes complexes. Dyes can be coated easily by spin coating method which will have good thermal stability, high refractive index and also complexes of the azo dyes are having extensive application in electro-photographic toners as charge controlling agents, developers in powder coating

materials, electric materials and in electrostatic separation processes, in-ink jets and in colour filters [1-6]. Mainly azo dyes of heterocyclic rings are structurally fashionable agents and have more number of applications such as fluorescence materials, second-order non-linear optical (NLO) materials, Liquid crystals components , Dye fastness, leather shining materials etc[7-10]. Azo dyes are also having various bio-medical applications. Now a days, sulphur and nitrogen containing heterocyclic molecules have been extensively used for various biological activities like anti fungal, anti bacterial, anti-inflammatory and anti cancer agents. [11-12]

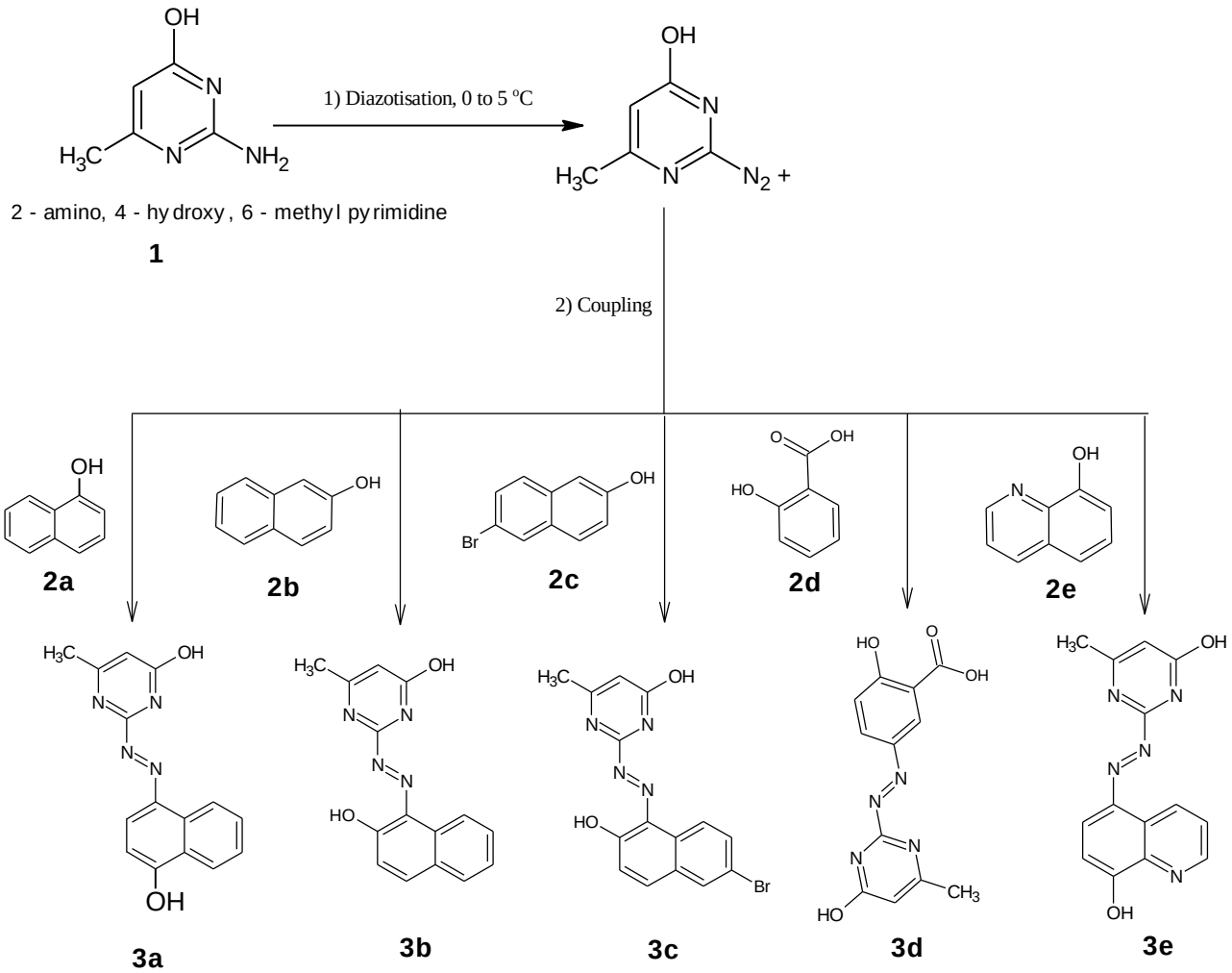
Since last two decades in our research laboratory, various azodyes and pigments were prepared, characterized, explored its various applications and reported in referred journals. In continuation of research in dyes and pigments area by keeping aforementioned synthetic and applications data of azo dyes in mind we have planned to synthesize new series of heterocyclic azo dyes of 2 - amino, 6 - methyl pyrimidine 4-ol . We have synthesized, characterized and explored few application in the research work including antimicrobial activities

Methods and materials

2 - Amino, 6 - methyl pyrimidine 4-ol, was purchased from Sigma Aldrich, India. It was directly used as such for diazotization reactions. The other chemicals used for the coupling purpose are of pure analytical reagent grade obtained from Himedia and Merk chemical suppliers. The melting point of the synthetic dyes was recorded using electrical instrument.The UV-Visible absorption spectra were recorded in, DMSO solvent with a ‘SHIMADZU UV-Visible 1650’ spectrometer in the wavelength range of 200-800 nm. Infrared spectra of azo dyes were recorded in the region of 400 cm^{-1} to 4000 cm^{-1} on a FTIR-460 Spectrometer in KBr pellets. The mass spectra were recorded with a LC-MS-trap-XCT_plus mass spectrometer. The ^{1}H NMR spectra were recorded in DMSO-d_6 at 400MHz using AMX-400 FT-NMR spectrophotometer using TMS as an internal standard.

Synthetic procedure for the Azo dyes

Diazotization and coupling: The 0.5g (0.003m) 2 - amino, 6 - methyl pyrimidine 4-ol was dissolved in a mixture of acetic acid(4mL) and conc. hydrochloric acid(2mL). An equimolar (1:1) mixture of $NaNO_2$, 0.45g (0.003m) was slowly added with stirring. The reaction mixture was stirred for 2 hrs maintaining the temperature of 0-5ºC. The pH of the reaction mixture was maintained at ~ 6 by adding chilled aqueous solution of sodium carbonate. A solution containing equimolar mixture of coupling component was taken in DMF (20 mL) and was added to reaction mixture and stirred well for 4hrs. The dye obtained was filtered, washed with water, dried, and recrystalised from ethanol. The purity of the compound was checked by thin layer chromatography. The synthetic path was shown in scheme-1



Scheme – 1: Synthesis of azo dyes (3a – 3e)

RESULTS AND DISCUSSIONS

As shown in Scheme – 1, the heterocyclic azo dyes 3(a-e) were prepared through the diazotization of 2 - amino, 6 - methyl pyrimidine 4-ol and and coupled with different coupling components 1-naphthol, 2-naphthol, 6 bromo 2- naphthol, 8-hydroxy quinoline and salicylic acids. The dyes were obtained in good yield and are partially soluble in acetone and readily soluble in DMF and DMSO. The % yield of the azo dyes, color, melting point, molecular formula and solubility data of all synthesized dyes are tabulated in Table 1.

Dye code	Colour	% Yield	Melting Point (^{o} C)	Molecular formula	Molecular weight	Solubility
3a	Orange red	60	190-192	$C_{15}H_{12}N_4O_2$	280.28	Acetone/DMF/DMSO
3b	Red	64	190-192	$C_{15}H_{12}N_4O_2$	280.28	Acetone/DMF/DMSO
3c	Red	58	188-190	$C_{15}H_{11}BrN_4O_2$	359.17	Acetone/DMF/DMSO
3d	Orange red	56	188-191	$C_{12}H_{10}N_4O_4$	274.23	DMF/DMSO
3e	Red	60	192-195	$C_{14}H_{11}N_5O_2$	281.26	DMF/DMSO

Table -1

UV-Visible absorption spectra of the synthesized azo dyes 3(a-e) were recorded in DMSO at a concentration of 10^{-6} mol L^{-1}. The results are summarized in the table. The infrared spectra of all the dyes (in KBr) were recorded, a broad band which has appeared in the spectra at 3500-3200 confirms the presence of hydroxyl group (-OH). The dyes 3(a-e) are showed 1416-1450 cm^{-1} was assigned for (N=N) group. The values at 3088-3000 cm^{-1} was assigned for (aromatic C-H) and at 2980-2855 cm^{-1} (aliphatic C-H) were also observed.

LC-MS data of the azo dye (3a) was recorded thereby confirmed the purity and structure of the azo dyes. The ^{1}H NMR spectra of the azo dyes were recorded in DMSO-d_6 at room temperature showed three set of signals for all dyes. The signals at 2.65 δ-ppm was assigned for aliphatic CH_3(3H, s) protons, the signals at ~4.9-5.1 δ-ppm was assigned for hydroxyl (-OH) proton/s and signals at 6.5-8.9 δ-ppm was assigned for aromatic protons. The data of electronic absorption, IR spectral and ^{1}H NMR spectra was tabulated in table -2

Compound	λ max in nm	IR(KBr)γ_{max} cm^{-1}	^{1}H - NMR data
Dye – 3a	478	OH (3300), Ar-CH (2984), N=N (1496).	2.65 δ-ppm, CH_3(3H, s) 5.0 δ-ppm-OH(2H, s), 6.2-8.0 δ-ppm (7H, m)
Dye – 3b	492	OH (3280), Ar-CH (2966), N=N (1506).	2.55 δ-ppm, CH_3(3H, s) 5.0 δ-ppm- OH(2H, s), 6.5-8.2 δ-ppm (7H, m)
Dye – 3c	490	OH (3321), Ar-CH (2887), N=N (1515).	2.65 δ-ppm, CH_3(3H, s) 5.0 δ-ppm- OH(2H, s), 6.5-8.9 δ-ppm (6H, m)
Dye – 3d	486	OH (3290), Ar-CH (2988), N=N (1490).	2.50 δ-ppm, CH_3(3H, s) 4.9-5.2 δ-ppm- OH(3H, s), 6.5-8.9 δ-ppm (4H, m)
Dye – 3e	492	OH (3304), Ar-CH (2983), N=N (1489).	2.65 δ-ppm, CH_3(3H, s) 4.9-5.1 δ-ppm- OH(2H, s), 6.5-8.0 δ-ppm (6H, m)

Table – 2: Spectral data of compounds 3a-e

Antibacterial activity

The antibacterial activity of the synthesized compounds was evaluated against E. coli and B. subtilis by disc diffusion assay and the results were listed in Table **1**. From the result, it is evident that all the compounds exhibited promising antibacterial activity against respective bacterial strains. Among the tested compounds, the compound 3d and 3e showing the highest zone of inhibition (2.3±0.26 for 50 mg/mL) against B. subtilis compared to the standard drug. The compounds 3a-3c are of same chemical type showing moderate activity against tested bacterial strains. The variation in the in the antimicrobial activity of the synthesized compounds may be explained by their functionality and charge density distribution

compounds	Zone of inhibition in mm			
	E. coli		B. subtilis	
	25 mg/mL	50 mg/mL	25 mg/mL	50 mg/mL
3a	1.3±02	1.7±0.5	1.4±0.12	2.1±0.12
3b	1.3±08	1.7±0.1	1.7±0.45	2.2±0.33
3c	1.4±02	1.6±0.9	1.6±0.64	2.0±0.18
3d	1.7±02	2.1±0.5	1.9±0.22	2.4±0.62
3e	1.6±72	1.9±0.5	1.8±0.12	2.3±0.32
Streptomycin	**1.9±0.25**	**2.5±0.28**	**2.0±0.32**	**2.6±0.35**

Table -3: Antibacterial activity of the synthesized compounds 3a-e

Conclusion

We have synthesized five novel heterocyclic azo dyes and were characterized by various analytical methods like UV-Visible, IR, ^{1}H-NMR, Mass spectral techniques. Their electrochemical behavior was studied by cyclicvoltametry and the synthesized dyes were screened for biological activity like antimicrobial activities.

Acknowledgements

The authors are very much thankful to Chairman, department of Chemistry, Kuvempu University for providing laboratory facilities. The authors are also much thankful to IISc, Bangalore for NMR and Mass spectral data.

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