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ANTIMICROBIAL ACTIVITY OF NEWLY SYNTHESIZED AND CHARACTERIZED MIXED BI-HETEROCYCLIC AZO COMPOUND (3-PYRIDYL-AZO-BENZIMIDAZOLE)

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ABSTRACT:
Azo-imine group containing Pyridine and benzimidazole in mixed bi-heterocyclic azo compound should have activities in biological fields. We are convinced from literature survey of pyridine and benzimidazole derivatives for synthesis of mixed bi-heterocyclic azo compound, 2-[(3′-pyridyl)azo]benzimidazole. Synthesis has been carried out by the reaction between diazonium salt of 3-aminopyridine and benzimidazole in alkaline solution at low temperature. After purification, structure of the newly synthesized compound has been characterized on the basis of IR, UV-Vis, ^{1}HNMR and Elemental analysis. Investigation of invitro anti-microbial activity of synthesized compound was done by well diffusion method against some common gram positive and gram negative bacteria. The successfully synthesized compound exhibited highest to moderate inhibitory effect against Gram-negative bacteria.

KEYWORDS: Pyridine, Benzimidazole, Bi-heterocyclic azo, Antimicrobial activity.

1. INTRODUCTION:

With change in environment microbes can change their activities on living things. Chemists are also deliberately engaged to discover new chemical compounds which are most effective to destroy such type of new microbes. Benzimidazole is one of the most important N-heterocyclic rings containing chemical compound which has valuable diverse activities in biological fields. We have searched out virtual activities of benzimidazole and its derivatives. Benzimidazole or its different derivatives have been successfully used as drugs in different fields, like: Anticancer[Ia-b] and Antidiabatic agents.[II] It has also different biological activities, such as: Antiviral,[III] Antifungal,[IV] Anthelmintic,[Va-b] Antibacterial,[VIa-b] Antagonist,[VII] and Selective Inhibitor.[VIIIa-b] Benzimidazole or its different derivatives also have ability to use as

ligand for complex formation with different metal ions.[IX] Some derivatives of benzimidazole shows catalytic activities.[Xa-b]

Another N-heterocyclic ring containing compound, pyridine, plays a key role in various purposes since it has versatile activities in biological and chemical fields. In many enzymes of living organism prosthetic pyridine nucleotide (NADP) is concerned in various oxidation and reduction processes. Pyridine derivatives are so much useful in existing drug as it has variable activities in biological fields like: Anti-microbial,[XIa-b] Anticancer,[XIIa-b] Antioxidant,[XIII] Antiinflammatory ,[XIV]Analgesic,[XV]Antihypertensive,[XVI] Antifungal,[XVII] Antitubercular.[XVIII]

Azo (-N=N-) group is a good chemical buster for changeable the biological activities of N-heterocyclic ring containing unsymmetrical mixed bi-heterocyclic azo compound though these found in literature are scarce[XIXa-b](**Figure-1**). Study of the versatile properties about pyridine and benzimidazole compounds in pharmaceutical field inspired us to synthesize a new type of mixed bi-heterocyclic azo compound. Henceforth we like to synthesize mixed bi-heterocyclic azo compound by joining two N-heterocyclic compound (Benzimidazole and Pyridine) with -N = N- spacer.

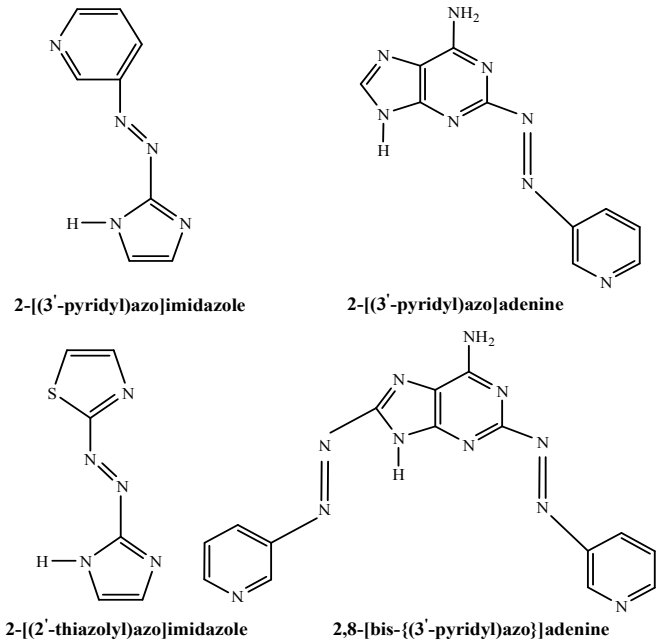


Figure-1:- Unsymmetrical bi-heterocyclic azo compounds

2.EXPERIMENTAL:

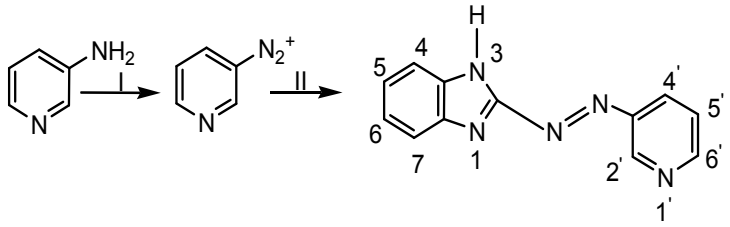
2.1. Material and Methods:

Reagents were all of analytical grade and purchased from Sigma Aldrich. The other chemicals were used without further refinement. IR spectra were recorded on a Shimadzu, FTIR Prestige-21 spectrometer in KBr pellets at Department of Chemistry, Burdwan University, Burdwan. UV-Vis spectra were examined on a UV-1800 Shimadzu spectrophotometer in MeOH solution. ^{1}HNMR spectra were recorded on 400MHz NMR

spectrometer by use DMSO-d_6 as solvent and reported relative to TMS as internal standard. Elemental analyses were recorded on an EL cube elemental analyzer. Both ^{1}HNMR and Elemental analysis were examined at SAIF, Cochin University, Kerala, India.

2.2. Synthetic Procedure of 3-Pyridyl-azo-benzimidazole:

Diazotization of 3-aminopyridine was performed by reaction of 3-aminopyridine (184mg, 2 mmol) with $NaNO_2$ (138mg, 2mmol) and Con.H_2SO_4 (0.83cc/5cc ; V/V) at nearly 0^0C. Then orange color diazonium salt solution was slowly added into KOH solution (210mg, 3.75mmol) of benzimidazole (236mg, 2mmol) with constant stirring until a red colour gummy precipitate appeared.(**Scheme:1**) The precipitate washed with 20ml water by rubbing with a glass rod, filtered and dried in to desiccators. The synthesized crude compound dissolved in small volume of MeOH and was performed TLC. Pure products were separated from crude synthesized compound by column chromatographic method and eluted as benzene, acetonitrile and methanol solvent containing compound. The benzene solvent containing compound was very unstable. Acetonitrile solvent containing gummy product was also unidentified. Finally, MeOH solvent eluted product was isolated after the evaporation of solvent as solid bright deep red compound.



I) $NaNO_2$-H_2SO_4, 0-2 ^{o}C; II) Benzimideazole in KOH solution, pH = 11-13

Scheme: 1

2.3. Spectral data of the synthesized compound:

Yield: (3.26g), 74%; Dark Brown solid; M.P.: 256^0C; IR spectrum, ν, cm^{-1}: 3086(-NH), 1575 (-C=N), 1417(-N=N-); UV-Vis (MeOH), λ_{max}, nm: 210(n-π^*), 250-260(π -π^*), 410-520(-N= N-); ^{1}HNMR Spectrum (400MHz, DMSO-d_6), δ, ppm (J, Hz): 8.41(1H, s, C_2′H-Py), 8.35 (1H, d, J=1.37, C_6′H-Py), 8.22(1H, d, J=1.03, C_4′H-Py), 7.42(1H, q, J=1.27, C_5′H-Py), 7.64 (2H, d, J=2.52, $C_{4,7}$H-Benzimidazole), 7.27(2H, q, J=1.0, $C_{5,6}$H-Benzimidazole); Elemental analysis: Found, %: C 55.12, H 5.98, N 21.50; Calculated, %: C 55.08, H 6.23, N 22.95.

2.4. Assay of Antibacterial Activity:

Antimicrobial activities of newly synthesized compound, 2-[(3′-pyridyl) azo]benzimidazole, in methanol solution was tested against some common Gram-positive bacteria like Streptococcus aureus, Bacillus subtilis and Gram-negative bacteria Pseudomonas fluorescence, Salmonella sp, Enterobacter aerogens and Escherichia coli. Potage culture of the above mentioned bacteria were spread on separate sterile Nutrient Agar (NA) plates and wells of 6mm diameter were made and methanol solution of the synthesized compound at 200µg/ml and 500µg/mL was added. The plates were then incubated in a B.O.D incubator at 37 ± 1^0C for 24hrs.

3. RESULTS AND DISCUSSION:

The designed mixed bi-heterocyclic azo compound is produced from N-heterocyclic ring containing pyridine and benzimidazole by the reaction between diazonium salt of 3-amino

pyridine and benzimidazole in KOH solution. We have obtained three products from the chromatographic separation of crude synthesized compound. Among them benzene solvent containing yellow product cannot be characterized because the compound is very unstable towards air or moisture. Probably this compound is N-[(3′-pyridyl)azo]benzimidazole. But dark-brown compound is effectively characterized from IR, UV-Vis, ^{1}HNMR and Elemental analysis data.

3.1. Spectral Studies:

FTIR spectra of the isolated compound gives broad absorption band in the region 3086cm^{-1} indicates the presence of -NH group. Strong absorption band appeared in the region 1575cm^{-1} and 1475cm^{-1} attributable to the stretching vibration of -C=N groups in two different heterocyclic rings. The synthesized compound provide medium intense absorption band at 1417cm^{-1} corresponds to the stretching vibration of -N=N- group.

UV-Vis spectra of synthesized compound were studied in CH_3OH and give three absorption bands at three different wavelengths. First, sharp intense absorption band appeared at 210nm reveals the n-π^* transition. Second, moderately broad absorption peak emerge at 250nm-260nm which indicates π-π^* transition. Third, broad peak observed at the region 410nm-520nm, which point out the transition for the presence of -N=N- group.

The ^{1}HNMR spectra of synthesized compound give comparatively strong sharp peak at the chemical shift position 8.35ppm related to C_6′-H proton in pyridine ring. It shows medium singlet peak, low doublet peak and medium broad doublet of doublet peak at the chemical shift position 8.41ppm, 8.22ppm and 7.38ppm-7.42ppm respectively which are point out C_2′-H, C_4′-H and C_5′-H protons in the pyridine ring. There was no such peak observed at the chemical shift position greater than 9.0ppm which indicates the absence of N-H proton because of the formation N-D, since DMSO-d_6 used as experimental solvent. NMR spectra of benzene part in benzimidazole gives quartet peak at the chemical shift position 7.24ppm - 7.26ppm indicates the existence of $C_{5,6}$-H protons. It gives doublet peak at the chemical shift position 7.62ppm-7.64ppm indicates the $C_{4,7}$-H protons.

3.2. Studies of Antibacterial Activity:

Azo-imine (-N=N-C=N-) group in mixed bi-heterocyclic azo compound[XIXa-b] can be used for achievement to control activities against bacteria. Antibacterial activities study of the synthesized compound was determined by well diffusion method at a concentration of 200µg/ml and 500µg/ml. A clear zone indicates the bacterial sensitivity to synthesize compound. The diameter of the zone of inhibition was measured by using antibiotic zone. The study compound did not show any inhibitory effect at the concentration level 200µg/ml against common bacteria. But at the concentration level 500µg/ml of the synthesized compound shows highest inhibitory effect against Gram-negative bacteria Pseudomonas fluorescence (27mm) followed by Salmonella sp (25mm) and medium effect against Escherichia coli(18mm). It did not show any antibacterial activity against Gram-positive bacteria such as Streptococcus aureus and Bacillus subtilis. Gram negative bacteria Enterobacter aerogens was also found to be activity resistant to this compound. These values were compared with standard drug Kanamycin(30µg/disc)(**Table-1**). We have also studied the antibacterial activity of 2-[(3′-pyridyl)azo]imidazole at concentration of 500µg/ml. But this compound failed to give any activity against common bacteria.

Table-1: Antibacterial activity of 2-[(3′-pyridyl)azo]benzimidazole in MeOH solution

Name of the bacteria		Zone of inhibition(mm)	
		Kanamycin(30µg/disc)	2-[(3-pyridyl)azo] benzimidazole (500µg/ml)
Gram positive	Streptococcus aureus	9	-
	Bacillus subtilis	7	-
Gram negative	Pseudomonas fluorescence	22	27
	Salmonella sp.	12	25
	Enterobacter aerogens	14	-
	Escherichia coli	15	18

Zone of inhibition values of the synthesized compound at 500µg/ml concentration."-" indicates no antimicrobial activity.

4. CONCLUSION:

We have successfully synthesized and characterized the objective compound as 2-[(3′-pyridyl)azo]benzimidazole and studied their antimicrobial activities. Then the result is compared with previously synthesized, 2-[(3′-pyridyl)azo]imidazole compound and the result also compared with standard drug. Benzene fused imidazole moiety has good antimicrobial activity compared with only imidazole is present in the synthesized bi-heterocyclic azo compound. The latter compound does not show any activity against common bacteria. The synthesized compound may have been established as drug by next step of investigation.

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