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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF UNSYMMETRICAL BI-HETEROCYCLIC-AZO-COMPOUND(3-PYRIDYL-AZO - ADENINE)

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Abstract:-
Adenine and its different derivatives have so many antibacterial activities which encouraged us to synthesize unsymmetrical bi-heterocyclic azo compound containing one N-heterocyclic nucleic acid as adenine and other pyridine spacer by azo group. Designed compound of 3-pyridyl azo adenine is to be synthesis by diazotization of 3-aminopyridine and then link with adenine in KOH solution. We search out two types of azo products by chromatographic separation: 2,8-[bis-{(3′-pyridyl)azo}]adenine (**1a**) and 2-[(3′-pyridyl)azo]adenine (**1b**). Synthesized compounds were characterized by elemental analysis, conductance, melting point, IR, UV-Vis and ^{1}HNMR spectral data. Both compounds were tested for antimicrobial activities against some common gram-positive and gram-negative bacteria. One of the synthesized compounds (**1a**) exhibits a little bit antimicrobial activity.

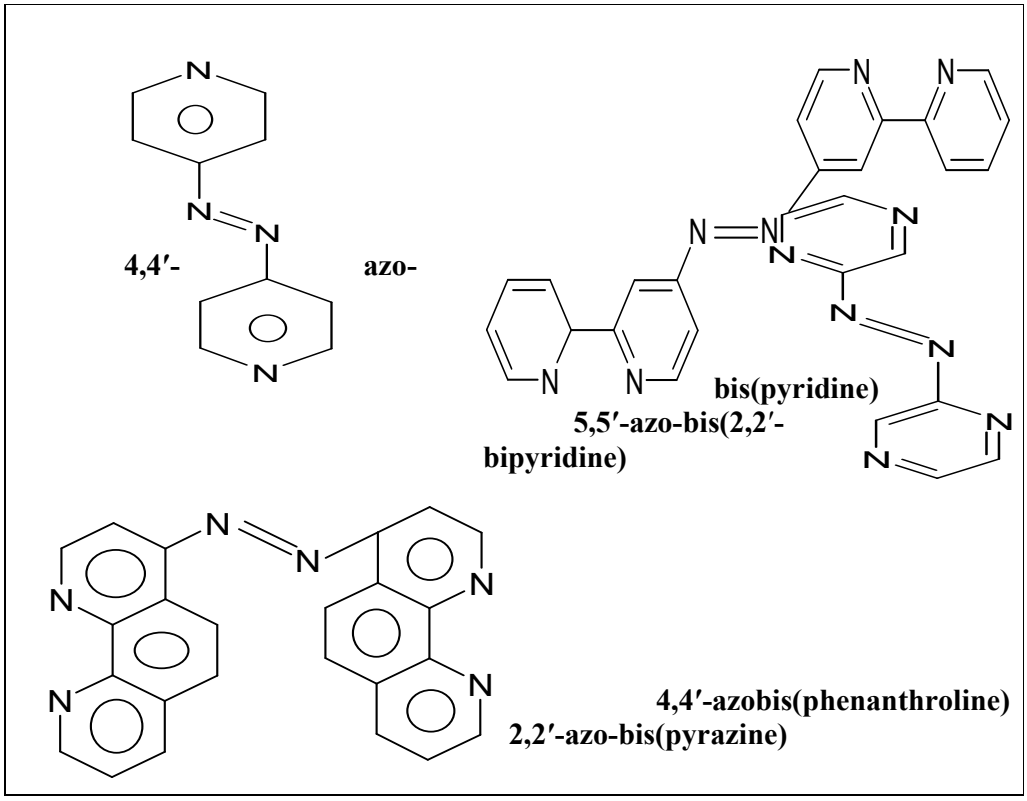
Keywords: Adenine**,** 3-aminopyridine, diazotization, Antimicrobial activity.

1. Introduction:

Symmetrical bi-heterocyclic compounds linked by azo-imine groups (-N=N-C=N-)[I,II] are found in literature(**Figure-1**) and such type of azo compounds have so many activities compared to other non-heterocyclic azo compounds[III,IV-VI]. Design and synthesis of unsymmetrical azo compounds containing N-heterocyclic rings are very much scarce [VII-VIII]. Nucleic acid related study has so much enthuse as seem to biological activities. Such nucleic acid component adenine and its derivative cover too much application in different fields like, biological activities [IX-XI], agrochemicals activities like: fungicides, insecticides and herbicides [XII] and also work as multimodal ligands [XIII-XV]. Another heterocyclic compound pyridine has too much potentiality in their different form with variable activities such as photo-physical, photochemical, antimicrobial, redox and anticancer [III, XVI-XVII]. The azo-imine group in 3-pyridyl-azo-adenine has controlling power over the two biologically active adenine and pyridine compounds. Synthesis of unsymmetrical azo compound containing biologically active N-heterocyclic rings are great confront to us. Finally, we were distinctly

obtained two different compounds of 3-pyridyl-azo-adenine. Henceforth the synthesize compounds were characterized by elemental analysis and IR, UV-Vis, NMR spectral data and also studies their antimicrobial activities.

Figure-1: Symmetrical Bi-heterocyclic Azo Compounds



2. EXPERIMENTAL SECTION:

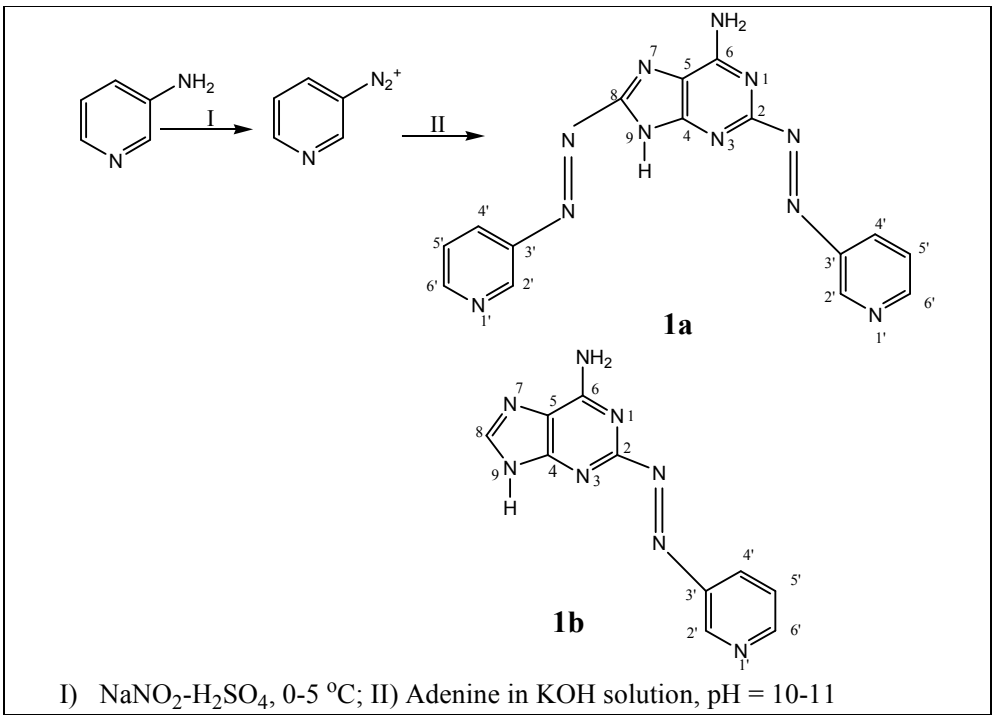
All reagents were purchased from Sigma Aldrich and the other chemicals used as analytical grade without further purification. Elemental analysis (C, H, N) was performed on a EL cube elemental analyzer at SAIF, Cochin, Kerala, India. Infrared spectra were recorded using FTIR Prestige-21 (Shimadzu) and U.V- Vis spectra were measured in ethanol using UV-1800 (Shimadzu) recorder spectrophotometer at Department of Chemistry, Burdwan University. ^{1}HNMR spectra were obtain on 300MHz NMR spectrometer using DMSO-d_6 and CD_3OD as solvent and reported relative to TMS as internal standard at IICB, Kolkata. An electro-thermal melting point apparatus was used for the determination of melting point. Conductivity also measures of the compounds were recorded at room temperature in ethanol solution.

2.1 Synthesis of 3-pyridyl azo adenine compound:

Cold solution of $NaNO_2$ (0.75g, 10.8mmol) was added to sulphuric acid solution (10ml) (1:1, V/V, prepared from commercially available $C.H_2SO_4$) of 3-aminopyridine (1g, 10.6mmol) under ice-salt both condition (maintained nearly at 0^o-2^oC temperature) until the solution colour totally changes to yellow. Adenine (1.45g, 10.7mmol) and potassium hydroxide (2.0g) was dissolved in 20ml water and cooled. To this cold solution 3-pyridyl-diazonium ion ($3\text{-}PyN_2^+Cl^-$) was added slowly with constant stirring under same temperature condition and finally orange-red compound was precipitated out. The mixture was left undisturbed for 4 hrs. Then the precipitate was filtered, washed with slight water and dried. Dry powder was suspended in 1N HCl solution (10ml) to produce an orange-red solution. Then pinch wise

solid $NaHCO_3$ was added to this solution until neutralize condition appear with an orange-red precipitate reappeared. The precipitate was filtered and washed with little water. The purified product was collected and dried. TLC of the isolated product was performed before using column chromatography containing 60-120 mesh silica gel for synthesized two distinctly products 2,8-[bis-{(3′-pyridyl)azo}]adenine (**1a**) and 2-[(3′-pyridyl)azo]adenine (**1b**). (**Figure-2**)

Figure-2: Synthetic Scheme



I) $NaNO_2$-H_2SO_4, 0-5 ^oC; II) Adenine in KOH solution, pH = 10-11

2,8-[bis-{(3′-pyridyl)azo}]adenine (1a):
Yield: 85%; M.P- 262^oC; ^{1}HNMR (CD_3OD) δ ppm: 8.13 & 8.19 (s, 1H, C_2H & $C_2'H$), 7.4-7.8 (m,1H,ArH); IR (KBr, υ/cm^{-1}): 3296 (NH_2, medium), 3095 (NH, broad), 1669 & 1597 (C=N), 1417 (N=N); UV-Vis (CH_3OH) λ nm: 205-242 (π to π*), 253-380 (n- π*), 405-513 (N=N); Elemental analysis Found (Calculated): C-47.32 (47.34), N- 44.30 (40.42), H- 4.20 (3.94).

2-[(3′-pyridyl)azo]adenine (1b):
yield: 75%; M.P-206^oC; ^{1}HNMR (CD_3OD) δ ppm: 8.07- 8.09 (s, 1H, C_8H), 7.4-7.8 (m,1H,ArH); IR (KBR, υ/cm^{-1}): 3296 (NH_2, medium), 3095 (NH, broad), 1669 & 1597 (C=N), 1417 (N=N); UV-Vis (CH_3OH) λ nm: 204-216 (m, π-π*), 219-275 (n- π*), 303-380 (N=N); Elemental analysis Found (Calculated): C-52.41 (52.20), N- 36.44 (37.48), H- 4.41 (4.35).

2.2 Antimicrobial activity studies of the synthesized compounds:
N-heterocyclic compounds such as pyridine and adenine derivatives have so many biological activity [IX-XI,XIV], hence forth we linked these compounds by azo (-N=N-) spacer to regulate the potentiality about their biological activities. We obtained 3-pyridyl azo adenine compounds in two different forms as **1a** and **1b** and their antimicrobial activities against common Gram-positive bacteria like *streptococcus aureus*, *Bacillus subtilis* and Gram-

negative bacteria as: *Pseudomonas fluorescence*, *Salmonella sp*, *Enterobacter aerogens* and *Escherichia coli*. Broth culture of the above mentioned bacteria were spread on separate sterile Nutrient Agar(NA) plates and wells of 6mm diameter were made and ethanol solution extract of the synthesized N-heterocyclic adenine containing azo compounds (**1a** & **1b**) at 500µg/ml was added. The plates were then incubated in a B.O.D incubator at 37±1^{o}C for 24hrs.

3. RESULT AND DISCUSSION:

3.1 Spectral property:

New kind of azo compound containing mixed N-heterocyclic rings (pyridine and adenine) have been synthesized by coupling diazonium salt of 3-aminopyridine with adenine in KOH solution (**Figure-2**). Chromatographic method has been applied to purify the product and isolated two compounds as **1a** and **1b**. They were characterized by IR, UV-Vis, ^{1}HNMR and elemental analysis.

FTIR spectra of synthesize compounds **1a** and **1b** gives a medium intense absorption band in the region 3296cm^{-1} which correspond to -NH_2 group of the adenine moiety. A little intense broad absorption band at 3095cm^{-1} represent for the presence of –NH group. Strong absorption band appear in the region1669cm^{-1} and 1597cm^{-1} due to the stretching vibration of –C=N- groups in two different heterocyclic rings. Both **1a** and **1b** compounds give medium intense absorption band at 1417cm^{-1} due to the stretching vibration correspond to –N=N- group.

It has been observed that the UV-Vis spectral data of adenine compound [XVII1] and its some derivatives give composite in nature [XIX]. UV-Vis spectra of synthesized compounds were studied in CH_3OH and they give three absorption bands at three different wavelengths. Compound **1a** give mild intense absorption band at the region 205nm to 242nm reveals the π to π^* transition whereas broad low intense bands show in the region 253nm to 380nm for the n to π^* transition. While compound **1b** give one mild intense peak at 204 nm to 216nm and medium band appeared in the region 219nm to 275nm correspond to the transitions π to π^* and n to π^* respectively. Both compounds give mild shoulder band at 405nm to 513nm and 303nm to 380nm which point out the transition for the presence of –N=N- group.

The ^{1}HNMR spectrum of compound **1a** in CD_3OD give comparatively strong peak and chemical shift moves at 8.19ppm - 8.13ppm related to two pyridine ring containing C_2-H and C_2'-H with different intensity indicating the presence of two rings with different chemical environment. We also find out multiples peak with very small intensities appeared at the chemical shift position 7.4ppm - 7.8ppm represent the presence of Ar-H. Here we can mention that the absence of peak at the chemical shift position 8.42ppm and 7.58ppm reveals the absence of C_2-H and C_8-H proton respectively [XX]. ^{1}HNMR spectrum of compound **1b** in DMSO-d_6 give low intense peak at the chemical shift position 8.07ppm to 8.09ppm indicate the presence of C_8-H proton. There was no such peak at 8.42 ppm which indicates the absence of C_2-H proton [V].

3.2. Antimicrobial studies:

Antibacterial activity of synthesized compounds **1a** and **1b** was determined by well diffusion method at a concentration of 500µg/ml and zone of inhibition was calculated using antibiotic zone scale. The synthesized compound **1a** shows highest inhibitory effect against Gram-negative bacteria *Pseudomonas fluorescence* (13mm) followed by *Salmonella sp* (11mm). It showed relatively smaller zone of inhibition against Gram-positive bacteria *Streptococcus aureus* (9mm) and *Bacillus subtilis* (8mm). *Enterobacter aerogens* and *Escherichia coli* were found to be resistant to this compound. No significantly inhibitory effect was seen by the

synthesized compound **1b**. These values were compared with the standard drug Kanamycin (30μg/disc). (**Table -1**)

5. CONCLUSION:

Two different N-heterocyclic rings are connected with the azo group (–N=N-) are limited in literature. In this work we used such N-heterocyclic compound which is the component of biological molecule for instance adenine. We satisfactorily synthesized the target compound and also studied their antimicrobial activities as application. The biological constituent containing bi-heterocyclic azo compound should be providing fewer side effects for used in medicinal purposes by further investigation.

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Table-1: Antimicrobial activity of 3-pyridyl-azo-adenine (3-paa) solution:

Name of the bacteria		**Zone of inhibition (mm)**	
		Kanamycin (30μg/disc)	2,8-[bis-{(3′pyridyl)azo}] adenine (**1a**) **(**500μg/ml)
Gram positive	*Streptococcus aureus*	9	9
	Bacillus subtilis	7	8
Gram negative bacteria	*Pseudomonas fluorescence*	22	13
	Salmonella sp	12	11
	Enterobacter aerogens	14	-
	Escherichia coli	15	-

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

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