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SYNTHESIS, SPECTRAL CHARACTERISATION AND ANTIMICROBIAL STUDIES OF A NOVEL SYMMETRICAL AZINE

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ABSTRACT

In this work, a novel symmetrical azine N,N′-bis(quinoxaline-2-carboxalidene)hydrazine (L) was synthesized from quinoxaline-2-carboxaldehyde and hydrazine hydrate by condensation reaction. The molecular structure of the azine was thoroughly confirmed by elemental microanalysis along with FT IR, UV visible, and ^{1}H NMR spectral techniques, all of which supported the proposed structural framework. Antimicrobial activity studies were carried out using the agar diffusion method, and the synthesized compound was evaluated against Escherichia coli as a representative Gram-negative bacterial strain. The experimental results revealed that the synthesized azine did not exhibit notable antibacterial activity under the tested conditions. The absence of significant activity may be attributed to several factors, including limited interaction with bacterial cell membranes, insufficient lipophilicity to penetrate the bacterial envelope, or the lack of key functional groups typically associated with antimicrobial efficacy. These findings suggest that further structural modification or derivatization may be necessary to enhance the biological potential of this class of compounds. It can be used as a ligand for the preparation of transition metal complexes, since metal coordination often enhances the biological activity of azine compounds.

Keywords: Symmetrical Azine, Quinoxaline-2-Carboxaldehyde, Hydrazine Hydrate, Condensation, Antimicrobial Activity, Escherichia Coli.

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INTRODUCTION

Azines constitute a unique subclass of conjugated Schiff bases.[1] Azines are an interesting class of compounds having the chemical formula RR'C=N–N=CRR" (R' = R" for symmetrical azines[2] and R' ≠ R" for unsymmetrical azines[3]). Because of their conjugated electronic systems and structural flexibility, they are closely related to aromatic heterocycles like pyridine, pyrimidine, triazine, and thiophene, which continue to be of significant scientific interest because of their structural and functional versatility.[4,5] Azines' inherent reactivity and adaptable molecular structure have made them useful in synthetic organic processes, coordination chemistry, and the development of pharmaceuticals and agrochemicals. The direct condensation of hydrazine with carbonyl compounds is the basis for conventional azines, and it is the most usual method for their preparation.[2,3] However, recent advancements in metal-catalysed processes have enabled more effective and environmentally friendly synthetic pathways.[4] Symmetrical azines are produced when identical carbonyl compounds are reacted, whereas unsymmetrical azines are produced when different carbonyl species react.[4,5,6] Azine derivatives are referred to as ketazines when derived from ketones and aldazines when derived from aldehydes, depending on the kind of carbonyl precursor. In an azine, an N-N bridge connects the two azomethine (-CH=N-) functionalities. That makes up the structurally distinctive C=N–N=C core of azines. This controls their chemical reactivity and permits various transformations and uses.[7] Strong polar and donor properties are conferred by their dual imine disposition and conformational flexibility with respect to the N–N single bond. Coordination and reactivity patterns are greatly influenced by the bonding arrangements of both symmetrical and asymmetrical variants, which are well documented. These ligands are inexpensive and simple to synthesize in addition to being effective and structurally adaptable. They are used in many industries and laboratory synthesises as both reagents and intermediates.[8,9] They are also appealing for use in advanced materials science because of their conformational flexibility, especially in liquid crystalline systems and

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nonlinear optical materials, such as twisted-nematic display technologies. Vibrant and stable colours are produced in the dye industry by aromatic-substituted azines, which function as chromophoric units with adjustable electronic properties.[10,11,12] Furthermore, with notable antibacterial, antimalarial, antiviral, antitumor, and anti-inflammatory properties against a variety of biological targets, azines are crucial scaffolds in medicinal chemistry.[2] In this work, a symmetrical azine derived from aromatic aldehyde, such as quinoxaline-2-carboxaldehyde, is synthesized by conventional condensation, yielding crystalline material suitable for in-depth structural and spectroscopic analysis. The synthesis and characterization of quinoxaline-2-carboxaldehyde azine are covered in detail in the following sections, with a focus on structural analyses. The quinoxaline core's steric and electronic effects give the compound unique polydentate ligand characteristics that make it useful for coordination chemistry applications. They can stabilize a variety of metal coordination environments by their conjugated π-systems and nitrogen lone pairs. Therefore, they can be essential building blocks for dinuclear metallohelicates. However, detailed investigations focusing specifically on the synthesis, spectral characteristics, and biological activity of azines and their derivatives are still ongoing. Therefore, the present study aims to synthesize a novel symmetrical azine from quinoxaline-2-carboxaldehyde. By examining its spectral properties and antimicrobial activity for a better understanding of this compound. Investigations into the synthesis, characterization, and electronic properties of this symmetric azine contribute to the development of novel compounds that may lead to improved pharmaceuticals, advanced functional materials, and innovative chemical processes. By improving our understanding of the structural and electronic behavior of these compounds, such research supports sustainable scientific progress and the discovery of molecules with potential benefits for healthcare and technological advancement.

EXPERIMENTAL

Material and Methods

Hydrazine hydrate (Merck) and quinoxaline-2-carboxaldehyde (Sigma Aldrich) were used as received. All other chemicals were of analytical grade purity and used without purification.

Synthesis of N,N′-bis(quinoxaline-2-carboxalidene)hydrazine (L)

A detailed synthetic pathway for the azine derivative L proceeds as follows: A fresh solution of quinoxaline-2-carboxaldehyde (0.3163g, 2 mmol) in anhydrous methanol (50 mL) is taken in a 100 mL round-bottom flask. Into the same round-bottom flask, a solution of hydrazine hydrate (0.05 mL, 1 mmol) in methanol (25 mL) is introduced dropwise via continuous, controlled stirring, ensuring optimal mixing of the reactant species. Immediately after mixing, the solution exhibits a yellow colour. As the solution stirs constantly, subsequently, the target yellow azine, L, precipitates spontaneously in accordance with Scheme-1. The precipitate is collected by filtration, and to ensure maximum purity and removal of occluded solvent or unreacted species, the isolated solid is sequentially washed with ice-cold methanol. Thorough desiccation over anhydrous calcium chloride yields the final product with an appreciable yield of 60% and a sharp melting point at 252°C.

Detection Method

Microanalyses of the compound was done with an Elementar Vario EL III CHNS elemental analyser. FT-IR spectrum was recorded in KBr pellets with a JASCO FTIR 4100 spectrophotometer from 4000 to 400cm^{-1}. The electronic spectrum of the azine L was recorded on a Thermoelectron Nicolet Evolution 300 UV-Vis spectrophotometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker 250 NMR spectrometer. The antimicrobial potential was tested by the well diffusion technique.

Antimicrobial Activity

Mueller-Hinton agar plates were seeded with 18–24-hour-old cultures of Escherichia coli (*E.coli*). Two wells were cut into the agar using a sterilised cork borer, and 100 µL of the sample (L) at a concentration of 100 mg/mL was added to each well. Gentamicin (100 µL per well) and methanol (100 µL per well) were used as the positive and negative controls, respectively. The inoculated plates were incubated at 37 °C for 24 hours, and the zones of inhibition were measured in millimetres (mm).[13]

RESULTS AND DISCUSSION

The discussions here, based on investigations into the synthesis, spectral properties, and antimicrobial activity of this symmetric azine, reveal intriguing insights about its structural and physicochemical behavior. Such studies contribute to the broader understanding of the compound and may support the development of new functional materials and potential applications in pharmaceutical chemistry. The synthesised symmetrical azine, L, N, N′-bis(quinoxaline-2-carboxalidene)hydrazine, is a non-hygroscopic, yellow-colored compound and stable in air at room temperature. The analytical data of the compound L confirm its molecular formula (Table-1), as shown in Scheme-1. The azine is soluble in common organic solvents such as methanol, ethanol, chloroform, DMF, and DMSO.

Table-1: Analytical Data of N,N′-bis(quinoxaline-2-carboxalidene)hydrazine, L

Azine	Empirical formula	Colour	Formula weight	Carbon (%)	Hydrogen(%)	Nitrogen(%)
L	$C_{18}H_{12}N_6$	Yellow	312	69.27 (69.22)	3.70 (3.87)	27.02 (26.91)

Electronic Spectral Study of N,N′-bis(quinoxaline-2-carboxalidene)hydrazine (L)

The electronic spectroscopic investigation of the azine, L, was carried out in methanol solution ($1x10^{-5}$ M) in the range 200-900 nm [Table-2]. The spectra of azine, L, exhibit three absorption maxima [Fig.-1]. These compounds exhibit bands of both π→π* and n→π* transitions and are assigned to quinoxaline and azomethine systems. The n→π* transition may be due to the nonbonding electrons present in the transition of the C=N group in the quinoxaline ring or in the azomethine group.[14,15,16,19] This transition is less intense than the π→π* transitions. The electronic spectral analysis of the synthesized azine revealed characteristic π→π and n→π* transitions, which are consistent with previously reported spectral behaviour of similar imine compounds.

Table-2: Electronic Spectral Data of Azine, L

Azine	λmax (nm)	λmax (cm^{-1})	Band assignment
L	202	49504	π → π*
	245	40810	π → π*
	316	31640	n → π*

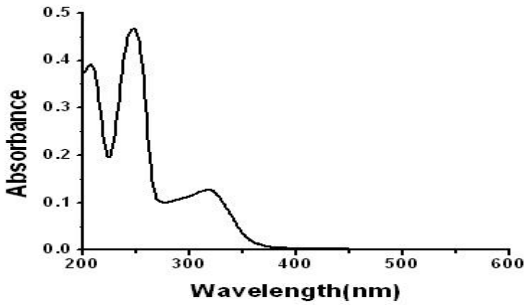

Fig.-1: Electronic Spectrum of Azine, L

IR Spectral Study of N,N′-bis(quinoxaline-2-carboxalidene)hydrazine (L)

The most significant IR spectral bands are given in Table-3. Examination of the $–NH_2$, –CH=O, and −C=N− regions of the infrared spectra provided valuable structural information related to the structure of the azine. The absence of the characteristic stretching frequency of C=O of the aromatic aldehyde and $–NH_2$ groups of hydrazine indicates that the condensation was complete. Although this compound, L, contains different types of C=N bonds, there are two from each quinoxaline ring and two from the azomethine linkage. The IR bands due to them are not well resolved in the infrared spectrum of the L. But this shows [Fig.-3] a very strong absorption band in the 1622 cm^{-1} range, which is characteristic of the azomethine ν(C=N) group.[15,16] The peak that appears in the range 1568 cm^{-1} could be due to the ν(C=N) stretches of the quinoxaline ring. The bands in the range 3050-2980 cm^{-1} could be assigned to the C-H stretching of aromatic and methylene groups, respectively. The medium-intensity bands observed in the

range 1220-923 cm^{-1} may be due to the in-plane bending of the aromatic C-H, and those in the 900-700 cm^{-1} range may be due to the out-of-plane bending vibration of the aromatic C-H.

Table-3: IR Spectral Data of Azine, L (ν in cm^{-1})

Azine	$\nu_{(C=N)}$ (Azomethine)	$\nu_{(C=N)}$ (Quinoxaline)	ν (C-H) (Aromatic)
L	1622	1568	3048

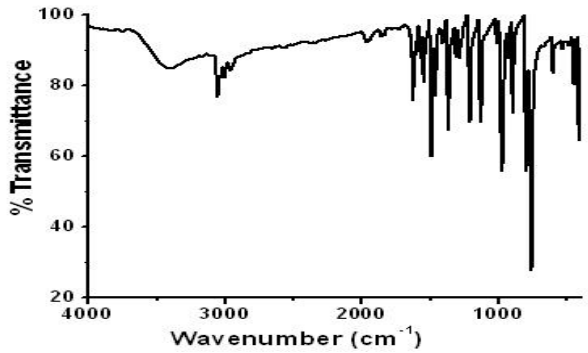

Fig.-3: IR Spectrum of Azine, L

NMR Spectral Study of N,N′-bis(quinoxaline-2-carboxalidene)hydrazine (L)

The proton NMR spectrum of azine compound L was recorded in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The spectrum displays a characteristic set of resonances that confirms the proposed structure. In the spectrum of azine, L, a signal around 1.6 ppm arises from water impurities in the deuterated solvent. The resonances for $CHCl_3$ and TMS are observed at approximately 7.2 ppm and 0 ppm, respectively. The NMR spectrum of L is shown in Fig.-4. The eight aromatic protons of the azine compound L consistently appear as a multiplet in the range of 7.80–8.20 ppm, revealing their aromatic environment. Distinct singlets corresponding to the two imine (-CH=N-) protons are clearly observed at δ 8.90 ppm and 9.70 ppm, confirming the formation of the azomethine functionalities.[17,18,19] These findings are listed in Table-4. The values are in strong agreement with classical azine NMR behavior reported in the literature[16], where multiplet patterns between 7.45 and 8.22 ppm are typical for aromatic protons, and singlets ranging from 8.0 to 9.7 ppm affirm the presence of imine groups. Thus, the NMR spectral analysis provides definitive evidence for the azine structure and integrity of the synthesized compound.

Table-4: NMR Spectral Data of Azine, L (ν in cm^{-1})

Azine	Chemical shift δ (ppm)	Assignment
L	9.70, 8.90 7.80-8.20	(s, 2H, CH azomethine) (m, 8H, aromatic protons)

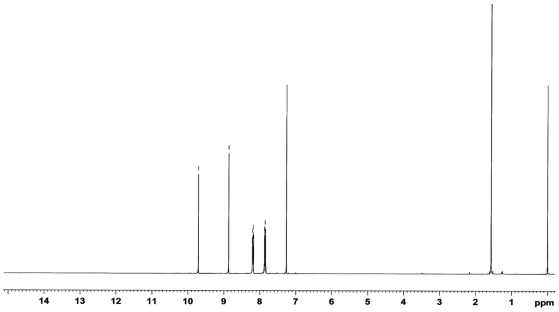

Fig.-4:^{1}H NMR Spectrum of Azine L

The Antimicrobial Activity of N,N′-bis(quinoxaline-2-carboxalidene)hydrazine (L)

The agar diffusion method was employed to evaluate the antimicrobial activity of test sample L against Escherichia coli. The absence of an inhibition zone in methanol, used as the negative control, confirmed

that the solvent had no antimicrobial effect. Gentamicin served as the positive control. The antimicrobial activity of the test sample was assessed by measuring the diameter of the inhibition zone; however, no zone was observed, indicating that sample L exhibited no antimicrobial activity against *E.coli*. The results were interpreted using standard and widely accepted methods. Overall, these findings indicate that compound L possesses no significant antimicrobial activity against E.coli.[5,20,21,22] The absence of significant antibacterial activity may be attributed to the limited interaction with bacterial cell membranes or the lack of key functional groups associated with antimicrobial efficacy. These findings show that further structural modification may be necessary to enhance the biological potential of this compound.

CONCLUSION

Through the condensation of quinoxaline-2-carboxaldehyde and hydrazine hydrate, a symmetrical azine, N,N′-bis(quinoxaline-2-carboxalidene)hydrazine (L), was purposefully molecularly assembled in this study, producing a yellow powder material of exceptional purity. Thorough analytical examination, including elemental analysis as well as UV-visible, FTIR, and NMR spectroscopy, confirmed the synthetic compound's structural authenticity and electronic profile. This methodical and comprehensive approach confirms the synthetic process and clarifies the quinoxaline moiety's basic role in altering the resulting azine architecture's physicochemical and coordination tendencies. The evaluation indicates that test sample L exhibits no antimicrobial activity against Escherichia coli, implying its ineffectiveness as an antibacterial agent for this strain.

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CONFLICT OF INTERESTS

The author declares that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-9: Industry, Innovation and Infrastructure*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the author can be verified from their ORCID ids, given below:

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