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SYNTHESIS AND CHARACTERIZATION OF AN AZO COMPOUND-BASED SCHIFF BASE DERIVED FROM RHODAMINE B

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ABSTRACT: Rhodamine-based Schiff bases are interesting functional materials because of their bright color, durability, and biomedical uses. The aim of this study was to synthesize and characterize the novel Rhodamine-based Schiff base (RSB) to investigate their structural characteristics. The synthesized dyes were characterized *via* FTIR, ^{1}H NMR and ^{13}C NMR to ensure their structural integrity and purity. This approach has many appealing advantages, including excellent yields, quick reaction times, and easy work-up.

INTRODUCTION: Schiff bases are a diverse category of chemicals distinguished by an imine (C=N) functional group, generally synthesized by the condensation process between a primary amine and an aldehyde or ketone. These chemicals function as intermediates in organic synthesis and functional materials owing to their facile production, adjustable properties, and extensive use in catalysis, medicinal chemistry, and materials research [1]. Azo compounds are characterized by one or more azo groups (-N=N-) and are classified as homogeneous or heterogeneous on the basis of the similarity or difference of the groups attached to the azo group, which offers optical, thermal, and electrical benefits for applications in sensing, imaging, and light-responsive materials [2].

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Rhodamine B, a xanthene dye noted for its robust fluorescence and photostability, has been incorporated into Schiff base frameworks, yielding multifunctional molecules with improved fluorescence, selective binding, and chemosensory capabilities [3]. Schiff bases demonstrate significant biological activities, including antibacterial, antifungal, anticancer, and anti-inflammatory properties, especially when they form metal complexes. These complexes, formed from stable metal ion interactions, serve as versatile chelating ligands with applications in biological and pharmaceutical chemistry [4].

Aromatic aldehydes are typically favored in Schiff base synthesis because of their stability and superior conjugation processes, whereas aliphatic aldehydes are susceptible to polymerization [5]. The structural diversity and flexibility of Schiff bases have been thoroughly investigated for their chemical and physical properties, facilitating their application in aldehyde or ketone detection, purification of carbonyl and amino compounds, and sensitive reactions [6, 7].

Schiff bases are widely utilized in numerous fields and play crucial roles in the formulation of pigments, dyes, catalysts, polymer stabilizers, and chemosensors. Rhodamine-based Schiff bases have attracted considerable interest because of their diverse uses in multiple domains. These compounds are widely utilized as selective chemosensors for the detection of metal ions, including Al^{3+}, Fe^{3+}, Cr^{3+}, Hg^{2+}, and Cu^{2+}, demonstrating their utility in evaluating water quality and monitoring biological samples [8]. The robust fluorescence and photostability of these materials render them optimal for bioimaging applications, facilitating cellular imaging and monitoring of biological processes in live systems, including zebra fish and mammalian cells [9]. In environmental monitoring, these Schiff bases are proficient in identifying heavy metal ions and harmful contaminants, tackling significant issues associated with heavy metal toxicity and contamination in aquatic ecosystems [10].

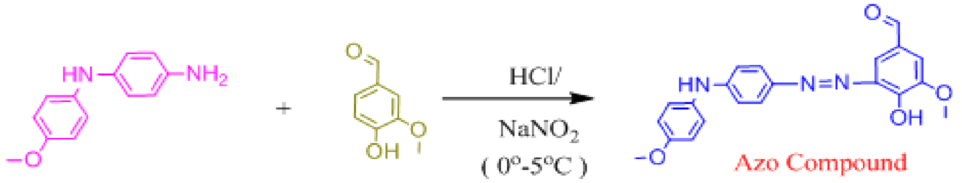
Additionally, several rhodamine-derived Schiff bases demonstrate potential anticancer and antibacterial properties. They engage with particular biological targets or microbial systems, exhibiting cytotoxic effects on cancer cells and suppressing pathogenic bacteria and fungi, especially in the presence of metal complexes [11, 12]. In molecular computing, these chemicals function as elements for constructing molecular logic gates, such as inhibit and or gates, by utilizing fluorescence alterations induced by particular inputs [13, 14, 15]. Moreover, rhodamine-based Schiff bases are employed in light-responsive materials, facilitating applications in photo switchable devices and energy-efficient coatings, as well as in drug administration systems where their fluorescence aids in the real-time monitoring of therapeutic agent release. They exhibit potential in photodynamic therapy for cancer treatment by producing reactive oxygen species (ROS) upon light activation, selectively attacking tumor cells while preserving healthy tissues [16]. Finally, in the field of green chemistry, rhodamine-based Schiff bases have been investigated as catalysts in organic processes, including C–C bond formation and oxidation, demonstrating their stability and adjustable features [17]. Finally, in the field of green chemistry, rhodamine-based Schiff bases have been investigated as catalysts in organic processes, including C–C bond formation and oxidation, demonstrating their stability and adjustable features [18]. The extensive uses underscore the versatile properties of rhodamine-based Schiff bases in the progression of science and industry. The characterization of rhodamine-azo Schiff bases (RSBs) utilizes sophisticated analytical methods to confirm the molecular structures and interactions of functional groups [19]. Fourier transform infrared spectroscopy (FTIR) delineates distinct vibrational modes, validating alterations in functional groups and interactions at binding sites [20]. Proton nuclear magnetic resonance (^1H NMR) elucidates hydrogen environments, revealing structural frameworks and molecular conformations, whereas ^{13}C NMR corroborates carbon skeleton configurations [21]. Liquid chromatography–mass spectrometry (LC–MS) enables accurate molecular weight determination and structural elucidation, facilitating the identification of fragments and byproducts [22, 23]. These thorough characterization techniques are essential for the development of dependable RSBs for environmental monitoring and biomedical diagnostics.

MATERIALS AND METHODS: All of the chemicals, reagents, and solvents used were obtained from Sigma–Aldrich, Thermo Fisher Scientific, and Hi-Media. We purchased analytical-grade ingredients, such as Rhodamine B (99.0%), vanillin (99%), variamine blue (99%), and other solvents, from Sigma–Aldrich and used them without any additional purification.

Synthesis of the Azo dye 4-hydroxy-3-methoxy-5-((4-((4-methoxyphenyl) Amino) Phenyl) diazenyl) Benzaldehyde and Rhodamine B Hydrazide: The azo compound was synthesized via the method reported by Rao *et al.* (2013) [24]. A precursor (0.837 g, 4.12 mmol) was dissolved in concentrated HCl, producing a green solution, which was stirred in an ice-salt bath at 5°C. Sodium nitrite (0.284 g, 4.12 mmol) was added, turning the solution light yellow. This diazo solution was mixed with a solution of vanillin (0.627 g, 4.12 mmol), sodium hydroxide (0.165 g, 4.12 mmol), and sodium carbonate (0.266 g, 2.51 mmol) in water. The mixture was incubated at room temperature, filtered, and treated with 10% sodium chloride. The resulting orange precipitate was washed, vacuum-dried, and confirmed *via* chromatography. The yield was 80%, with a melting point of 110°C. Rhodamine B hydrazide (RBH) was synthesized following the methods described by Xiang *et al.* (2007) and Gupta **et al.** (2015) [25, 26]. Rhodamine B (1.20 g, 2.5 mmol) was dissolved in ethanol, and hydrazine hydrate (85%) was added dropwise while stirring. The mixture was refluxed for 2 hours, turning from purple to bright orange. After cooling, the solvent was removed, and 1 M HCl was added, resulting in the formation of a red solution. Sodium hydroxide was then added to adjust the pH to 9.0, precipitating a peach-colored solid (0.83 g, 75% purity). The solid was dried via infrared radiation, and the purity was increased through CH_3CN/H_2O crystallization.

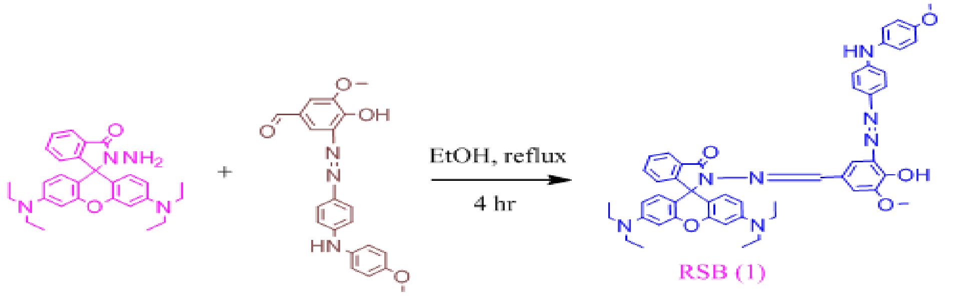
Synthesis of the Azo compound-based Schiff base 3',6'-bis (diethylamino)-2-((4-hydroxy-3-methoxy-5-((4((4methoxyphenyl) amino) phenyl) diazenyl) benzylidene) amino) spiro[iso indoline-1,9'-xanthen]-3-one: The Schiff base was synthesized via the method of Mabhai *et al.* (2018) [27], with modifications involving a reflux reaction between Rhodamine B hydrazide (0.536 g, 1.175 mmol) and 4-hydroxy-3-methoxy-5-((4-((4-methoxyphenyl) amino) phenyl) diazenyl) benzaldehyde (0.430 g, 1.175 mmol) in 20 mL of ethanol (Scheme 1). The mixture was refluxed for 4 hours with a $CaCl_2$ guard tube, forming a maroon solid. After cooling, TLC was used to monitor the progress of the reaction. The volume was reduced by one-third by heating, and the mixture was filtered. Washing with cold ethanol-ether (1:1) yielded a reddish-brown precipitate with a 65% yield and a melting point of 210°C.

STEP 1:



4-hydroxy-3-methoxy-5-((4-((4-methoxyphenyl)amino)phenyl)diazenyl)benzaldehyde
Chemical Formula: $C_{21}H_{19}N_3O_4$
Exact Mass: 377.14
Molecular Weight: 377.40
m/z: 377.14 (100.0%), 378.14 (22.7%), 379.14 (2.5%), 378.13 (1.1%)
Elemental Analysis: C, 66.83; H, 5.07; N, 11.13; O, 16.96

STEP 2



3',6'-bis(diethylamino)-2-((4-hydroxy-3-methoxy-5-((4-((4-methoxyphenyl)amino)phenyl)diazenyl)benzylidene)amino)spiro[isoindoline-1,9'-xanthen]-3-one
Chemical Formula: $C_{49}H_{49}N_7O_5$
Exact Mass: 815.38
Molecular Weight: 815.98
m/z: 815.38 (100.0%), 816.38 (53.0%), 817.39 (13.8%), 816.38 (2.6%), 818.39 (2.3%), 817.38 (1.4%), 817.38 (1.0%)
Elemental Analysis: C, 72.13; H, 6.05; N, 12.02; O, 9.80

SCHEME 1: SYNTHESIS OF AN AZO COMPOUND-BASED SCHIFF BASE DERIVED FROM RHODAMINE B

Characterization of Synthesized Rhodamine-Based Schiff Base: Infrared (IR) spectra were recorded using a Bruker Alpha FT-IR spectrophotometer at a scanning range from 4000 to 500 cm^{-1} to identify functional groups and structural features. Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were obtained in DMSO-d6 and $CDCl_3$ solutions, with tetramethylsilane (TMS) serving as an internal reference. These spectra were recorded via a 400 MHz Varian NMR instrument to determine the chemical environment of the hydrogen and carbon atoms in the compound.

RESULTS AND DISCUSSION: The present study was carried out to synthesize, characterize, and investigate an azo compound-based Schiff base derived from Rhodamine B. The synthesized Schiff base (SB) was thoroughly characterized via a range of analytical techniques, including Fourier transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance (^1H NMR), carbon-13 nuclear magnetic resonance (^{13}C NMR), and liquid chromatography–mass spectrometry (LC–MS). These methods were employed to confirm the structural integrity, purity, and functional group identification of the synthesized compounds, ensuring their suitability for further applications. The comprehensive characterization provided a clear understanding of the molecular composition and structural features of the Schiff base, facilitating its potential use in various scientific and industrial applications.

Characterization of Synthesized Rhodamine-Based Schiff base: The radionuclide-based Schiff base was systematically evaluated via FTIR, ^1H NMR, and ^{13}C NMR. The characterization of the Rhodamine-based Schiff base azo compound was carried out via FTIR, ^1H NMR, and ^{13}C NMR spectroscopic techniques under standard conditions. FTIR analysis was conducted in the range of 4000–400 cm^{-1} to evaluate the identification of functional groups through their characteristic vibrational frequencies. For ^1H NMR and ^{13}C NMR spectroscopy, deuterated solvents, such as DMSO-d_6 or $CDCl_3$, were used as the media to dissolve the compounds, with tetramethylsilane (TMS) as an internal standard. The NMR spectra were recorded at room temperature on a high-resolution spectrometer operating at frequencies of 400 MHz for ^1H and 100 MHz for ^{13}C nuclei. These conditions facilitated the precise identification of the proton and carbon environments in the compound, confirming its structural attributes and functional group connectivity.

FTIR Spectra of the Rhodamine-Based Schiff Base: The FTIR analysis revealed several characteristic peaks corresponding to different functional groups and interatomic bonds **Fig. 1**. The prominent peak at 2351.40 cm^{-1} was attributed to the stretching vibration of the hydroxyl group (O-H). The peak observed at 1609.72 cm^{-1} was attributed to the stretching vibration of C–H bonds, which are commonly found in alkanes. The significant peak at 1512.43 cm^{-1} was assigned to the stretching of carbonyl groups (C=O), highlighting the presence of ketones, aldehydes, or other related compounds. The peak at 1258.07 cm^{-1} corresponded to the stretching of C=C bonds, indicating the presence of alkenes or aromatic compounds. Another peak at 1112.02 cm^{-1} was attributed to C-H bending vibrations, which are likely associated with aromatic or alkane groups. The peak at 691.91 cm^{-1} was attributed to the stretching vibrations of C–O bonds, suggesting the presence of alcohols, esters, or ethers. Furthermore, the peak at 619.85 cm^{-1} was indicative of out-of-plane bending vibrations of C-H bonds, which are often found in aromatic or alkane structures. Finally, the peak at 526.71 cm^{-1} suggested possible low-frequency vibrations, which may be related to specific structural characteristics within the compound. The FTIR peak positions and interatomic bonds of the rhodamine-based Schiff base are depicted in **Table 1.**

TABLE 1: FTIR PEAK POSITIONS AND INTERATOMIC BONDS OF THE RHODAMINE-BASED SCHIFF BASE

Peak Position (cm^{-1})	Interatomic Bond
2351.40	O-H (Hydroxyl group, stretching)
1609.72	C-H (Alkane, stretching)
1512.43	C=O (Carbonyl group, stretching)
1258.07	C=C (Alkene or aromatic, stretching)
1112.02	C-H (Aromatic or alkane, bending)
691.91	C-O (Alcohols, esters, or ethers, stretching)
619.85	C-H (Out-of-plane bending, aromatic or alkanes)
526.71	Stretching vibration of alkyl halides (C-Br).

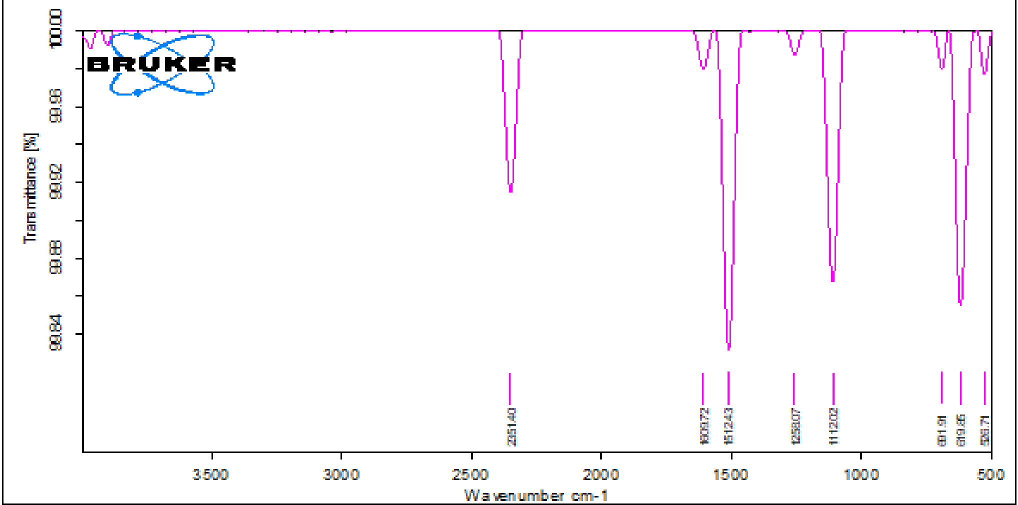


FIG. 1: FTIR PEAK POSITIONS AND INTERATOMIC BONDS OF THE RHODAMINE-BASED SCHIFF BASE

^{1}H NMR Profiling of the Rhodamine-Based Schiff Base: The ^{1}H NMR data of the Rhodamine-based Schiff base azo compound provided detailed insights into the proton environment within the molecule. The peak at 8.188 ppm (doublet of triplets, J = 7.8 Hz, 1H) was attributed to an aromatic proton, followed by a signal at 8.112 ppm (doublet, J = 7.8 Hz, 1H), which also corresponded to an aromatic proton. Additional aromatic protons were observed at 7.986 ppm (multiplet, J = 7.5 Hz, 1H), 7.943 ppm (multiplet, J = 7.5 Hz, 1H), 7.844 ppm (multiplet, J = 7.5 Hz, 1H), 7.644 ppm (multiplet, J = 7.5 Hz, 1H), 7.606 ppm (multiplet, J = 7.5 Hz, 1H), 7.312 ppm (multiplet, J = 7.2 Hz, 1H), and 7.206 ppm (multiplet, J = 7.2 Hz, 1H). The presence of two additional aromatic protons was confirmed by peaks at 6.642 ppm (multiplet, J = 6.8 Hz, 1H) and 6.577 ppm (multiplet, J = 6.8 Hz, 1H). Exchangeable protons were identified at 6.212 ppm (singlet, 1H) and 6.002 ppm (singlet, 1H), which corresponded to NH or OH groups, suggesting the presence of hydrogen-bonding interactions or labile protons. The signals at 3.926 ppm (triplet, J = 6.0 Hz, 1H), 3.896 ppm (triplet, J = 6.0 Hz, 1H), and 3.856 ppm (triplet, J = 6.0 Hz, 1H) were assigned to CH_2 groups adjacent to electronegative atoms such as nitrogen or oxygen. Furthermore, CH_2 groups in proximity to aromatic systems were observed at 2.967 ppm (multiplet, J = 5.8 Hz, 1H) and 1.988 ppm (multiplet, J = 5.5 Hz, 1H). Terminal CH_3 groups were identified at 1.274 ppm (doublet, J = 7.0 Hz, 3H), 1.122 ppm (doublet, J = 7.0 Hz, 3H), and 1.071 ppm (doublet, J = 7.0 Hz, 3H), whereas isolated CH_3 groups were detected at 0.940 ppm (singlet, 3H), 0.870 ppm (singlet, 3H), and 0.813 ppm (singlet, 3H). A trace impurity or additional isolated CH_3 group was indicated by a signal at 0.093 ppm (singlet, 1H). ^{1}H NMR profiling of the Rhodamine-based Schiff base depicted in **Fig. 2**.

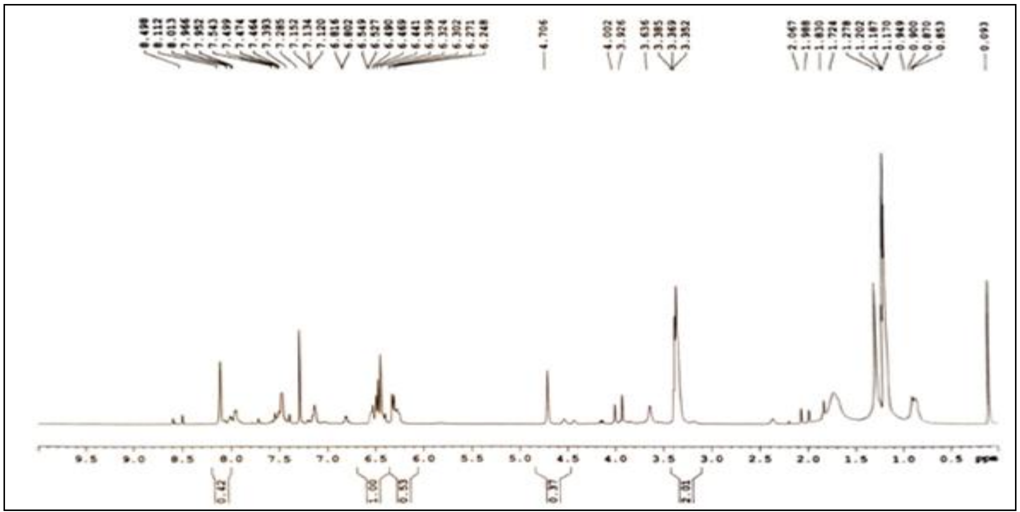


FIG. 2: ^{1}H NMR PROFILING OF THE RHODAMINE-BASED SCHIFF BASE

These findings confirmed the structural integrity of the synthesized Schiff base azo compound, highlighting the presence of aromatic protons, exchangeable NH or OH groups, CH_2 groups adjacent to electronegative atoms, and terminal as well as isolated CH_3 groups. The comprehensive proton distribution validated the expected molecular framework, supporting the successful synthesis of the compound.

^{13}C NMR Profiling of the Rhodamine-Based Schiff Base: The ^{13}C NMR spectrum of the rhodamine-based Schiff base azo compound is shown in **Fig. 3.** The signal at 166.17 ppm was attributed to the carbonyl carbon (C=O) in the amide or ester group, confirming the presence of this functional moiety. The peaks at 156.45 ppm and 153.87 ppm were assigned to aromatic carbons bonded to electron withdrawing and electronegative groups, respectively, highlighting the substitution pattern in the aromatic ring system. Quaternary aromatic carbons were identified at 141.92 ppm and 138.69 ppm, indicating the presence of nonhydrogenated aromatic carbons in the structure. Several signals, including those at 133.17 ppm, 131.17 ppm, 130.75 ppm, 128.58 ppm, 128.12 ppm, 122.84 ppm, and 122.08 ppm, were assigned to aromatic CH carbons, confirming the presence of multiple aromatic rings. The peaks observed at 111.60 ppm and 110.06 ppm were attributed to aromatic or heteroaromatic CH carbons, whereas the signals at 106.26 ppm, 100.61 ppm, and 97.84 ppm corresponded to heteroaromatic carbons, which are likely associated with nitrogen or oxygen-containing rings. The solvent peak ($CDCl_3$) was observed in the range of 77.23–76.92 ppm, and the signal at 65.85 ppm indicated the presence of CH_2 groups adjacent to electronegative atoms, such as oxygen or nitrogen. The peak at 55.94 ppm suggested the presence of methoxy groups or CH_3 groups attached to oxygen. The signal at 44.38 ppm was assigned to aliphatic CH_2 groups adjacent to nitrogen atoms, supporting the presence of secondary or tertiary amines. Furthermore, peaks in the range of 31.13–29.37 ppm were assigned to aliphatic CH_2 groups, whereas signals at 22.70 ppm and 12.62 ppm corresponded to terminal methyl (CH_3) groups. The final peak at 1.02 ppm indicated an isolated methyl group. These findings confirmed the presence of aromatic, aliphatic, and heteroaromatic structural features in the Schiff base azo compound. The presence of carbonyl, methoxy, and heteroaromatic carbons was consistent with the expected molecular framework, confirming the successful synthesis and structural integrity of the Rhodamine-based compound.

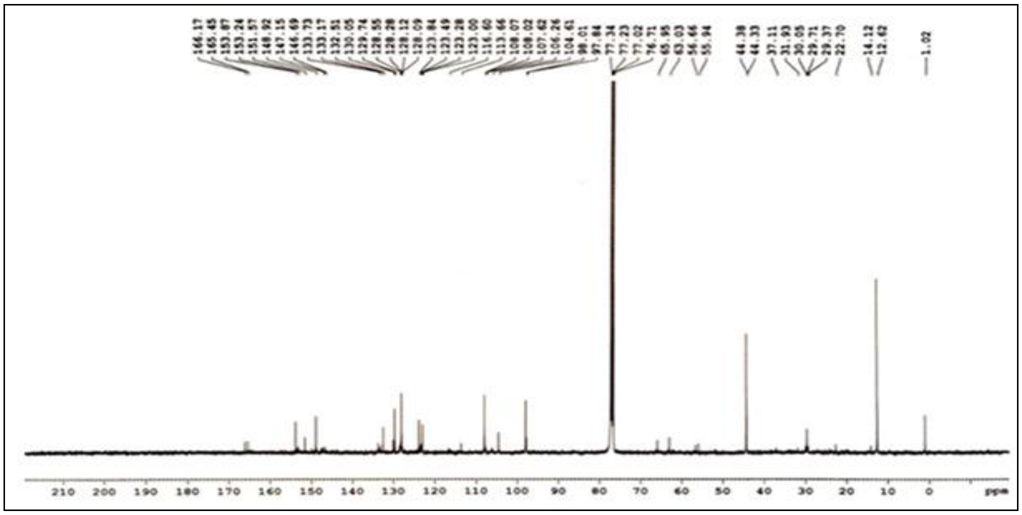


FIG. 3: 13C NMR PROFILING OF THE RHODAMINE-BASED SCHIFF BASE

These findings confirmed the presence of aromatic, aliphatic, and heteroaromatic structural features in the Schiff base azo compound. The presence of carbonyl, methoxy, and heteroaromatic carbons was consistent with the expected molecular framework, confirming the successful synthesis and structural integrity of the Rhodamine-based compound.

CONCLUSION: The synthesis of the Rhodamine B-based azo Schiff base compound was successfully accomplished via a well-established protocol, followed by detailed characterization through FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopy. The characterization results confirmed the structural integrity and purity of the synthesized Schiff base, with precise identification of functional groups and molecular features. The compound demonstrated promising properties, suggesting its potential applications in diverse fields, such as sensing, bioimaging, and materials science. This study offers valuable insights into the synthesis and characterization of Rhodamine B-based Schiff bases, providing a strong foundation for future research and development, particularly in the design of advanced functional materials with enhanced performance.

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