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Synthesis, characterization, and study of biological and laser activity of Schiff bases derived from 1,3,5-triazine-2,4,6-triamine

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Abstract

This investigation concentrates on the synthesis of Schiff base derivatives (F1-F5) by reacting a single mole of 1,3,5-triazine-2,4,6-triamine with three moles of various benzaldehyde derivatives. The compounds that were synthesized underwent characterization via a variety of physical and spectroscopic methodologies, encompassing Infrared (IR) spectroscopy, Proton Nuclear Magnetic Resonance (^1H NMR), carbon nuclear magnetic resonance (^{13}C NMR), and elemental analysis. The evaluation of melting points and purity levels was conducted, while the progression of the reaction was monitored utilizing Thin-Layer Chromatography (TLC). The antibacterial efficacy of the synthesized compounds was assessed against two distinct bacterial strains: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) were used, with standard antibiotics such as amoxicillin, ampicillin, and ciprofloxacin serving as reference controls. Importantly, several of the synthesized derivatives demonstrated pronounced antibacterial properties. Additionally, the interaction of the synthesized compounds with a visible helium-neon laser was explored using exposure durations of 15, 30, 45, and 60 seconds. The study also examined the physical properties and changes in the compounds resulting from laser irradiation.

Keywords: 1,3,5-Triazine-2,4,6-triamine, Schiff bases, laser activity, biological activity

Introduction

1,3,5-triazine-2,4,6-triamine, commonly known as melamine, is a nitrogen-rich organic compound ^[1]. It is a white crystalline solid that is mostly known for its exceptional thermal stability, high nitrogen content, and wide range of industrial applications ^[2]. The melamine structure consists of a heterocyclic six-atom ring with three alternating carbon and three nitrogen atoms within the ring and three amine groups (NH_2) attached to the carbon atoms at the 2, 4, and 6 positions ^[3]. This unique chemical structure gives melamine distinct properties that make it valuable in a variety of fields, including the production of resins, laminates, coatings, and adhesives ^[4]. Schiff bases represent an important and diverse class of organic compounds that play a pivotal role in many chemical and biological applications ^[5]. These compounds are named after the German chemist Hoko Schiff, who first discovered them in the late 19th century ^[6]. Schiff bases are also known as imine amines ^[7] and are typically formed by a condensation reaction between a compound containing a carbonyl group (such as an aldehyde or ketone) and a compound containing a primary amine ^[8]. This process leads to the creation of a double bond linking the carbon and nitrogen atoms, producing a compound known as a Schiff base ^[9]. Schiff bases exhibit unique chemical properties that make them of wide interest in a variety of fields ^[10]. For example, they can form metal complexes with many transition elements ^[11], making them important materials in coordination chemistry ^[12]. Schiff bases are also used as intermediates in the synthesis of many important organic compounds ^[13], including pharmaceuticals, polymers, and pesticides ^[14]. In addition to their industrial applications, Schiff bases are of particular interest in biochemistry ^[15]. They exhibit diverse biological activities including antibacterial ^[16], antifungal ^[17], antioxidant ^[18], and anticancer properties ^[19]. Moreover, these compounds are used in the design of chemical sensors and biological detection devices due to their ability to

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selectively interact with specific types of ions or molecules [20]. The study of Schiff bases is not limited to exploring their chemical and biological properties only [21], but also extends to understanding their reaction mechanisms and how to improve their efficiency and stability in various applications [22]. The development and continuous diversity of these compounds reflects the enormous potential they can provide for the future of chemistry and related sciences [23].

Materials and Methods

All reagents employed in the present investigation were procured from Aldrich, Fluka, and BDH, and were utilized in their received form without any additional purification processes. The ^1H -NMR and ^{13}C -NMR spectra were acquired utilizing a Bruker AVANCE NEW-400 spectrometer functioning at a frequency of 400 MHz. FT-IR spectra were obtained using KBr pellets on a SHIMADZU FT-IR spectrometer. The ultraviolet-visible (UV-Vis) absorption spectra were recorded with a T92+double-beam spectrophotometer, utilizing a quartz cell with a path length of 1.0 mm.

Procedure

Preparation of Schiff base derivatives (F1-F5)

A solution consisting of benzaldehyde derivatives (0.03 mol) was formulated by dissolving the respective compounds in 30 mL of absolute ethanol. Following complete dissolution, four drops of CH_3COOH were introduced, succeeded by the incorporation of 1,3,5-triazine-2,4,6-triamine (0.01 mol, 1.26 g). The resultant mixture was subjected to continuous stirring for a duration of 7-9 hrs, and the advancement of the reaction was assessed via TLC. Upon reaching completion, the reaction mixture was

gradually permitted to cool, and the formed precipitate was subsequently filtered, washed, and dried until a constant weight was achieved. The crude product underwent recrystallization utilizing benzene and was dried once more. The physical characteristics of the synthesized Schiff base compounds are delineated in Table 1 [24, 25].

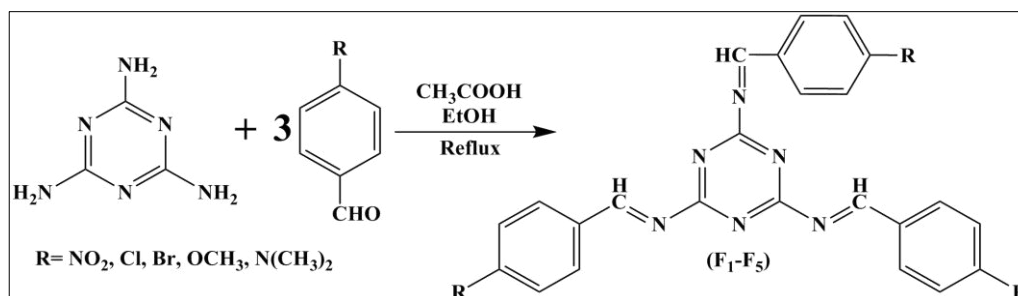
Measuring the laser effectiveness of prepared compounds

The laser effectiveness of prepared compounds (F1-F5) was measured using a visible helium-neon laser. Compounds were irradiated for various time intervals (15, 30, 45, 60 seconds), and physical properties and changes in the prepared compounds were observed [26, 27].

Biological activity study

The biological activity of the synthesized compounds was evaluated against two pathogenic bacterial strains: *Staphylococcus aureus* and *Escherichia coli* [28-30]. The study involved determining the Minimum Inhibitory Concentration (MIC) of the compounds (F1-F5). Solutions of the compounds were prepared at concentrations of 25, 50, and 75 mg/mL using dimethyl sulfoxide (DMSO) [31-33] as a solvent. The results were assessed after 24 hours, evaluating the sensitivity of the bacterial strains to the derivatives based on the diameter of the inhibition zones formed around the wells in the agar plates. A larger inhibition zone diameter indicated higher biological activity of the compounds. The inhibition zones of the synthesized compounds were compared to those of standard antibiotics for reference [34].

Results and Discussion



In this study, Schiff bases derivatives were synthesized by reacting one mole of 1,3,5-triazine-2,4,6-triamine with three moles of different benzaldehyde substitutes, as illustrates in Scheme 1

Scheme 1: Pathway of synthesized compounds (F1-F5)

Characterization of Schiff bases derivatives (F1-F5)

The Infrared (IR) spectra of the Schiff base derivatives exhibited significant changes indicative of successful reaction progress. The characteristic (NH_2) band of 1,3,5-triazine-2,4,6-triamine was no longer present, confirming its participation in the reaction [35]. Similarly, the aldehyde carbonyl band of the benzaldehyde derivatives disappeared. A novel absorption band was identified within the spectral range of $3042\text{--}3097\text{ cm}^{-1}$, which is ascribed to the stretching vibration of the aromatic (C-H) bond [36]. Furthermore, a band corresponding to the azomethine (C=N) stretching vibration was detected within the range of $1633\text{--}1643\text{ cm}^{-1}$, alongside another band in the range of $1622\text{--}1628\text{ cm}^{-1}$,

which was due to the (C=N) bond of the triazine ring [37]. Additionally, two more absorption bands were observed in the ranges of $1575\text{--}1597\text{ cm}^{-1}$ and $1483\text{--}1499\text{ cm}^{-1}$, which are linked to the aromatic (C=C) stretching vibrations [38]. These spectral findings are detailed in Table 2 and demonstrates in Figures 1 and 2.

The ^1H -NMR spectrum of compound (F2) in DMSO-d_6 solvent revealed several characteristic signals. A multiplet appeared in the range of 6.89-7.74 ppm, corresponding to the protons of aromatic rings [39]. A singlet at $\delta = 6.21$ ppm was attributed to the proton of the azomethine groups (HC=N) [40]. Additionally, a signal at $\delta = 2.48$ ppm was associated with the protons of the solvent (DMSO-d_6) [41], and another singlet at $\delta = 1.87$ ppm corresponded to the protons of water (H_2O) [42]. The spectrum is shown in Figure 3.

During the examination of the ^{13}C -NMR spectrum of (F2), it was observed that a resonance was detected at $\delta = (191.40)$ ppm, which is ascribed to the carbon atoms within the (C=N) moieties of the azine framework [43]. Additionally, a

signal was noted at $\delta = (172.69)$ ppm, corresponding to the carbon atoms of the azomethine functionalities ($\text{HC}=\text{N}$) [44]. Furthermore, a resonance was identified at $\delta = (167.16)$ ppm, which is attributed to the carbon atoms associated with the (C-Cl) groups [45]. A multiplet signal was also recorded within the range of $\delta = (116.30-163.82)$ ppm, relating to the carbon atoms of the aromatic system. Lastly, a signal was detected at $\delta = (39.02-40.69)$ ppm, which is attributed to the solvent (DMSO- d_6) [46], with the spectral representation depicted in Figure 4.

Laser activity measurement results for prepared compounds

Certain prepared compounds (F1-F5) were irradiated using a helium-neon laser device at various time intervals (15, 30, 45, 60 seconds) for each compound. Subsequently, the physical properties (color, melting point, and Rf) [47]. The study revealed that during the time intervals of 15, 30, and 45 seconds, no changes in the physical properties of the

chemical compounds were observed [48]. After a 60-second exposure, noticeable changes in physical properties were observed, with a significant decrease in melting points for all the studied compounds [49]. Additionally, changes in the Rf values and slight alterations in color were noted [50]. These changes can be attributed to the occurrence of bond breakage in some of the compounds. It is possible that new compounds were formed because of prolonged and continuous exposure to high-energy laser radiation [51], as illustrated in Table 3.

Evaluation of the biological activity of prepared compounds

The compounds had remarkable inhibitory efficacy against *S. aureus* and substantial inhibitory activity against *E. coli* [52-55]. As shown in Table 4 and Figure 5, the relationship between conc. and inhibition was found to be dose-dependent, exhibiting greater inhibition percentages at a conc. of 75 mg per mL [56].

Table 1: Some physical properties of Schiff bases derivatives

Comp.	R	Molecular-Formula/ M.Wt g/mol	Color	Time(h)	M.P °C	Rf	Yield%
F ₁	NO ₂	C ₂₄ H ₁₅ N ₉ O ₆ / 525.44	Dark yellow	8	229-230	0.80	82
F ₂	Cl	C ₂₄ H ₁₅ N ₆ Cl ₃ / 493.78	Light yellow	9	179-181	0.89	83
F ₃	Br	C ₂₄ H ₁₅ N ₆ Br ₃ / 627.14	White	7	192-193	0.90	80
F ₄	OCH ₃	C ₂₇ H ₂₄ N ₆ O ₃ / 480.53	Brown	7	217-219	0.87	86
F ₅	N(CH ₃) ₂	C ₃₀ H ₃₃ N ₉ / 519.66	Dark brown	8	204-206	0.77	81

Table 2: FT-IR absorption results for Schiff bases derivatives by KBr(cm^{-1})

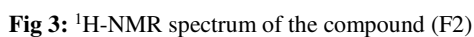
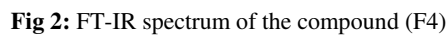
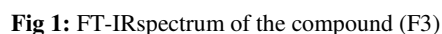
Comp.	R	$\nu\text{C-H}_{\text{Arom.}}$	$\nu\text{HC}=\text{N}$	$\nu\text{C}=\text{N}$	$\nu\text{C}=\text{CArom.}$	Others
F ₁	NO ₂	3078	1639	1628	1597, 1496	$\nu(\text{NO}_2)$ 1541, 1372
F ₂	Cl	3042	1633	1625	1587, 1490	$\nu(\text{C-Cl})$ 767
F ₃	Br	3097	1635	1627	1581, 1494	$\nu(\text{C-Br})$ 615
F ₄	OCH ₃	3053	1643	1622	1575, 1483	$\nu(\text{CH}_3)$ 2964, 2850
F ₅	N(CH ₃) ₂	3085	1637	1626	1590, 1499	$\nu(\text{CH}_3)$ 2936, 2838

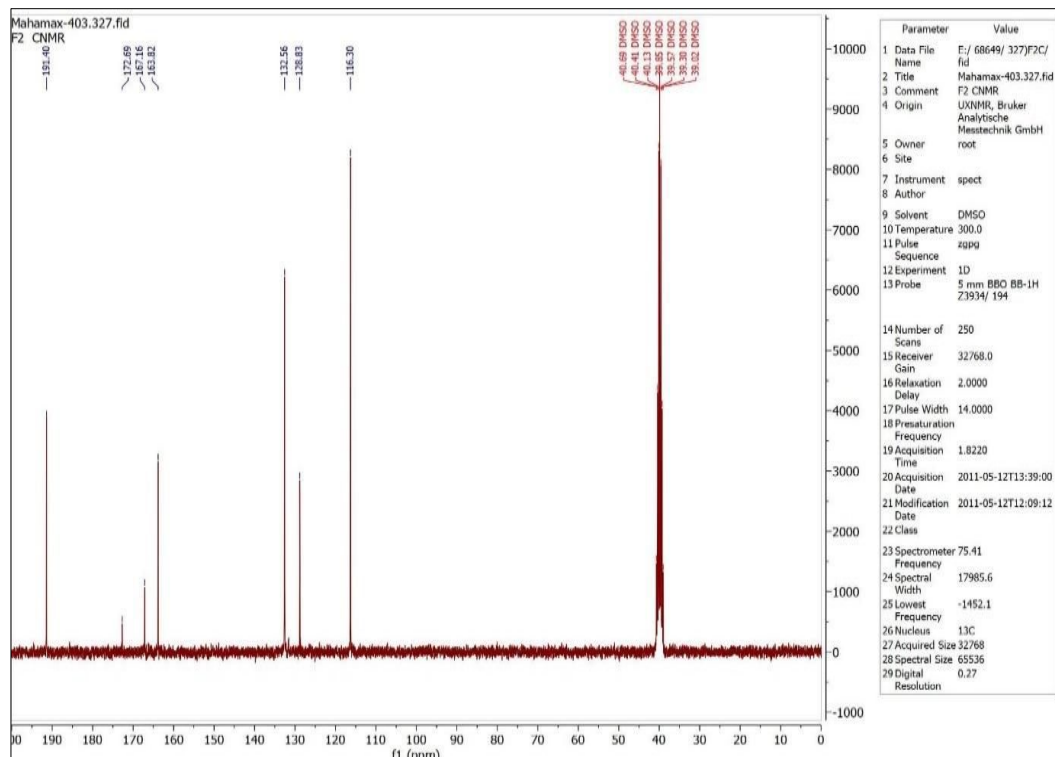
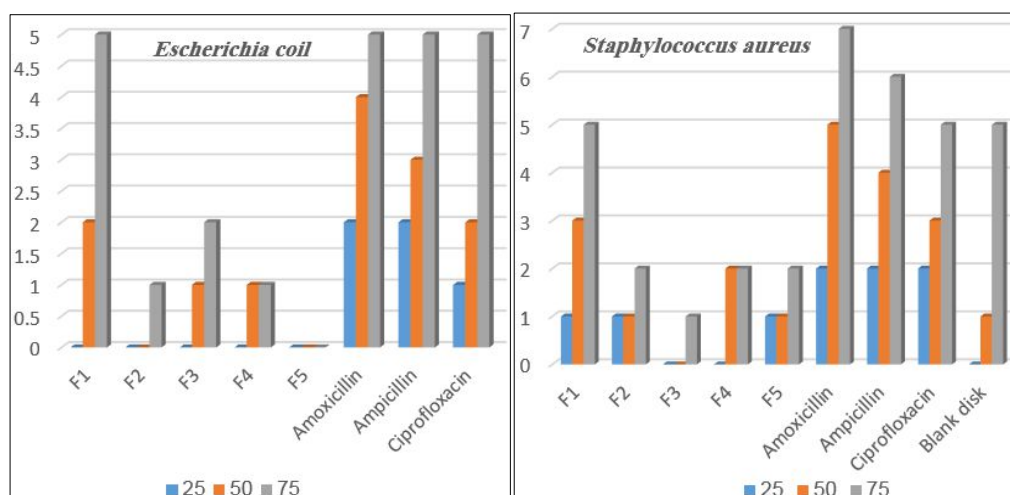
Table 3: Results of measuring the laser effectiveness of some prepared compounds

Comp.	15 S			30 S			45 S			60 S		
	Color	M.P °C	Rf	Color	M.P °C	Rf	Color	M.P °C	Rf	Color	M.P °C	Rf
F ₁	Dark yellow	229-230	0.80	Dark yellow	229-230	0.80	Dark yellow	229-230	0.80	Yellow	210-212	0.67
F ₂	Light yellow	179-181	0.89	Light yellow	179-181	0.89	Light yellow	179-181	0.89	Yellow	164-166	0.42
F ₃	White	192-193	0.90	White	192-193	0.90	White	192-193	0.90	White	177-179	0.73
F ₄	Brown	217-219	0.87	Brown	217-219	0.87	Brown	217-219	0.87	Brown	196-198	0.79
F ₅	Dark brown	204-206	0.77	Dark brown	204-206	0.77	Dark brown	204-206	0.77	Brown	182-184	0.85

Table 4: Efficacy of synthesized compounds and reference treatments (measured inhibition in mm).

Comp. No.	<i>Escherichia coil</i>			<i>Staphylococcus aureus</i>		
Conc. mg/ml	25	50	75	25	50	75
F ₁	0	2	5	1	3	5
F ₂	0	0	1	1	1	2
F ₃	0	1	2	0	0	1
F ₄	0	1	1	0	2	2
F ₅	0	0	0	1	1	2
Amoxicillin	2	4	5	2	5	7
Ampicillin	2	3	5	2	4	6
Ciprofloxacin	1	2	5	2	3	5
Blank disk	0	0	0	0	0	0



Fig 4: ^{13}C -NMR spectrum of the compound (F2)Fig 5: Inhibitory activity values of the prepared compounds against *E. coli* and *S. aureus*

Conclusion

The bioassay results revealed that the majority of the synthesized compounds presented significant antibacterial activity, effectively inhibiting bacterial growth. Notably, some compounds demonstrated superior biological efficacy compared to the standard antibiotics used as controls. Additionally, the stability of certain prepared compounds under helium-neon laser radiation was confirmed. The structural accuracy of the synthesized nanocompounds was validated through comprehensive physical and spectroscopic analyses.

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