

Synthesis, Characterization, and Bioactivity Studies of the Schiff Base Ligand and its Zinc(II) Complex

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Abstract—One of the largest concerns to global health in recent decades has been identified as the growth of bacteria resistance to antibiotics. The Schiff base (SBs) and the zinc(II) SBs complex compounds category have attracted a lot of interest because of their function in chemical syntheses and their potential for bioactive and pharmacological effects. The present study includes the synthesis of various SBs with different substituents. Equimolar mixtures of benzaldehyde derivatives (1, 2) and aniline derivatives (3, 4) are used to carry out a series of condensation reactions to get compounds (5-7). By stoichiometrically combining Zn (II) acetate and ZnCl₂ separately with the SBs ligand (7) in ethanol, it has been possible to prepare the SBs zinc(II) complex (8). The structure of the ligand and its metal complex are analyzed using (Fourier-transform infrared spectroscopy, ¹H-NMR, ¹³C-NMR) spectroscopy, scanning electron microscopy, and liquid chromatography–mass spectrometry. Moreover, the synthesized compounds are verified *in vitro* against *Escherichia coli* Gram negative, *Staphylococcus aureus* Gram positive, and fungi (*Candida albicans*). Compounds (5, 7, and 8) indicated significant growth inhibition against *E. coli* Gram negative and fungi (*C. albicans*) with different inhibition zones starting from 7 to 17.5 mm.

Index Terms—Bioactive studies, Condensation reaction, Microorganism, Schiff base, Zinc(II) complex.

I. INTRODUCTION

Schiff bases (SBs) and their metal complexes have been widely investigated because of their biological activity (Liang, et al., 2023), (Shammout, et al., 2021). It is an important class of compounds containing the azomethine (>C=N-) linkage as a functional group, nitrogen bonds to the aryl or alkyl group but not to the hydrogen atom, and are excellent ligands that can coordinate and stabilize metal ions with various oxidation states (Yousif, et al., 2017), (Shah, et al., 2020). Hugo Schiff, a German chemist, reported these chemicals in 1864 (Scheme 1), hence his name was used to refer to them (Omidi and Kakanejadifard, 2020). Most of the SBs are represented by the generic formula R₁R₂C=NR₃

(Muzammil K et al., 2015), whereas some of these have the general formula R₁CH=NR₂, wherein a hydrogen atom rather than an alkyl or aryl group attaches to the carbon (Kolapwar, 2017).

When carbonyl compounds, such as ketones or aldehydes, condense with primary amines (aliphatic, aromatic, or heteroaromatic), SBs are created. Because of conjugation, aromatic aldehyde-based SBs compounds are stable, whereas corresponding aliphatic aldehyde-based SBs compounds are unstable because they spontaneously polymerize. (Sadia, et al., 2021), (Mahdi and Ismail, 2022).

In literature, various techniques have been employed to prepare SBs: Gopalakrishnan et al., have reported CaO-catalyzed synthesis of SBs under microwave conditions (Gopalakrishnan et al., 2007). Devidas, et al., synthesized SBs catalyzed by P₂O₅ under free solvent conditions (Devidas et al., 2011). Furthermore, Bendale, Bhatt, and Narkhede reported the synthesis of SBs using mortar and pestle, sonicator bath (without catalyst or with a catalyst), and in the presence of U.V. Rays (Bendale, Bhatt, and Narkhede, 2011).

The SBs have been reported to be one of the most active classes of the compound possess remarkable biological activities such as antiviral, antibacterial, antifungal and anticancer, antitumor activities (Journal and Al-daffaay, 2022), (Jaber, 2023). SBs find many applications including reduction catalysts, oxidation catalysts, acid catalyst, and dye, and display a unique reactivity to metal ions (Vhanale, Deshmukh and Shinde, 2019).

Many metal complexes of SBs have been reported in literature possessing bioactivity including insecticides, pesticides antibacterial, antimalarial, antifungal, antiviral, anti-inflammatory, anticancer, antitumor, antioxidant, and anti-human immunodeficiency virus activity (Vhanale, Deshmukh and Shinde, 2019), (Pinchaipat, et al., 2021).

Zinc(II) is a component of many different enzymes and is involved in numerous physiological processes in living organisms, therefore zinc(II) is an essential element that is crucial to biological systems (Bazhina, et al., 2023), (Poole, 2017). Through research on Zn(II) coordination compounds with organic molecules, compounds with therapeutic (antifungal, antibacterial, anti-inflammatory, and anticancer) action can be obtained that may be used as building blocks for novel medications and chemo/biosensors (Slassi, et al., 2020). While some chemical compounds may be highly



biologically active on their own, they may also be quite poisonous to the organism or have poor solubility. These compounds can be coordinated with transition metal ions, such Zn(II), to increase the therapeutic effect provided by the organic part and enhance the resultant molecules' bioavailability (Mathews, Begum and Kurup, 2020), (Bazhina, et al., 2023).

II. EXPERIMENTAL

Materials and Methods

All the starting materials compounds (1-4), reagents, as well as solvents, were obtained commercially and used without further purification. Thin layer chromatography (TLC) (*silica* gel on aluminum plates) was used to track the reactions' progress; ethyl acetate with toluene (1:9) was used as a solvent system, and the result was observed by UV light. The purification of the products was done by recrystallization in ethanol. ¹H and the ¹³C-NMR spectrum was observed by 400 and 100 MHz (Ascend) respectively. Fourier-transform infrared spectroscopy (FT-IR) Affinity-1S spectrometer Shimadzu), and melting points were taken by Stuart Scientific melting point apparatus 3. Mol.Wt. where taken by liquid chromatography–mass spectrometry Shimadzu UFLC-AB Sciex 3200 QTRAP. Finally, scanning electron microscopy (SEM) where taken by SEM Quanta agent 450.

General procedure A: Preparation of the SBs ligand (5-7) (Hajiashtafi, Zekriazadeh and Kubicki, 2020)

In a flask with a flat bottom, an equimolar mixture of benzaldehyde and aniline derivatives was added. In an acidic medium, the reaction mixture was refluxed for 7 h. TLC was used to keep track of the reaction's development. After a reaction has been completed, the solvent is slightly evaporated, the precipitate is washed with ethanol, filtered out, and the product is recrystallized in warm ethanol.

General procedure B: Preparation of the complex (8)

To a flask with a flat bottom, an equimolar mixture of compound (7) (0.0013 mol and 0.0006 mol) respectively, and zinc salt (0.0013 mol and 0.0006 mol) was added. The mixture was then heated at 78°C under reflux in ethanol for 3 h to produce a colored precipitate (8). The precipitates were afterward removed by filtering, rinsed with distilled water, and then crystallized again from ethanol.

(E)-1-(4-(2-nitrostyryl)phenyl)ethan-1-one (5)

General procedure A: 2-nitrobenzaldehyde 3 (0.72 g, 0.0099 mol, 1 equivalent), glacial acetic acid (2 drops) was dissolved in EtOH (20 mL), then compound 1 (0.65 g, 0.0048 mol, 1.0 equivalent) was added; gave the product 5 as a yellow color, (72%) yield, m.p./154–156°C. ¹H-NMR (400 MHz, DMSO-d⁶) δ ppm: 2.52 (quintet, *J* = 1.9 Hz, DMSO), 2.6 (s, CH₃CO, 3H), 3.41 (H₂O), 6.58 (d, *J* = 7.7 Hz, 2Ar-H), 7.36 (d, *J* = 7.6 Hz, 2Ar-H), 7.79 (t, *J* = 12.3 Hz, 1Ar-H), 7.86–7.92 (m, 1Ar-H), 8.05 (d, *J* = 7.6 Hz, 1Ar-H), 8.19 (d, *J* = 7.3 Hz, 1Ar-H), 8.9 (s, CH=N); ¹³C-NMR (100 MHz, DMSO-d⁶) δ ppm: 22.7, 124.7, 130.3, 130.9, 131, 132, 134.3, 134.6, 134.7, 137, 148.9, 155.9, and 190.4; FT-IR (cm⁻¹):

1697 (C=N) stretching, 1651 (C=O) stretching, 1517, 1338 (NO₂) stretching (symmetric and asymmetric), *m/z* (ES+) found: MH+ 269.

(E)-1-(4-(4-methylstyryl)phenyl)ethan-1-one (6)

General procedure A: 4-methylbenzaldehyde 4 (0.57 g, 0.0048 mol, 1 equivalent), glacial acetic acid (2 drops) was dissolved in EtOH (20 mL), then compound 1 (0.65 g, 0.0048 mol, 1.0 equivalent) was added; gave the product 6 as a light orange, (65%) yield, m.p./139–140°C. ¹H-NMR (400 MHz, DMSO-d⁶) δ ppm: 2.5 (quintet, *J* = 1.9 Hz, DMSO), 2.39 (s, Ar-CH₃, 3H), 2.58 (s, CH₃CO, 3H), 3.38 (H₂O), 7.32 (d, *J* = 8.6 Hz, 2Ar-H), 7.35 (d, *J* = 7.35 Hz, 2Ar-H), 7.87 (d, *J* = 8 Hz, 2Ar-H), 8 (d, *J* = 8.5, 2Ar-H), 8.59 (s, CH=N), 9.95 (impurities); ¹³C-NMR (100 MHz, DMSO-d⁶) δ ppm: 21.5, 26.9, 121.5, 129.5, 129.9, 131, 133.6, 134.4, 142.5, 156.34, 162.4, and 197.3; FT-IR (cm⁻¹): 1699 (C=N) stretching, 1670 (C=O) stretching, *m/z* (ES+) found: M+ 237.

(E)-2-(2-nitrostyryl)phenol (7)

General procedure A: 2-nitrobenzaldehyde 3 (2.72 g, 0.018 mol, 1 equivalent), glacial acetic acid (2 drops) was dissolved in EtOH (20 mL), then compound 2 (2 g, 0.018 mol, 1.0 equivalent) was added; gave the product 7 as a dark yellow solid, (76%) yield, m.p./106–108°C. ¹H-NMR (400 MHz, DMSO-d⁶) δ ppm: ¹H-NMR (400 MHz, DMSO-d⁶) δ ppm: 2.51 (quintet, *J* = 1.9 Hz, DMSO), 3.4 (H₂O), 6.87 (t, *J* = 7.5 Hz, 1Ar-H), 6.94 (d, *J* = 8 Hz, 1Ar-H), 7.14 (t, *J* = 7.7 Hz, 1Ar-H), 7.18 (d, *J* = 7.8 Hz, 1Ar-H), 7.76 (t, *J* = 7.7 Hz, 1Ar-H), 7.87 (t, *J* = 7.5 Hz, 1Ar-H), 8.11 (d, *J* = 8.1 Hz, 1Ar-H), 8.43 (d, *J* = 7.8 Hz, 1Ar-H), 8.98 (s, CH=N), 9.26 (s, =COH); ¹³C-NMR (100 MHz, DMSO-d⁶) δ ppm: 116.8, 120, 120.3, 124.8, 128.7, 130.2, 130.8, 132, 134, 137.8, 149.7, 151.8, 155.5; FT-IR (cm⁻¹): 3388 (OH) stretching, 1699 (C=N) stretching, 1514, 1336 (NO₂) stretching (symmetric and asymmetric), *m/z* (ES+) found: MH+ 243.

Bis(2-(((Z)-2-nitrobenzylidene)amino)phenoxy)zinc (8)

General procedure B: Compound 7 (0.15 g, 0.0006 mol, 1 equivalent), was dissolved in EtOH (10 mL), then zinc acetate (0.11 g, 0.0006 mol, 1.0 equivalent) was added; gave product 8 as a mustard color, (80%) yield. ¹H-NMR (400 MHz, DMSO-d⁶) δ ppm: 2.51 (quintet, *J* = 1.9 Hz, DMSO), 3.41 (H₂O), 6.68 (bro.s, 1Ar-H), 6.85 (t, *J* = 7.4 Hz, 1Ar-H), 7.1 (t, *J* = 7.1 Hz, 1Ar-H), 7.27 (bro.s, 1Ar-H), 7.77 (t, *J* = 8.3 Hz, 1Ar-H), 7.87 (t, *J* = 7.4 Hz, 1Ar-H), 8.18 (d, *J* = 7.8 Hz, 1Ar-H), 8.27 (bro.s, 1Ar-H), 9.1 (s, CH=N); ¹³C-NMR (100 MHz, DMSO-d⁶) δ ppm: 113.6, 117.8, 120.4, 124.7, 124.9, 130.3, 130.9, 131.2, 132.2, 134.5, 134.7, 148.7, 164.5; FT-IR (cm⁻¹): 1590 (C=N) stretching, 1516, 1334 (NO₂) stretching (symmetric and asymmetric).

General procedure B: Compound 7 (0.3 g, 0.0013 mol, 1 equivalent), KOH (0.07 g, 0.0013 mol) was dissolved in EtOH/H₂O (10 mL, 3 mL), then zinc chloride (0.17 g, 0.0013 mol, 1.0 equivalent) was added; gave the product 8 as a dark mustard color, (77%) yield. ¹H-NMR (400 MHz, DMSO-d⁶) δ ppm: 2.51 (quintet, *J* = 1.9 Hz, DMSO), 3.41 (H₂O), 6.4 (bro.s, 1Ar-H), 6.6 (bro.s, 1Ar-H), 7.05

(bro.s, 1Ar-H), 7.4 (bro.s, 1Ar-H), 7.7–7.9 (m, 2Ar-H), 8.2 (bro.s, 1Ar-H), 8.4 (bro.s, 1Ar-H), 9.26 (s, CH=N); FT-IR (cm^{-1}): 1589 (C=N) stretching, 1517, 1338 (NO_2) stretching (symmetric and asymmetric).

III. RESULTS AND DISCUSSION

One of the largest risks to world health in recent decades has been the evolution of germ resistance to antibiotics. Therefore, we decided to synthesize some new expected bioactive SB derivatives.

In the present work, SBs were synthesized by the condensation of different substituted aromatic amines with a series of aromatic aldehydes (Scheme 2):

The synthesis of SBs undergoes according shown mechanism below (Scheme 3):

Furthermore, the synthesized SB (7) utilized in the creation of Zn (II) complex (8) by stoichiometrically Zn (II) acetate and ZnCl_2 separately (Scheme 4):

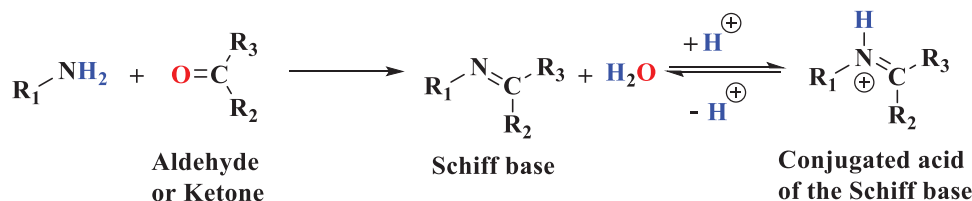
FT-IR Spectroscopy

The IR spectra of the SB and the Zn (II) complex are shown in Figs. 1 and 2. The IR stretching frequencies of free

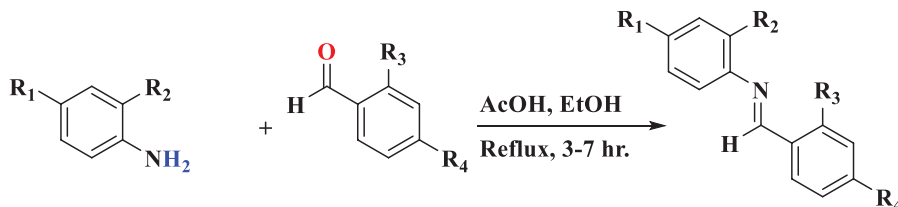
SB and SB Zn-complex have been reported in Table I. Distinct bands appear at (1651, 1670) and (1690, 1699) cm^{-1} were attributed to (C=O) stretching and azomethine (CH=N) group in compounds (5 and 6), respectively. The IR spectrum of compound (7) indicated a band at (3388) cm^{-1} for the (O-H) group, but when compound (7) was used to make complex (8a and 8b) (OH) peak disappeared and this shows that the zinc ion is coordinated over oxygen of (OH) group. The azomethine (CH=N) group in compound 7 also produces a unique band at 1699 cm^{-1} , but in the complex (8a and 8b), this peak is displaced to lower frequencies (1590, 1589) cm^{-1} respectively, demonstrating that the nitrogen atom of the azomethine group is coordinated to the metal ion too.

TABLE I
IR SPECTRA FOR SYNTHESIZED COMPOUNDS IN (cm^{-1}). 8A, WHEN $\text{Zn}(\text{CH}_3\text{CO}_2)_2$ AND 8B WHEN ZnCl_2 USED

Compounds	(OH) str.	(C=N) str.	(C=O) str.	(C=C) Str.	(NO_2) str. assym. and symm.
5	---	1690	1651	1485 1560	1338 1517
6	---	1699	1670	1512 1589	---
7	3388	1699	---	1480 1581	1336 1514
8a	---	1590	---	1485 1570	1516 1334
8b	---	1589	---	1485 1570	1517 1334



Scheme 1. General route for synthesis Schiff base (Pooja et al., 2018).



In compound 1 = $\text{R}_1 = \text{CH}_3\text{CO}$, $\text{R}_2 = \text{H}$

In compound 2 = $\text{R}_1 = \text{H}$, $\text{R}_2 = \text{OH}$

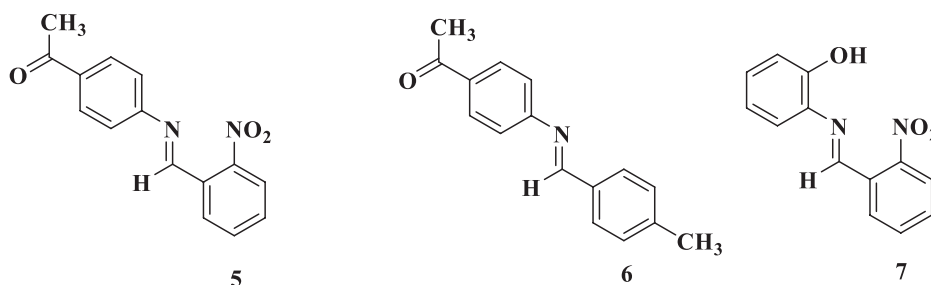
In compound 3 = $\text{R}_3 = \text{NO}_2$, $\text{R}_4 = \text{H}$

In compound 4 = $\text{R}_3 = \text{H}$, $\text{R}_4 = \text{Me}$

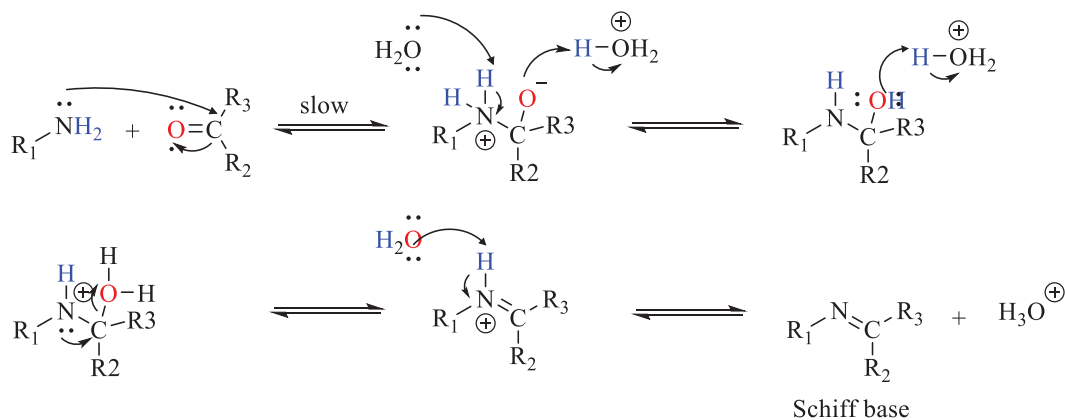
In compound 5 = $\text{R}_1 = \text{CH}_3\text{CO}$, $\text{R}_2 = \text{H}$, $\text{R}_3 = \text{NO}_2$, $\text{R}_4 = \text{H}$

In compound 6 = $\text{R}_1 = \text{CH}_3\text{CO}$, $\text{R}_2 = \text{H}$, $\text{R}_3 = \text{H}$, $\text{R}_4 = \text{Me}$

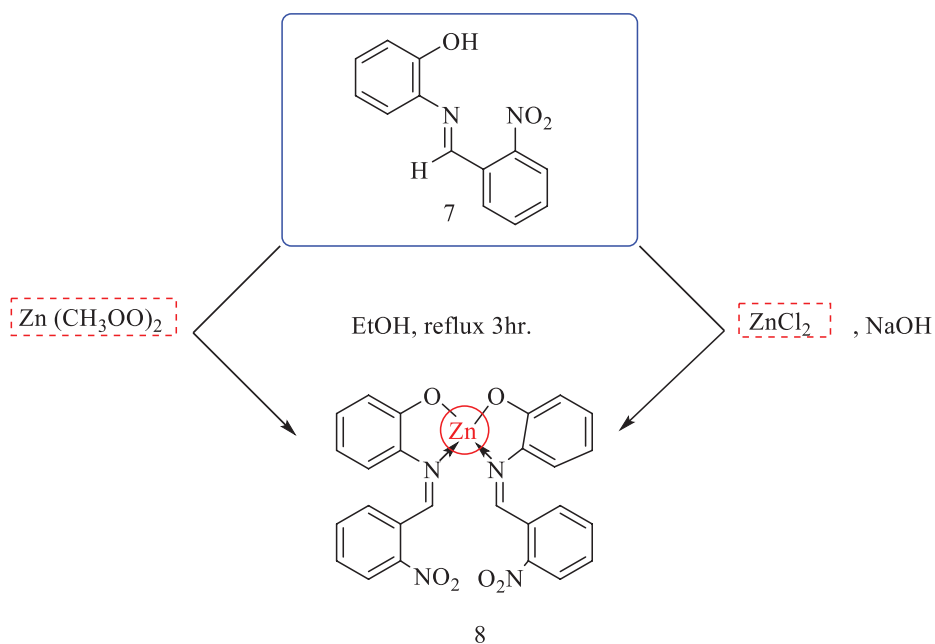
In compound 7 = $\text{R}_1 = \text{H}$, $\text{R}_2 = \text{OH}$, $\text{R}_3 = \text{NO}_2$, $\text{R}_4 = \text{H}$



Scheme 2. Synthesis of Schiff base.



Scheme 3. Suggested mechanism for synthesis of Schiff base.



Scheme 4. Synthesis of Schiff base zinc complexes.

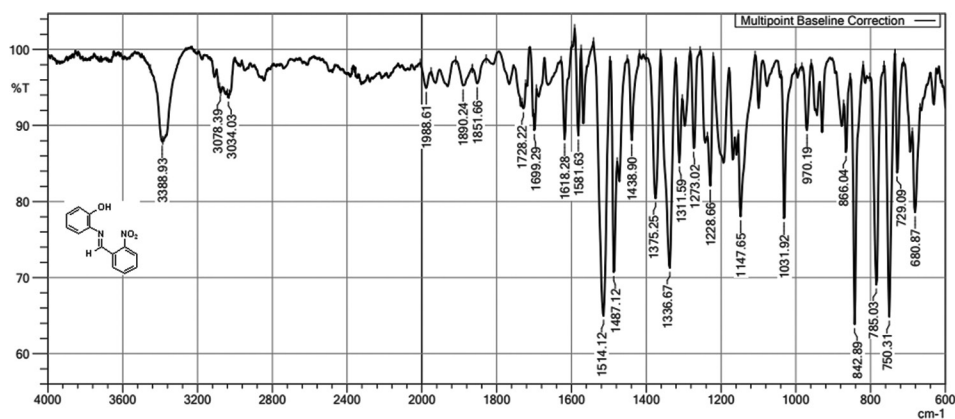


Fig. 1. Fourier-transform infrared spectroscopy for Schiff base 7.

The structures of the compounds (5-8) were evaluated by ¹H-NMR spectra Figs. 3 and 4. Imine protons (CH=N) in ¹H-NMR spectra of compounds (5 and 6) appeared as a

singlet at (8.9) and (8.59) ppm, respectively. Furthermore, the imine proton in the compound (7) appears at (8.98) ppm, while the azomethine proton of the zinc complex

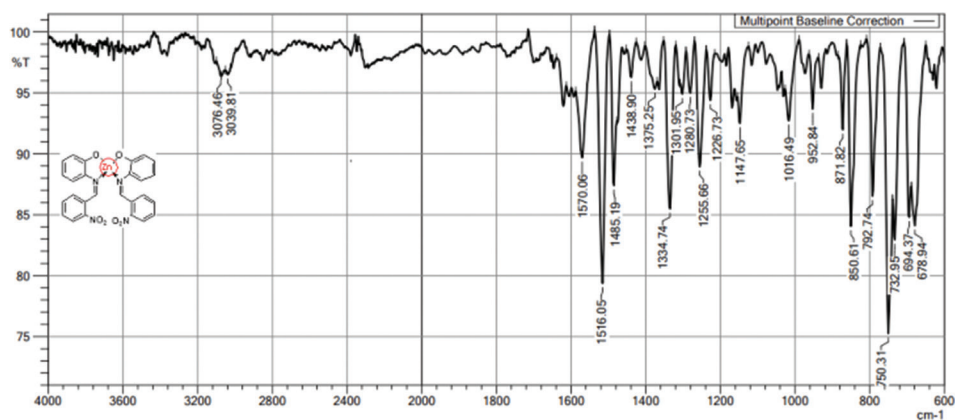


Fig. 2. Fourier-transform infrared spectroscopy of Schiff base zinc complex 8 When $Zn(CH_3CO_2)_2$ used as the reagent.

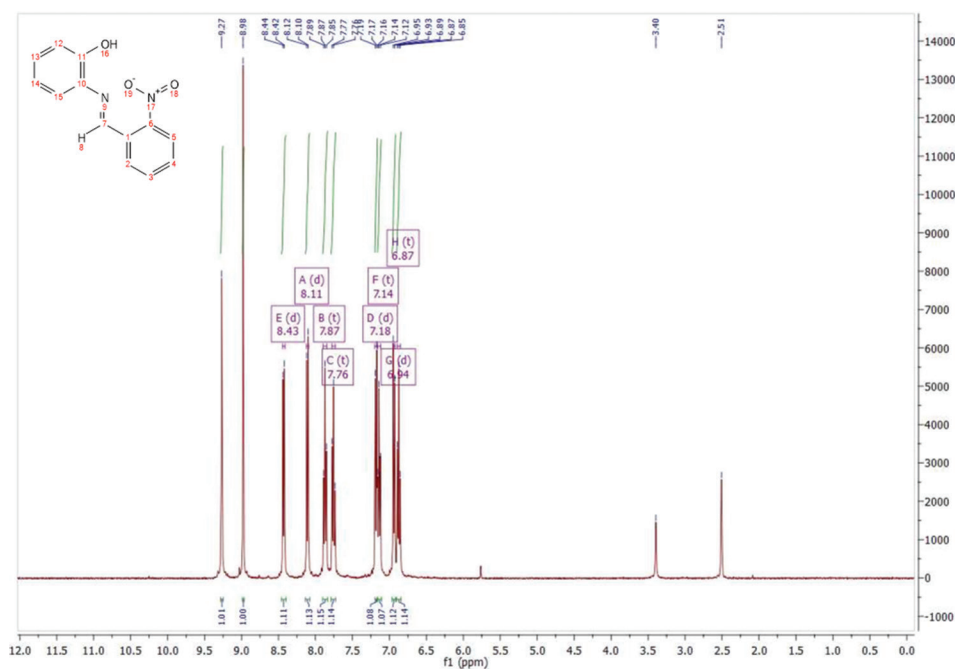


Fig. 3. 1H -NMR spectroscopy for Schiff base 7.

TABLE II

ILLUSTRATES THE PHYSICAL PROPERTIES AND YIELDS OF THE SYNTHESIZED COMPOUNDS

Yield %	Color	m.p. in °C	Compounds
72	Yellow color onion	154–156	5
65	Light orang	139–140	6
76	Dark yellow-solid	106–108	7
80	Mustard color	---	8a
77	Dark Mustard color	---	8b

TABLE III

BIOLOGICAL ACTIVITY OF SYNTHESIZED COMPOUNDS IN MM

	Bacterial species			10 mg/1 mL CH_2Cl Samples
	<i>Candida albicans</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	
-	-	10	-	5
-	-	-	-	6
15	-	7	-	7
17.5	-	8	-	8

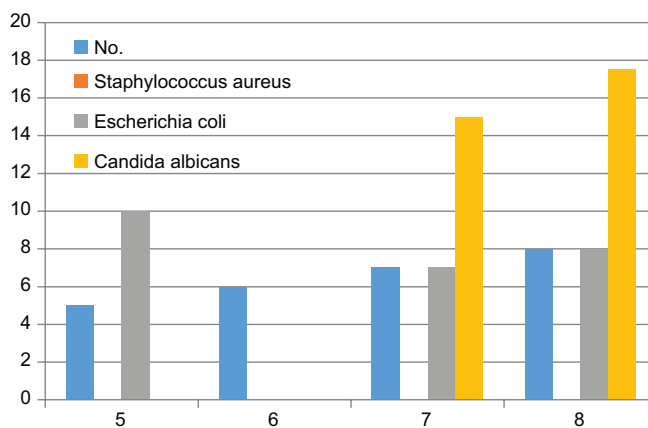


Diagram 1. Biological activity of synthesized compounds.

appears at (9.1) ppm. The predicted range of (6.4–8.4) ppm is where the aromatic ring protons are found.

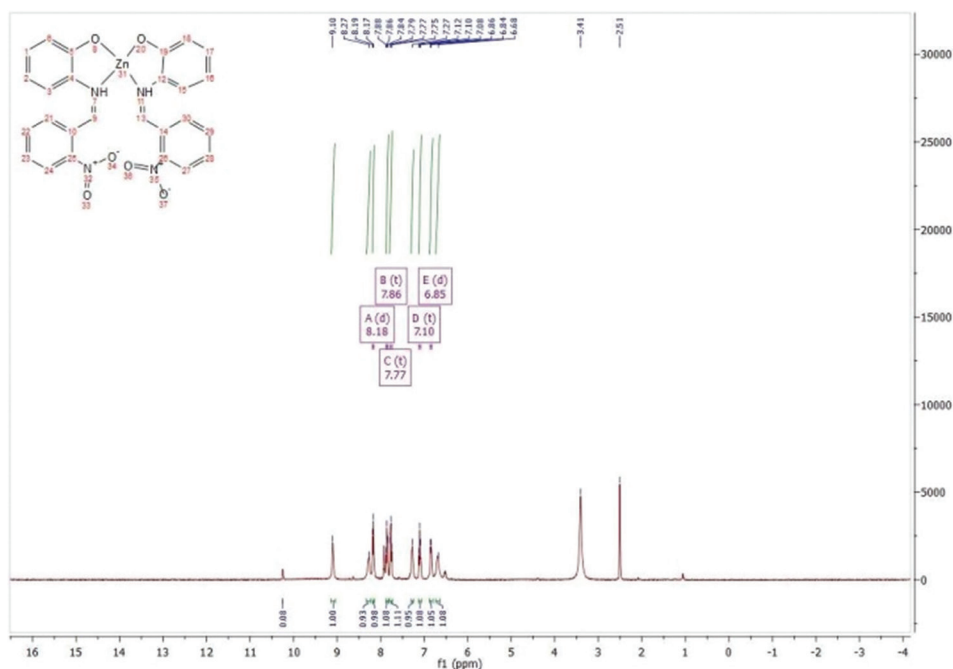


Fig. 4. $^1\text{H-NMR}$ of Schiff base zinc complex 8 When $\text{Zn}(\text{CH}_3\text{CO}_2)_2$ used.

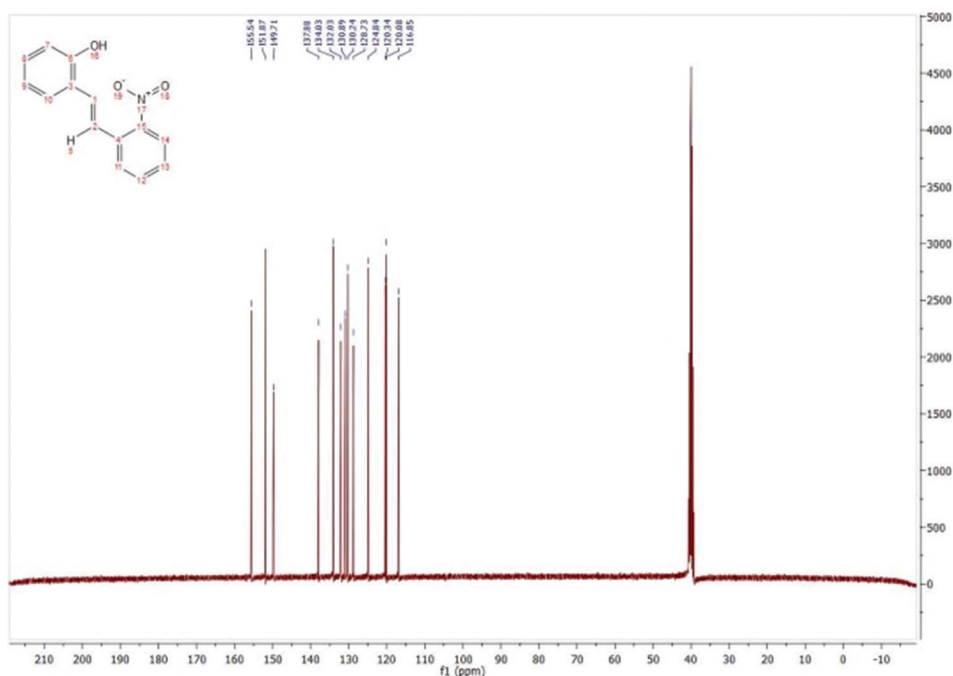


Fig. 5. $^{13}\text{C-NMR}$ spectroscopy for Schiff base 7.

Furthermore, using $^{13}\text{C-NMR}$ the signal at (155.9), (162.4) and (155.5) were attributed to azomethine carbon in compounds (5-7) Fig. 5 for compound 7.

Table I IR spectra for synthesized compounds in (cm^{-1}). 8a, when $\text{Zn}(\text{CH}_3\text{CO}_2)_2$ and 8b when Zn used

Table II illustrates the physical properties and yields of the synthesized compounds.

B. SEM Analysis

The SEM method was employed to examine the morphology of the SB no. 7 and zinc(II) complex no. 8.

The SEM picture of the SB (7) has a sheet mass structure; however, Zn(II) complex (8) has appeared as a sphere on a sheet is shown in Fig. 6.

Table III shows the results of the study on the antibacterial and antifungal properties of synthetic compounds against *Staphylococcus aureus*, *Escherichia coli* bacteria and *Candida albicans* fungus.

Diagram 1 clarifies the inhibition activity of the compounds (5, 7, and 8) against *S. aureus*, *E. coli* bacteria, and *C. albicans* fungus with different inhibition zones starting from 7 to 17.5 mm.

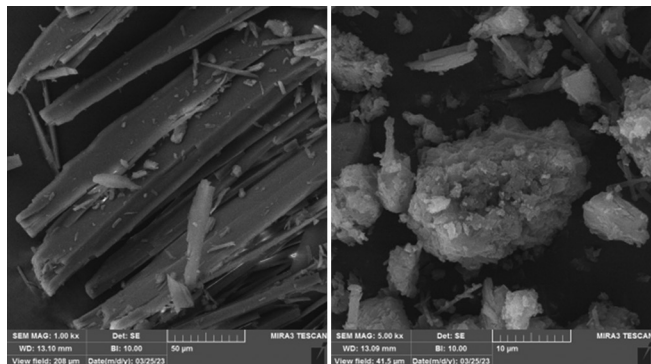


Fig. 6. Scanning electron microscopy of compounds 7 and 8.

IV. CONCLUSION

The study has demonstrated a straightforward method for synthesizing biologically active and non-toxic SB compounds and zinc(II) SB complexes. These newly developed chemicals exhibited inhibitory effects against *E. coli* and *C. albicans*. Furthermore, the ligand was found to coordinate with the metal ion via phenolic oxygen and azomethine nitrogen.

V. ACKNOWLEDGMENT

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