

Ultrasonic Assisted Synthesis, Characterization and Bioactivity Assessment of Novel Piperonal Based Schiff Base and Its Metal Complexes

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ABSTRACT: A novel Schiff base was synthesized by the reaction of piperonal and anthranilic acid, which was further utilized in the synthesis of five novel complexes by reaction with different metal salts ultrasonically. Time for the reaction was greatly reduced through ultrasound irradiations and the yield of reactions was also high as compared to the conventional methods using reflux conditions. The synthesized Schiff base and its metal complexes were characterized by spectroscopic techniques like UV-Visible, IR, and ¹H NMR. The synthesized compounds were tested for their antibacterial and anti-oxidant activity. Good results were obtained in the case of antibacterial activities.

KEYWORDS: Schiff base; Antibacterial; Anti-oxidant; Anthranilic acid.

INTRODUCTION

Ultrasonic assisted organic synthesis is used as a modern and environment-friendly method to increase the rate of organic synthesis [1]. The effect of ultrasound on the rate of chemical reactions was first studied by Loomis and Richards in 1917 [2]. Cavitation is the main reason for the effect of ultrasound on the rate of reaction.

The temperature and pressure inside the bubble is very high due to which the bubble act as a microreactor for the conversion of sound energy into useful chemical energy. Ultrasonically assisted synthesis is a very important tool of green chemistry because it has reduced the waste product formation and minimized energy requirement.

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Ultrasonic assisted synthesis is used in those reactions where very expensive reagents, high temperature and prolonged reaction conditions are required. This technique is also used to increase the yield of the reaction. [3-6].

Schiff bases are very important class of organic compounds also known as azomethines because of the carbon nitrogen double bond existence. Schiff bases are synthesized from the condensation reaction of primary amine with the aldehyde or ketone using an acid or base catalyst. Schiff bases have gained remarkable interest among the synthetic chemists because of their vast applications as anticancer, antiviral, antifungal, antibacterial and antioxidant agents [7-14]. Schiff bases act as good ligands in coordination chemistry since lone pair of electrons on the nitrogen of azomethine group is involved in coordination with transition metals. Schiff bases metal complexes are widely used in industry in catalysis, dying and as analytical reagents [15]. Some Schiff base complexes are also used as chemotherapeutic agents and are attracting the attentions of the biochemists. Literature study shows that Schiff bases metal complexes show higher activity than Schiff bases [16]. *Maihub et al.* prepared osmium (III) and iron (III) complexes from Schiff base synthesized from anthranilic acid and salicylaldehyde and characterized them by various spectroscopic techniques like IR, NMR, and UV etc. [17]. Similarly *Morad et al.* also prepared Schiff base from anthranilic acid and salicylaldehyde. They synthesized Schiff base complex of the above Schiff base with Ni (II), characterized it by various techniques and also studied the antibacterial activity of the above Schiff base and its metal complex [18].

The literature study revealed that no work has been reported till date for synthesis and applications of Schiff base from the piperonal and anthranilic acid. Therefore the current work aimed ultrasonic assisted synthesis, characterization and biological evaluation of the Schiff base derived from piperonal and anthranilic acid and its metal complexes.

EXPERIMENTAL SECTION

Piperonal and anthranilic acid were obtained from Aldrich. Metal salts like $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Pb}(\text{OAc})_2 \cdot 3\text{H}_2\text{O}$, $\text{SnCl}_2 \cdot \text{H}_2\text{O}$, and MnCl_2 were purchased from Merck. Solvents like methanol ethanol and acetic acid were taken from Fluka. All the chemicals used were of analytical grade.

The melting points of the synthesized compounds were recorded in capillary tubes on melting point apparatus,

model Stuart SMP3 (UK). Infrared spectra were recorded by Perkin-Elmer 1000 FT-IR spectrophotometer as KBr disks from 400 to 4000 cm^{-1} . ^1H NMR spectra were recorded on a Bruker AM-300MHz FT-NMR spectrometer (Germany) using TMS as internal standard. The mass spectrum (EI-MS) was recorded by JEOL MS 600H-1(USA). Thin layer chromatography was performed on precoated silica gel 60 HF254 aluminum sheet (Merck, Germany). Antibacterial activities of the synthesized compounds were performed in (Sanyo, Germany) incubator and sterilized in autoclave (Omron, Japan). The minimum inhibitory concentrations and antioxidant activities were determined in a Micro Quant machine (USA). Ultrasonic bath used was Elmasonic S 30 H, 100 1955(Germany).

Synthesis of Chief Base (I)

Schiff base was synthesized from the condensation reaction of piperonal (15 mmol) and anthranilic acid (15 mmol) in the presence of acetic acid as catalyst in methanol solvent. The mixture was sonicated at 37 KHz for 10 mints at 25 °C in ultrasonic bath. The reaction was monitored using TLC. After completion the reaction mixture was concentrated, cooled and filtered. Precipitates were washed with methanol, yellow product was obtained. The structure of Schiff base was confirmed by the physical and spectroscopic data.

2-(benzo[d][1,3]dioxol-5-ylmethyleneamino) benzoic acid (I)

Yellow solid (94%); R_f : 0.34 (n-hexane: acetone, 1:1); IR (pure, cm^{-1}): 3340 (OH), 3100 (sp^2CH), 1705 (C=O), 1616 (C=N), 1591, 1492 (C=C), 1296 (C-O), 1238 (C-N). ^1H NMR (CDCl_3 , 300 MHz); δ = 14.89 (s, 1H, acidic OH), 8.61 (s, 1H, H_4), 8.36 (dd, 1H, $J=7.8, 1.2$ Hz, H_6), 7.63 (dt, 1H, $J=7.8, 1.2$ Hz, H_8), 7.47-7.43 (m, 3H, $\text{H}_{3,7,9}$), 7.39 (dd, 1H, $J=8.1, 1.5$ Hz, H_2), 6.97 (d, 1H, $J=7.8$ Hz, H_1), 6.13 (s, 2H, H_5); EIMS (m/z, %): 269 (54), 224 (100), 195 (22), 167 (22), 148 (41), 119 (25), 92 (5), 65 (6), 51 (3). UV-Visible (CHCl_3) (200-1100): Aromatic ($\pi \rightarrow \pi^*$) 225 nm, Immine ($\pi \rightarrow \pi^*$) 264 nm, Immine ($n \rightarrow \pi^*$) 285 nm.

Synthesis of Schiff base complexes (4-8)

Different metal complexes were synthesized by treating the above synthesized Schiff base with metal salts like $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Pb}(\text{OAc})_2$,

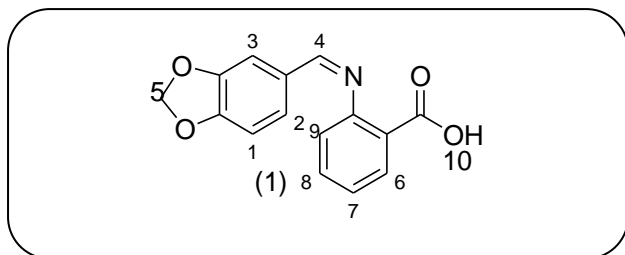


Fig. 1: Structure of the synthesized Schiff base showing different protons.

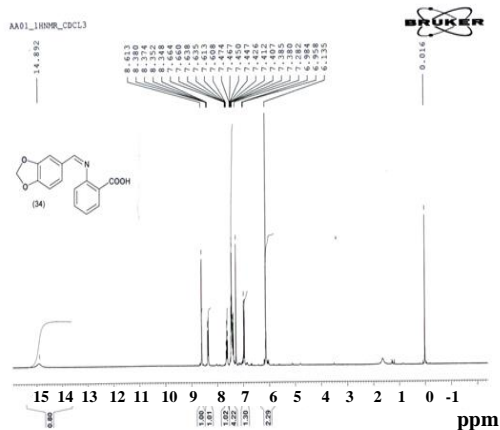


Fig. 2: ¹H-NMR Spectrum of Schiff base.

SnCl₂.H₂O, MnCl₂.H₂O. Schiff base and metal salts were mixed in 2:1 (2 mmol:1 mmol) in methanol. In case of metal chlorides, trimethylamine (0.2 mmol) was also used. Methanolic solution of both Schiff base and metal salts were mixed, sonicated with 37 kHz in ultrasonic bath for 25-30 minutes at 60 °C. Reaction monitoring was done by TLC. After completion the reaction mixture was allowed for cooling and settling. Finally precipitates were filtered and washed with methanol. The synthesis of the complexes was confirmed by their spectroscopic data. General structure of the complexes showing different protons is given below.

Copper complex (4)

Light blue solid (78%), R_F: 0.24 (n-hexane: acetone, 2:3); IR (pure, cm⁻¹): 1612 (C=O), 1537 (C=N), 3122 (Sp² C-H), 1491-1402 (C=C), 1327 (C-N), 527 (Cu-O), 480 (Cu→N); ¹H NMR (DMSO-d₆, 300 MHz) δ(ppm): 8.73 (s, 2H, H_{4,4'}), 8.23 (d, 2H, J=7.8 Hz, H_{6,6'}), 7.89 (dt, 2H, J=7.8, 1.2 Hz, H_{8,8'}), 7.49 (d, 2H, J=7.8Hz, H_{9,9'}), 7.35 (dt, 2H, J=7.8, 1.2 Hz, H_{7,7'}), 7.26-7.20 (m, 4H, H_{2,3,2',3'}),

6.90 (d, 2H, J=7.8Hz, H_{1,1'}), 6.24 (s, 4H, H_{5,5'}); UV-Visible (DMSO) Aromatic (π → π*) 227nm, Immine (π → π*) 275nm, Immine (n → π*) 290nm.

Nickel complex (5)

Gray solid (85%), R_F: 0.38 (n-hexane: acetone, 2:3); IR (pure, cm⁻¹): 1630 (C=O), 1535 (C=N), 3102 (Sp² C-H), 1490-1402 (C=C), 1322 (C-N), 545 (Ni-O), 513 (N→Ni); ¹H NMR (DMSO), 300 MHz); δ 8.70 (s, 2H, H_{4,4'}), 8.21 (d, 2H, J=7.8 Hz, H_{6,6'}), 7.83 (dt, 2H, J=7.8, 1.2 Hz, H_{8,8'}), 7.46 (d, 2H, J=7.8Hz, H_{9,9'}), 7.39 (dt, 2H, J=7.8, 1.2 Hz, H_{7,7'}), 7.18-7.11 (m, 4H, H_{2,3,2',3'}), 6.73 (d, 2H, J=7.8Hz, H_{1,1'}), 6.20 (s, 4H, H_{5,5'}); UV-Visible (DMSO) Aromatic (π → π*) 229nm, Immine (π → π*) 274nm, Immine (n → π*) 293nm.

Lead complex (6)

Pink solid (80%), R_F: 0.31 (n-hexane: acetone, 2:3); IR (pure, cm⁻¹): 1621 (C=O), 1530 (C=N), 3110 (Sp² C-H), 1481-1408 (C=C), 1332 (C-N), 537 (Pb-O), 492(N→Pb); ¹H NMR (DMSO), 300 MHz); δ 8.69 (s, 2H, H_{4,4'}), 8.21 (d, 2H, J=7.8 Hz, H_{6,6'}), 7.85(dt, 2H, J=7.8, 1.2 Hz, H_{8,8'}), 7.46 (d, 2H, J=7.8Hz, H_{9,9'}), 7.41 (dt, 2H, J=7.8, 1.2 Hz, H_{7,7'}), 7.22-7.15 (m, 4H, H_{2,3,2',3'}), 6.82 (d, 2H, J=7.8Hz, H_{1,1'}), 6.29 (s, 4H, H_{5,5'}); UV-Visible (DMSO) Aromatic (π → π*) 225nm, Immine (π → π*) 262nm, Immine (n → π*) 294nm.

Tin complex (7)

White solid (75%), R_F: 0.21 (n-hexane: acetone, 2:3); IR (pure, cm⁻¹): 1638 (C=O), 1532 (C=N), 3130 (Sp² C-H), 1400-1380 (C=C), 1312 (C-N), 520 (Sn-O), 510 (N→Sn); ¹H NMR (DMSO), 300 MHz); δ 8.76 (s, 2H, H_{4,4'}), 8.31(d, 2H, J=7.8 Hz, H_{6,6'}), 7.80(dt, 2H, J=7.8, 1.2 Hz, H_{8,8'}), 7.48 (d, 2H, J=7.8Hz, H_{9,9'}), 7.43 (dt, 2H, J=7.8, 1.2 Hz, H_{7,7'}), 7.18-7.12 (m, 4H, H_{2,3,2',3'}), 6.91 (d, 2H, J=7.8Hz, H_{1,1'}), 6.21 (s, 4H, H_{5,5'}); UV-Visible (DMSO) Aromatic (π → π*) 227nm, Immine (π → π*) 267nm, Immine (n → π*) 291nm.

Manganese complex (8)

Brown solid (83%), R_F: 0.36 (n-hexane: acetone, 2:3); IR (pure, cm⁻¹): 1675 (C=O), 1553 (C=N), 3108 (Sp² C-H), 1490-1402 (C=C), 1330 (C-N), 571 (Mn-O), 590 (N→Mn); ¹H NMR (DMSO), 300 MHz); δ 8.78 (s, 2H, H_{4,4'}), 8.29 (d, 2H, J=7.8 Hz, H_{6,6'}), 7.86 (dt, 2H, J=7.8, 1.2 Hz, H_{8,8'}), 7.44 (d, 2H, J=7.8Hz, H_{9,9'}), 7.34 (dt, 2H, J=7.8, 1.2 Hz, H_{7,7'}), 7.11-7.06 (m, 4H, H_{2,3,2',3'}),

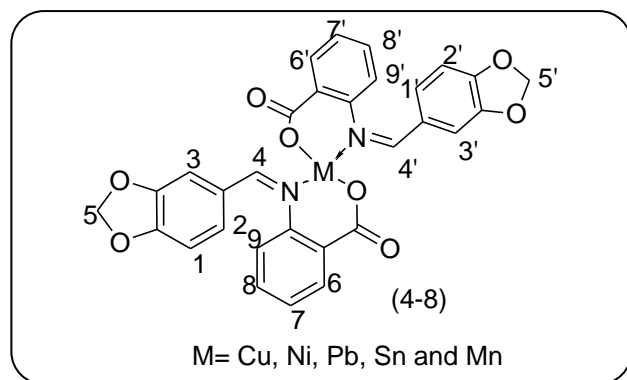


Fig. 3: Structure of the synthesized Schiff base metal complexes showing different protons position.

6.97 (d, 2H, $J=7.8\text{Hz}$, $H_{1,1'}$), 6.24 (s, 4H, $H_{5,5'}$); UV-Visible (DMSO) Aromatic ($\pi \rightarrow \pi^*$) 226nm, Immine ($\pi \rightarrow \pi^*$) 266nm, Immine ($n \rightarrow \pi^*$) 302nm.

Antibacterial activity

Antibacterial activity of the synthesized Schiff base and its metal complexes were tested by agar well diffusion method [20] against gram positive (*S.Aureus*) and gram negative (*E.coli*) bacterial strains. The zones of inhibition were measured in millimeter. The standard drug used for comparison of the antibacterial activity was Vancomycin.

Antioxidant activity

The antioxidant activity of the synthesized Schiff base and its metal complexes was evaluated by DPPH method [21] and the results show that all of the compounds are inactive. The standard used for the comparison of antioxidant activity was Gallic acid and N-acetyl-cysteine.

RESULTS AND DISCUSSION

Chemistry

In the current research we have synthesized Schiff base and five metal complexes. Schiff base was synthesized from the condensation of piperonal (1) and anthranilic acid (2) in the presence of glacial acetic acid as a catalyst in ultrasonic bath (Scheme 1). Synthesis of Schiff base was confirmed by the physical constants and spectroscopic data (IR, ^1H NMR and Mass spectrometry). The appearance of peak in IR spectra of Schiff bases at 1616cm^{-1} and 3340cm^{-1} confirms formation of Schiff base; these two peaks were assigned to imine bond and OH. Similarly in ^1H NMR the appearance of peaks at 8.61 ppm and 14.89 ppm confirmed the formation of the

Schiff base as the peaks belong to imine and OH (acidic) protons. Further confirmation was done through EI-MS where the molecular ion peak at 269 m/z confirmed the synthesis of the Schiff base.

Schiff base metal complexes were synthesized in very good yields by the reaction of Schiff base with different metal salts in ultrasonic bath (Scheme 1). Synthesis of Schiff base metal complexes was confirmed by IR spectroscopic data where the peaks for hydroxyl group were absent in the complexes spectra, similarly the peaks for imine group in the complexes spectra shifted toward lower value which confirmed that the lone pair of electrons on the nitrogen of imine group is involved in coordination with the metal [19]. The syntheses of complexes was also confirmed by the UV-Visible spectroscopy because the peak due to the imine group ($n \rightarrow \pi^*$) transition in complexes shifted towards longer wavelength which also shows that the lone pair of imine is involved in coordination with metal atom. The synthesis of the Schiff base complexes was also confirmed by the ^1H NMR where absence of acidic OH proton and presence of all other relevant peaks confirmed desired product structure.

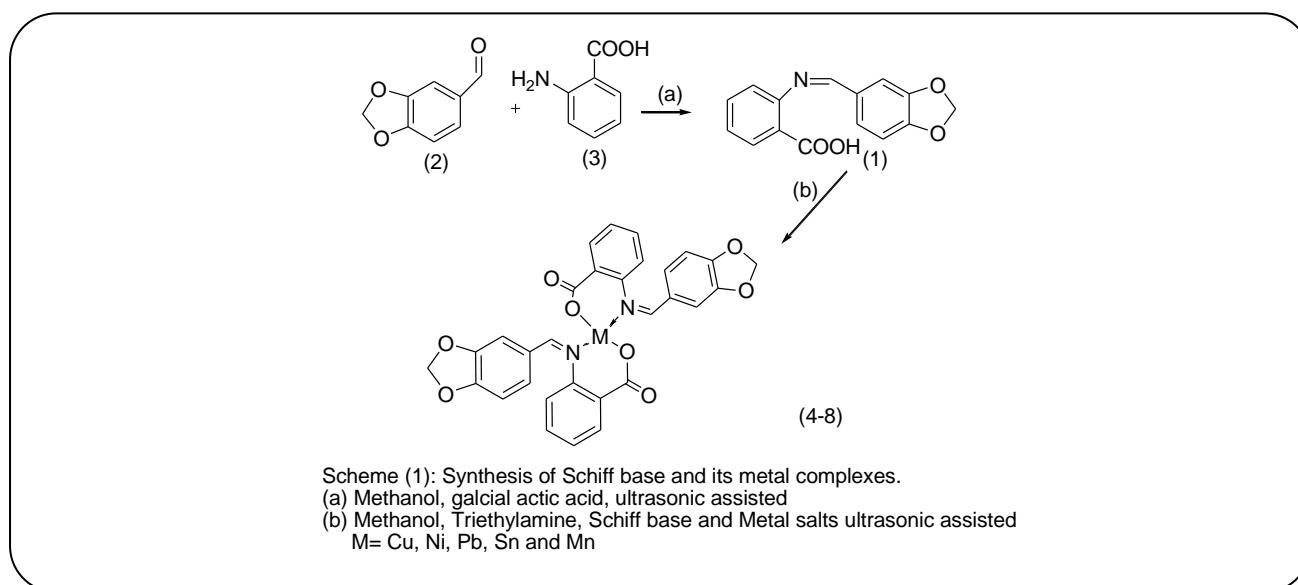
Biological activity

Antibacterial activity

The synthesized Schiff base and its metal complexes were screened for their antibacterial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacterial strains. The zones of inhibition for test samples were measured in millimeter and compared with the standard drug vancomycin. All the compounds were found active against the analyzed bacterial strains; however the complexes showed higher activity than the Schiff base. Among complexes, lead complex (6) exhibited best activity against both strains *S. aureus* and *E. coli* with zones of inhibition 37.9mm and 33.4mm respectively compared to standard vancomycin with zones of inhibition 29mm and 36mm respectively. Activity of the lead complex was even higher than the standard drug against the gram positive bacteria with (*S. aureus*). Manganese complex (8) was found least active among complexes. Difference in activity potential of the same compound against used strains is due to the difference in cell wall composition of the gram positive and gram negative bacteria. Present study also supports the fact that the antibacterial activity not only depends

Table 1: Antibacterial activity of the synthesized Schiff base and its metal complexes.

Compound	Zone of inhibition (mm)	
	S. aureus	E. coli
(3)	20.6	16.9
Cu-complex (4)	21.8	27.5
Ni-complex (5)	27.7	23.5
Pb-complex (6)	37.9	33.4
Sn-complex (7)	26.2	22.8
Mn-complex(8)	21.5	18.7
Vancomycin	29	36

**Fig. 4: Synthesis of Schiff base and its metal complexes.**

on the organic part of the complex but also on the nature of the metal present in molecule. The following Table 1 shows the antibacterial activity of the synthesized compounds. The following graph shows the comparison of antibacterial activity of different compounds.

Antioxidant activity

The synthesized Schiff base and its metal complexes were also screen for their antioxidant activity. The standards used for the comparison of antioxidant activity were Gallic acid and N-acetyl cysteine. The results in this case were not encouraging as all of the synthesized compounds were inactive. The following table shows the limited antioxidant activity of the Schiff base and its metal complexes.

CONCLUSIONS

A novel Schiff base was synthesized from piperonal and anthranilic acid and then its metal complexes with

different metal salts in ultrasonic bath. In comparison to the other conventional methods the time of reaction was greatly reduced and yields of the products were high as compared to the other methods used for similar kind of reactions. The synthesized compounds were screened for their antibacterial activity where very good results were obtained. Complexes show higher antibacterial activity as compared to the Schiff base (lead complex being most active). However all the synthesized products were found inactive when evaluated for their antioxidant activity.

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Table 2: Anti-oxidant Activity of Schiff base and its metal complexes (3-8).

Compound	IC ₅₀ ± SEM [μM]	% RSA (Radical Scavenging Activity)
(3)	Inactive	9.1207
Cu-complex (4)	Inactive	17.558
Ni-complex (5)	Inactive	10.02
Pb-complex (6)	Inactive	-ive
Sn-complex (7)	Inactive	13.452
Mn-complex(8)	Inactive	17.981
Standard: Gallic acid	23.436±0.43	93.93
Standard: N-acetyl-cysteine	111.44±0.7	95.95

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