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Syntheses, Spectroscopy, Thermal Analysis, Antioxidant Activity of Two Novel Schiff bases 3-(*m*-tolylimino)indolin-2-one and (*E*)-1-((*m*-tolylimino)methyl)naphthalen-2-ol

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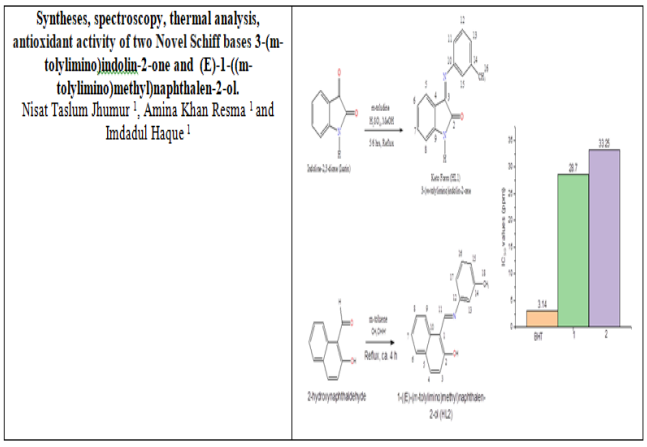
Abstract:- Reaction of 1H-Indole-2,3-dione (Isatin) and 2-hydroxy-1-naphthaldehyde with *m*-toluidine provided the corresponding 3-(*m*-tolylimino)indolin-2-one (HL1) and (*E*)-1-((*m*-tolylimino)methyl)naphthalen-2-ol (HL2). IR and ^{1}HNMR spectra demonstrate the Keto-tautomer in the solid state. The little molar conductance (Λ_m = 0.12 (HL1), 0.24 (HL2)) S m^2 mol^{-1} suggests non-electrolyte behaviour of the Schiff base in DMF at room temperature. Differential scanning calorimetry (DSC) results suggest an irreversible phase transformation from solid crystals to isotropic liquids and provides estimates of the thermal stability for HL1 and HL2. Different analytical techniques including Fourier transform infrared (FTIR), 1H-NMR, thermal analysis, electronic spectra, antioxidant testing and molar conductance techniques were used to characterize the compounds (HL1) and (HL2).

GRAPHICAL ABSTRACT



Keywords:- *Schiff Bases; Thermal Analysis and Antioxidant Activity.*

I. INTRODUCTION

A chemical molecule containing a functional group that has a carbon-nitrogen double bond (C=N) is referred to as a "Schiff base, where the nitrogen atom is not coupled to hydrogen but is instead attached to an aryl or an alkyl group. The synthesis of schiff bases, which are adaptable ligands, involves the condensation of primary amines with carbonyl groups in a variety of environments and solvents with the removal of water molecules[1]. They are utilized to create pharmaceutical molecules and are well-known to be vital in medicine. In addition to their use as antibacterial agents, the Schiff bases are therapeutically effective and exhibit cytotoxic, anti-inflammatory, anti-tumorous, antioxidant and anti-tubercular action[2].The indoline-2,3-dione (Isatin) and 2-hydroxy-1-naphthaldehyde, a cheap starting material for drug synthese derived Schiff bases are particularly relevant due to their pharmacological activity as anticancer, antibacterial, antifungal, antiviral, antileukemic, and antiprotozoal agents[3,4,5]. In this regard, isatin was condensed with arylamine[6,7], hydrazine[8,9], semicarbazide[10,11], thiosemicarbazide[12,13], and dithiocarbazate[14], producing several isatin-Schiff bases and their derivatives. Similar Schiff bases with Ar = o/p-tolyl and their Cu/Ni/Zn(II) complexes were also described[15,16] with the predicted square planer geometry around the metal ion.

The current project, which is a continuation of an earlier one, describes the outcomes of syntheses, spectroscopy, and antioxidant investigations on the isatin-Schiff base 3-(*m*-tolylimino)indolin-2-one (HL1) and 2-hydroxy-1-naphthaldehyde-Schiff base (*E*)-1-((*m*-tolylimino)methyl)naphthalen-2-ol (HL2), respectively. We have investigated biological Activity (Antioxidant) test. In future, we will try to do metal complex and further researched.

II. MATERIALS AND METHODS

At room temperature, IR spectra were captured using a Nicolet iS10 (Thermo Scientific) spectrometer. Shimadzu UV 1800 spectrophotometer data were collected in chloroform at 25°C. In DMSO-d6 at 20°C, 1H NMR spectra were taken using a Bruker Avance DPX 400 spectrometer. On a Shimadzu DSC-60, differential scanning calorimetry (DSC) was carried out at 30-300°C (about 5°C over the melting point) with a heating rate of 10 K min^{-1}.

A. Synthesis of the Schiff Base Ligands (HL1)

A solution of 1-H-Indole-2,3-dione (Isatin) (0.74 g, 5.0 mmol) was dissolved in 10 mL of methanol before 3–4 drops of concentrated H_2SO_4 were added. The mixture was then stirred for approximately 10 minutes at RT. In this

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solution, m-toluidine was gradually added in an equimolar amount (0.54 g, 5.0 mmol, diluted in 5 mL of methanol). After that, the reaction mixture was refluxed for approximately 5 to 6 hours. To track the reaction's development, thin-layer chromatography (TLC) was used. After the reaction was complete and no ppt was generated, a rotary evaporator was used to decrease the volume of the solution to around 50%. Precipitate was then observed and filtered out. Three times (2 mL each) of methanol were used to wash the products. To get light-yellow products of 3-(*m*-tolylimino)indolin-2-one (HL1), the products were dried in open air.

3-(*m*-tolylimino)indolin-2-one (HL1)**:** Yield: 0.896 g (70 %, based on 1-H-Indole-2,3-dione (Isatin)). IR (KBr): ν =3458 (H_2O) 3166, 3118 (N-H), 3066, 3016, 2991, 2976 w (H-Ar),1745, 1724,1658 (C=O), 1614,1598 vs (C=N) and 1581 vs (C=C) cm^{-1}. UV-Vis (0.10 mM, MeOH): λ_{max}/nm (ε_{max}/L mol^{-1} cm^{-1}) = 296 (14110) and 418.5 (7190). ^{1}HNMR (400MHz, DMSO-d_6): δ/ppm = δ2.34 ppm (s, 3H, H_{16}), δ6.39 ppm (d, J_{HH} = 7.6 Hz, 1H, H_{15}), δ6.71-6.80 ppm (m, 3H, $H_{11,12,13}$), δ686–6.94 ppm (m, 1H, H_6), δ7.06 ppm (t, J_{HH} = 7.2 Hz, 1H, H_7), δ7.32 – 7.37 ppm (m, 2H, $H_{5,8}$), δ10.97 ppm (s, br, NH). (for hydrogen atom numbering see (Fig.2).

B. Synthesis of the Schiff Base Ligands (HL2)

A solution of 2-hydroxy-1-naphthaldehyde (0.861 g, 5.0 mmol) was dissolved in 10 mL of methanol before 3–4 drops of concentrated H_2SO_4 were added. The mixture was then stirred for approximately 10 minutes at room temperature. In this solution, m-toluidine was gradually added in an equimolar amount (0.54 g, 5.0 mmol, diluted in 5 mL of methanol). After that, the reaction mixture was refluxed for approximately 4 hours. After the reaction was complete and ppt was generated, a rotary evaporator decreased the volume of the solution to around 50%. Precipitate then filtered out. Three times (2 mL each) of methanol were used to wash the products. To create light-yellow orange microcrystals of (*E*)-1-((*m*-tolylimino)methyl)naphthalen-2-ol (HL2), the products were dried open air.

(*E*)-1-((*m*-tolylimino)methyl)naphthalen-2-ol(HL2): Yield 0.91 g (65%).– IR (KBr, cm^{-1}): ν = 3059, 3037, 2914w (C-H), 1625, 1589vs (C=N) and 1544vs (C=C). UV-Vis (0.101 mM, MeOH): λ_{max}/nm (ε_{max}/L mol^{-1} cm^{-1}) = 322 (6790), 378 (5380) and 467 (4430). ^{1}HNMR (400MHz, DMSO-d_6): δ/ppm = δ2.40 ppm (s, 3H, H_{18}), δ6.98 ppm (d, J_{HH} = 9.2 Hz, 1H, H_{13}), δ7.13 ppm (d, J_{HH} = 7.2 Hz, 1H, H_{15}),δ7.31-7.46 ppm (m, 3H, $H_{7,16,17}$), δ7.51 ppm (d, J_{HH} = 9.2 Hz, 1H, H_3), δ7.50–7.59 ppm (m, 1H, H_8), δ7.78 ppm (dd, J_{HH} = 7.9, 1.4 Hz, 1H, H_9), δ7.92 ppm (d, J_{HH} = 9.2 Hz, 1H, H_6), δ8.48 ppm (d, J_{HH} = 8.4 Hz, 1H, H_4), δ9.63 ppm (d, J_{HH} = 5.6 Hz, 1H, CHN), δ15.84 ppm (d, J_{HH} = 5.8 Hz, 1H, OH). (for hydrogen atom numbering).

C. Antioxidant Assay

According to one of the most popular antioxidant methods, DPPH assays, the Scavenging Capacity of HL**1** and HL**2** were examined. As a standard, butylated hydroxytoluene (BHT) was used. Using 80% methanol, a 1000 ppm stock solution of the standard, samples (HL1 and HL2), and 0.004% DPPH were created. The standard and samples were then prepared at various concentrations using 80% methanol. The standards and samples were combined with 2.5 mL of 0.004% DPPH in exactly the same amounts. The mixtures were measured for absorbance at 517 nm using a spectrophotometer after 30 minutes of incubation in a dark environment. The following equation was used to calculate the percentage of DPPH inhibition:

$$\text{DPPH scavenging activity (\%)} = \frac{(\textbf{Abs control} - \textbf{Abs sample})}{\textbf{Abs control}} \times 100$$

Where **Abs** control and **Abs** sample, respectively, represent the absorbance of the control and sample. The concentration of a sample required to decrease DPPH activity by 50% is known as the IC_{50}, which is used to express DPPH scavenging capacity. By constructing an equation of line from a graph of concentration (g/mL) vs percentage of inhibition, the IC_{50} value was calculated.

III. RESULTS AND DISCUSSION

Reaction of 1H-Indole-2,3-dione (Isatin) and 2-hydroxy-1-naphthaldehyde with *m*-toluidine provided the corresponding 3-(m-tolylimino)indolin-2-one (HL1) and (E)-1-((m-tolylimino)methyl)naphthalen-2-ol(HL2).
Vibrational spectra feature very strong bands at 1625, 1614, 1589, 1588 cm^{-1} for νC=N and at 1560, 1544 cm^{-1} for νC=C. The very low molar conductance (Λ_m = 0.12 (HL1), 0.24 (HL2) suggests non-electrolyte behaviour of the ligands in DMF at 25°C.

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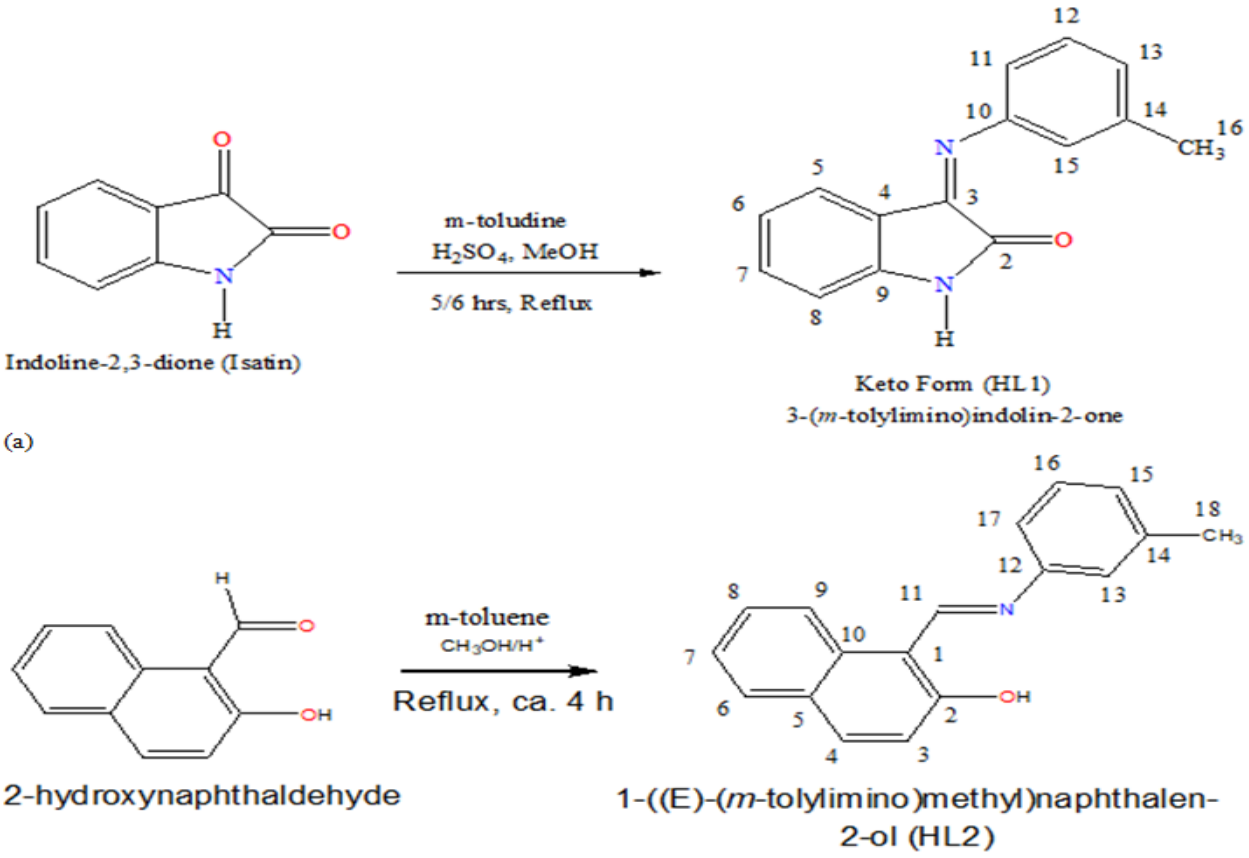


Scheme 1. Synthetic method to the formation of (a) 3-(*m*-tolylimino)indolin-2-one (HL1) and (b) (*E*)-1-((*m*-tolylimino)methyl)naphthalen-2-ol (HL2).

➢ *^{1}H NMR spectra (HL1) and (HL2)*

- *For HL1 :*

The ^{1}H-NMR spectra for HL1 (Fig. 1) displays a singlet peak for the methyl protons (CH_3) at = 2.34 ppm, while the aromatic protons are predominately represented by several doublet, triplet, and multiplet peaks at 6.84–7.57 ppm (see Experimental section). The NH proton exhibits a broad singlet at = 10.97 ppm (peak assignments are in the experimental section).

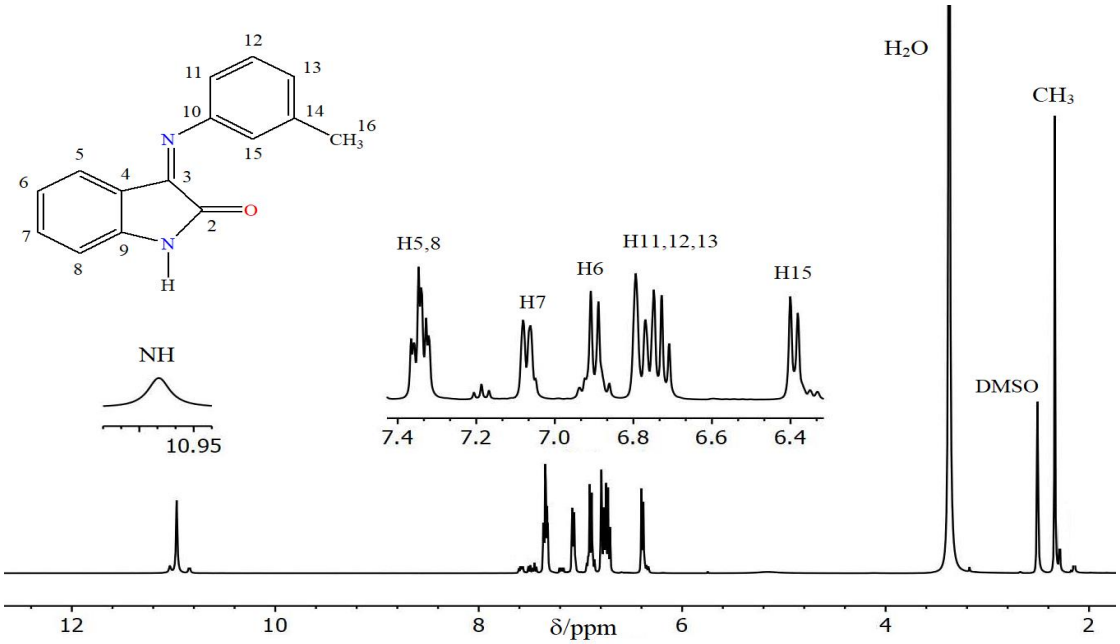


Fig 1 ^{1}H NMR spectrum for HL1 in DMSO-d_6 at 20°C

- *For HL2:*

The signal for the methyl protons (CH_3) and imine proton (CHN) appear as singlet at = 2.40 and 9.63 ppm, respectively, in the ^{1}H-NMR spectrum for HL2 (Fig. 2). Due to de-shielding from the main N atom and the benzene ring next to the carbon atom, the imine proton peak is seen somewhat downfield. The aromatic protons (Ar-H) exhibit multiplet peaks in the range of = 6.98-8.48 ppm, whereas the phenolic proton exhibits a broad singlet at = 15.84 ppm (see experimental section for specific peak assignments).

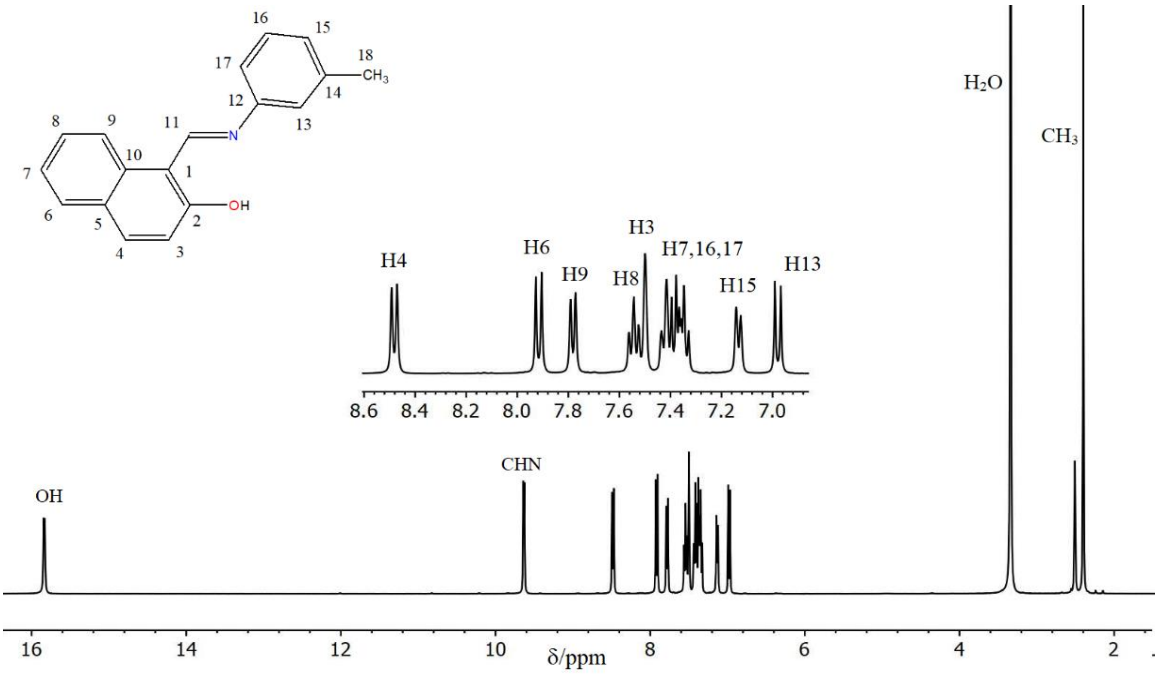


Fig 2 ^{1}H NMR Spectrum for HL2 in DMSO-d_6 at 20 °C

➢ *Experimental Electronic Spectra (UV-Vis.)*

The electronic absorption (UV-Vis.) spectra for HL1 and HL2 were measured in $CHCl_3$ at RT (Figure-3 (a,b)). The spectra are identical through out the measured spectral range. The spectra feature several bands such as (i) Two or one very strong band below *ca.*400 nm with absorption maxima at *ca.*296 nm (HL1) and 332 and 380 nm for intra-ligand $\pi \rightarrow \pi^*$ transitions called ligand to ligand charge transfer (LLCT)[15,16], (ii) a strong band at 400-500 nm with absorption maxima at *ca.*417 nm (HL1) and 445 nm (HL2) for intra-ligand $n \rightarrow \pi^*$ transitions (LL) (figure-3).

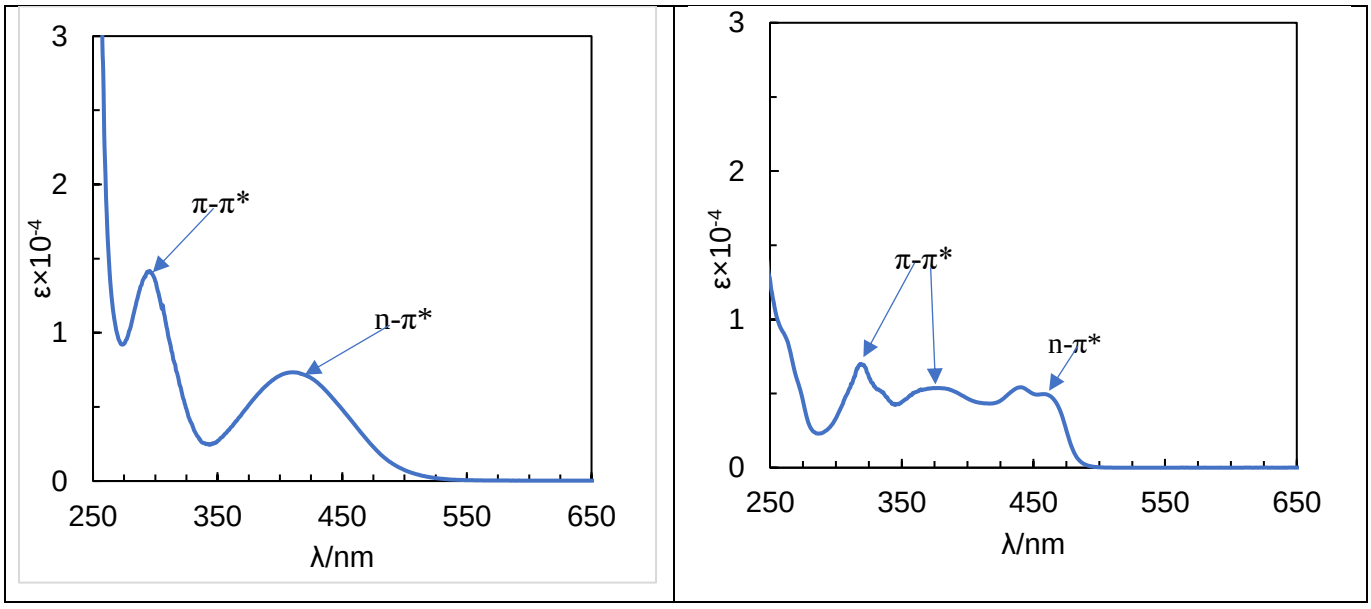


Fig 3 Experimental Spectrum for HL1 (a) and HL2 (b) (ca. 0.10 mM) in chloroform at 25°C

➢ *Thermal Analysis*

Thermal analysis for **HL1 and HL2** were investigated by differential scanning calorimetry (DSC) following the phase transformation and enthalpy changes (ΔH)[16, 17a,b]. The DSC heating curve for the HL1 and HL2 show an endothermic peak at ca. 243°C (ΔH = -32.91 kJ mol^{-1}) and ca. 110°C (ΔH = -28.57 kJ mol^{-1}) corresponds to transformation from crystalline-solid (Cr) to isotropic-liquid phase (I or m.p., Cr ⇆ I) (Fig. 6a and Table 3). The cooling curve shows no peak on the reverse direction, corresponding an irreversible phase transformation. The 2nd heating curve shows no peak on the opposite direction.

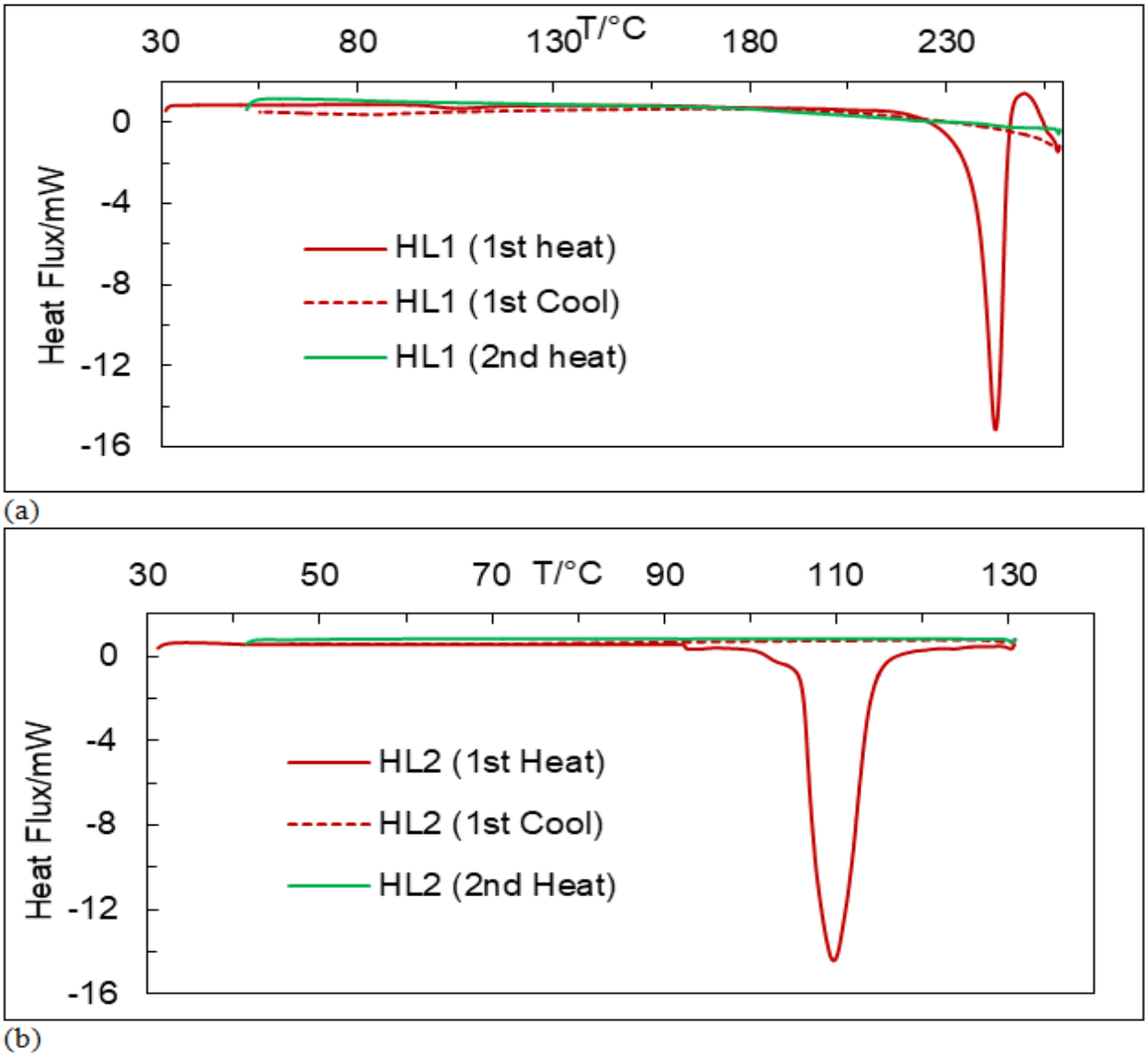


Fig 4 Differential Scanning Calorimetry (DSC) Heating curve for HL1 (a) and HL2 (b).

Table 1 Phase Transformation and Thermal Stability for the Compounds

Compounds	Peak temperature (°C)/ΔH (kJ mol^{-1}) * (1st cycle)	Peak temperature (°C)/ΔH (kJ mol^{-1}) * (2nd cycle)
HL1	ca. 243/ -32.91 (Cr ⇆ I) (heat) No peak (cool)	No peak (heat) No peak (cool)
HL2	ca. 110/ -28.57 (Cr ⇆ I) (heat)	No peak (heat)

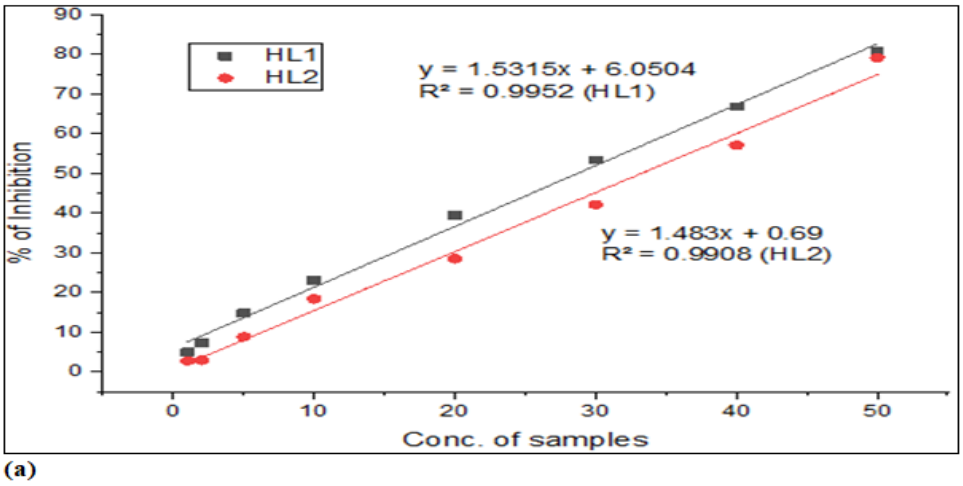
* ΔH = Heat of transformation.

➢ *Antioxidant Activity*

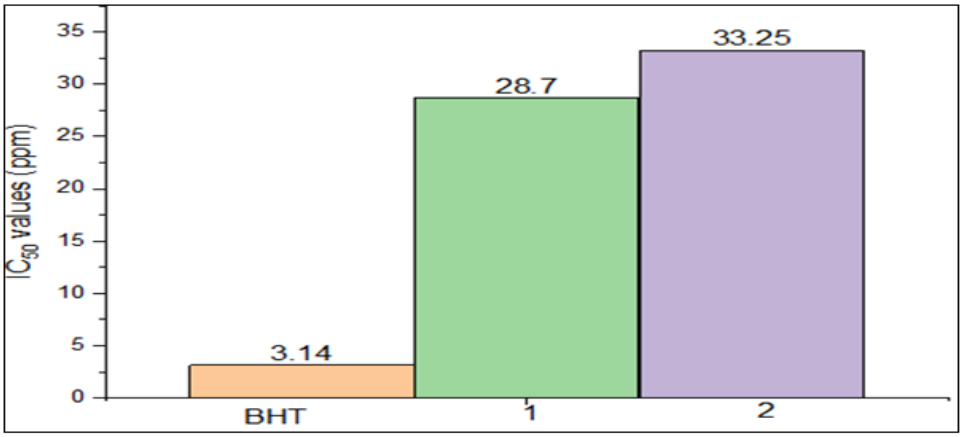
Additionally, the value of IC_{50} (the sample concentration necessary to suppress DPPH absorbance by 50%) is shown in Fig.5.[18] The IC_{50} values of HL1 and HL2 were 28.70 µg/mL and 33.25 µg/mL respectively. The lower the IC_{50} value, the higher is the antioxidant activity [19]. Compounds HL1 and HL2 showed good scavenging activity with IC_{50} values of 28.70 and 33.25 ppm respectively. These substances operate as natural antioxidants to eliminate harmful free radicals produced by normal cellular processes. The IC_{50} values of the Schiff bases (HL1 and HL2) are given in Table.2.

Table 2 Antioxidant Activities of the 1 and 2 Complexes

Compound	IC_{50} Value (ppm)	Inference
HL1	28.70	Antioxidant
HL2	33.25	Antioxidant



(a)



(b)

Fig 5 DPPH Radical Scavenging Capacity (a) and IC_{50} Values (b) of BHT and Schiff bases (HL1) and (HL2)

IV. CONCLUSION

Reaction of 1H-Indole-2,3-dione (Isatin) and 2-hydroxy-1-naphthaldehyde with *m*-toluidine provided the corresponding 3-(*m*-tolylimino)indolin-2-one (HL1) and (*E*)-1-((*m*-tolylimino)methyl)naphthalen-2-ol (HL2). Molecular structures are confirmed by IR, ^{1}HNMR spectroscopy. Differential scanning calorimetry (DSC) results suggest an irreversible phase transformation from solid crystals to isotropic liquids and provides estimates of the thermal stability for HL1 and HL2. Compounds HL1 and HL2 showed good scavenging activity.

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➢ *Conflicts of Interest*:

The authors affirm that none of the known financial or personal conflicts between them could potentially have impacted the research presented in this study.

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