

CHAPTER 3

EXPERIMENTAL

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Six Schiff bases were prepared from the reaction of 4-phenyl-1,3,5-triazine-2,6-diamine with 5-chlorosalicylaldehyde, 5-bromosalicylaldehyde, 5-nitrosalicylaldehyde, 3-hydroxysalicylaldehyde, 4-hydroxysalicylaldehyde, and 3,5-di-*tert*-butylsalicylaldehyde. These Schiff bases were labeled as H₂L1 – H₂L6 respectively (Figure 3.1).

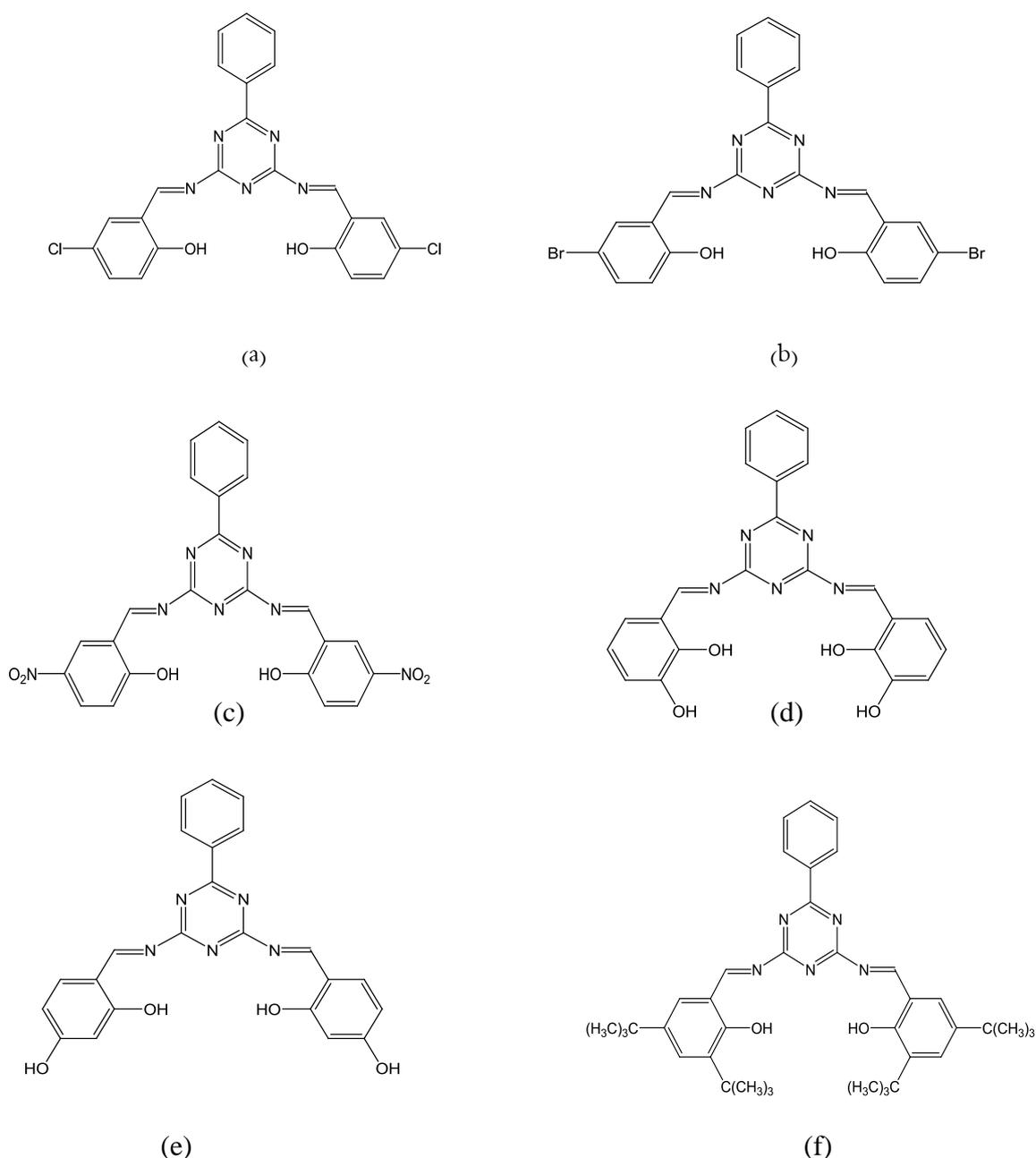


Figure 3.1 Structural formula of Schiff bases: (a) H₂L1; (b) H₂L2; (c) H₂L3; (d) H₂L4 (e) H₂L5 and (f) H₂L6

The above Schiff bases were then used to prepare nickel(II), copper(II) and zinc(II) complexes (a total of eighteen). The methods used to prepare the Schiff bases and metal(II) complexes were as reported in the literature [67,68].

The bases were characterized by elemental analysis (CHN), Fourier transform infrared spectroscopy (FT-IR), ^1H - and ^{13}C - nuclear magnetic resonance spectroscopy (NMR), ultraviolet-visible spectroscopy (UV-vis), while the complexes were additionally characterized by CHN, FT-IR, UV-vis and thermogravimetric analysis (TGA).

3.1 Materials

The chemicals used in the synthesis were 4-phenyl-1,3,5-triazine-2,6-diamine ($\text{C}_9\text{H}_9\text{N}_5$, FW 187.21), 5-chlorosalicylaldehyde (5- $\text{ClC}_6\text{H}_3(\text{OH})\text{CHO}$; FW 156.57), 5-bromosalicylaldehyde (5- $\text{BrC}_6\text{H}_3(\text{OH})\text{CHO}$; FW 201.03), 5-nitrosalicylaldehyde ($\text{C}_7\text{H}_5\text{O}_4\text{N}$, FW 167.12), 3-hydroxysalicylaldehyde ($\text{C}_6\text{H}_3(\text{OH})_2\text{CHO}$; FW 138.12), 4-hydroxysalicylaldehyde ($\text{C}_6\text{H}_3(\text{OH})_2\text{CHO}$; FW 138.12), 3,5-di-*tert*-butylsalicylaldehyde ($\text{C}_{15}\text{H}_{22}\text{O}_2$, FW 234.34), triethylamine, copper(II) acetate monohydrate ($\text{CuC}_4\text{H}_6\text{O}_4\cdot\text{H}_2\text{O}$; FW 199.65), nickel(II) acetate tetrahydrate ($\text{NiC}_4\text{H}_6\text{O}_4\cdot 4\text{H}_2\text{O}$; FW 248.86) and zinc acetate dihydrate ($\text{C}_4\text{H}_6\text{O}_4\text{Zn}\cdot 2\text{H}_2\text{O}$, FW 219.50). These chemicals and common organic solvents were commercially available and used as received.

3.2 Preparation of H_2L1 and its metal complexes

3.2.1 H_2L1

A solution of 5-chlorosalicylaldehyde (2 g, 12.77 mmol) in ethanol (40 cm³) was mixed with a solution of 4 phenyl-1,3,5 triazine-2,6-diamine (1.19 g, 6.35 mmol) in ethanol (40 cm³). The mixture was stirred under reflux for 2 hours. The pale yellow powder formed was filtered and recrystallized from ethanol. It was dried in an oven (80°C) for 30 min. The yield was 2.14 g (72%). Anal. Calc. for [C₂₃H₁₅O₂N₅Cl₂; FW 464.35]: C, 59.43; H, 3.23; N, 15.07. Found: C, 59.62; H, 3.11; N, 15.51%. Selected FTIR data (KBr, cm⁻¹): 3444 (m, -OH), 2374 (m, C-H), 1622 (s, C=N), 1275 (s, C-O).

3.2.2 NiL_1

A solution of H_2L1 (0.60 g, 1.29 mmol) in ethanol (40 cm³) was added to a solution of nickel(II) acetate tetrahydrate (0.32 g, 1.28 mmol) in ethanol (30 cm³). A few drops of triethylamine were then added, and the mixture was magnetically stirred and refluxed for 3 hours. The light green powder formed was filtered and recrystallized from DMSO. The yield was 0.52 g (77%). Anal. Calc. for NiC₂₃H₁₇O₄N₅Cl₂; FW 559.04]: C, 49.50; H, 3.05; N, 12.57; Found: C, 50.20; H, 2.94; N, 12.98%. Selected FTIR data (KBr, cm⁻¹): 2873 (m, C-H), 1616 (s, C=N), 1319 (s, C-O), 542 (w, Ni-O)

3.2.3 $CuL1$

The method is the same as for $NiL1$, using H_2L1 (0.50 g, 1.07 mmol) and Cu(II) acetate monohydrate (0.21 g, 1.05 mmol). The product was a dark green powder, and the yield was 0.44 g (78%). Anal. Calc. for [CuC₂₃H₁₅O₃N₅Cl₂; FW 543.89]: C, 50.75; H, 2.75; N, 12.87. Found: C, 50.05; H, 2.92; N, 13.10%. Selected FTIR data (KBr, cm⁻¹): 2851 (m, C-H), 1616 (s, C=N), 1317(s, C-O), 565 (w, Cu-O).

3.2.4 ZnL1

The method is the same as for NiL1, using H₂L1 (0.50 g, 1.07 mmol) and Zn(II) acetate dihydrate (0.23 g, 1.04 mmol). The product was a yellow, and the yield was 0.42 g (75%). Anal. Calc. for [ZnC₂₃H₁₇O₄N₅Cl₂; FW 563.74]: C, 48.95; H, 3.01; N, 12.41. Found: C, 49.54; H, 2.91; N, 13.02%. Selected FTIR data (KBr, cm⁻¹): 2857 (m, C-H), 1616 (s, C=N), 1314 (s, C-O), 542 (w, Zn-O).

3.3 Preparation of H₂L₂ and its metal complexes

3.3.1 H₂L₂

The method is the same as for H₂L1, using 5-bromosalicylaldehyde (2g, 9.94 mmol) and 4 phenyl-1,3,5-triazine-2,6-diamine (0.93 g, 4.96 mmol). The product was a pale yellow powder, and the yield was 2.25 g (82%). Anal. Calc. for [C₂₃H₁₅O₂N₅Br₂; FW 535.25]: C, 49.88; H, 2.71; N, 12.65. Found: C, 50.03; H, 2.22; N, 12.94%. Selected FT-IR data (KBr, cm⁻¹): 3407 (m, -OH), 2371 (m, C-H), 1622 (s, C=N), 1278 (s, C-O).

3.3.2 NiL2

The method is the same as for NiL1, using H₂L2 (0.50 g, 0.90 mmol) and Ni(II) acetate tetrahydrate (0.22 g, 0.88 mmol). The product was a light green powder, and the yield was 0.42 g (76%). Anal. Calc. for [NiC₂₃H₁₇O₄N₅Br₂; FW 645.94]: C, 42.72; H, 2.63; N, 10.83. Found: C, 43.56; H, 2.59; N, 11.14%. Selected FTIR data (KBr, cm⁻¹): 2374 (m, C-H), 1617 (s, C=N), 1321 (s, C-O), 537 (w, Ni-O).

3.3.3 Preparation of CuL2

The method is the same as for NiL1, using H₂L2 (0.50 g, 0.90 mmol) and Cu(II) acetate monohydrate (0.18 g, 0.90 mmol). The product was a dark green, and the yield was 0.39 g (70%). Anal. Calc. for [CuC₂₃H₁₅O₃N₅Br₂; FW 632.79]: C, 43.61; H, 2.37; N, 11.06. Found: C, 43.07; H, 2.37; N, 11.06%. Selected FTIR data (KBr, cm⁻¹): 2346 (m, C-H), 1616 (s, C=N), 1320 (s, C-O), 563 (w, Cu-O).

3.3.4 ZnL2

The method is the same as for NiL1, using H₂L2 (0.50 g, 0.90 mmol) and Zn(II) acetate dihydrate (0.19 g, 0.86 mmol). The product was a yellow powder, and the yield was 0.41 g (74%). Anal. Calc. for [ZnC₂₃H₁₇O₄N₅Br₂; FW 652.64]: C, 42.28; H, 2.60; N, 10.72. Found: C, 43.06; H, 2.24; N, 11.12%. Selected FTIR data (KBr, cm⁻¹): 2346 (m, C-H), 1617 (s, C=N), 1315(s, C-O), 536 (w, Zn-O).

3.4 Preparation of H₂L3 and its metal complexes

3.4.1 H₂L3

The method is the same as for H₂L1, using 5-nitrosalicylaldehyde (2 g, 11.96 mmol) and 4-phenyl-1,3,5-triazine-2,6-diamine (1.12 g, 5.98 mmol). The product was an orange powder, and the yield was 2.21 g (76%). Anal. Calc. for [C₂₃H₁₅O₆N₇; FW 485.45]: C, 56.85; H 3.08; N 20.18. Found: C, 57.87; H, 2.98; N, 21.48%. Selected FT-IR data (KBr, cm⁻¹): 3445 (m, -OH), 2834 (m, C-H), 1625 (s, C=N), 1282 (s, C-O).

3.4.2 NiL3

The method is the same as for NiL1, using H₂L3 (0.60 g, 1.23 mmol) and Ni(II) acetate tetrahydrate (0.30 g, 1.20 mmol). The product was a pale green powder, and

the yield was 0.48 g (71%). Anal. Calc. for $[\text{NiC}_{23}\text{H}_{17}\text{O}_8\text{N}_7]$; FW 578.14]: C, 47.73; H, 2.94; N, 16.95. Found: C, 48.01; H, 2.58; N, 17.34%. Selected FTIR data (KBr, cm^{-1}): 2975 (m, C-H), 1619 (s, C=N), 1320 (s, C-O), 595 (w, Ni-O).

3.4.3 *CuL3*

The method is the same as for NiL1, using H₂L3 (0.60 g, 1.23 mmol) and Cu(II) acetate monohydrate (0.25 g, 1.25 mmol). The product was a green powder, and the yield was 0.50 g (74%). Anal. Calc. for $[\text{CuC}_{23}\text{H}_{15}\text{O}_7\text{N}_7]$; FW 564.99]: C, 48.85; H, 2.65; N, 17.34. Found: C, 48.13; H, 2.55; N, 17.31%. Selected FTIR data (KBr, cm^{-1}): 2801 (m, C-H), 1622 (s, C=N), 1332(s, C-O), 594 (w, Cu-O).

3.4.4 *ZnL3*

The method is the same as for NiL1, using H₂L3 (0.60 g, 1.23 mmol) and Zn(II) acetate dihydrate (0.27 g, 1.23 mmol). The product was a yellow powder, and the yield was 0.52 g (77%). Anal. Calc. for $[\text{ZnC}_{23}\text{H}_{17}\text{O}_8\text{N}_7]$ FW 548.84]: C, 47.19; H, 2.90; N, 16.75. Found: C, 48.05; H, 2.42; N, 17.38%. Selected FTIR data (KBr, cm^{-1}): 2380 (m, C-H), 1622 (s, C=N), 1311(s, C-O), 520 (w, Zn-O).

3.5 *Preparation of H₂L4 and its metal complexes*

3.5.1 *H₂L4*

The method is the same as for H₂L1, using 3-hydroxysalicylaldehyde (2 g, 14.48 mmol) and 4-phenyl-1,3,5-triazine-2,6-diamine (1.35 g, 7.21 mmol). The product was a dark grey powder, and the yield was 2.36 g (76%). Anal. Calc. for $[\text{C}_{23}\text{H}_{17}\text{O}_4\text{N}_5]$; FW 427.45]: C, 64.56 H 3.97; N16.37. Found: C, 64.98; H, 3.10; N, 17.04%. Selected FT-IR data (KBr, cm^{-1}): 3446 (m, -OH), 2985 (m, C-H), 1627 (s, C=N), 1255 (s, C-O).

3.5.2 NiL4

The method is the same as for NiL1, using H₂L4 (0.50 g, 1.16 mmol) and Ni(II) acetate tetrahydrate (0.29 g, 1.16 mmol). The product was a dark brown powder, and the yield was 0.45 g (72%). Anal. Calc. for [NiC₂₃H₁₉O₆N₅; FW 520.14]: C, 53.06 H, 3.65; N, 13.45. Found: C, 54.15; H, 2.98; N, 13.94%. Selected FTIR data (KBr, cm⁻¹): 2246 (m, C-H), 1622 (s, C=N), 1255(s, C-O), 565 (w, Ni-O).

3.5.3 CuL4

The method is the same as for NiL1, using H₂L4 (0.50 g, 1.16 mmol) and Cu(II) acetate monohydrate (0.23 g, 1.15 mmol). The product was a dark brown powder and the yield was 0.42 g (73%). Anal. Calc. for [CuC₂₃H₁₇O₅N₅; FW 506.99]: C, 54.43 H, 3.35; N, 13.80. Found: C, 53.43; H, 2.95; N, 13.80%. Selected FTIR data (KBr, cm⁻¹): 2218 (m, C-H), 1624 (s, C=N), 1258 (s, C-O), 565 (w, Cu-O).

3.5.4 ZnL4

The method is the same as for NiL1, using H₂L4 (0.50 g, 1.16 mmol) and Zn(II) acetate dihydrate (0.26 g, 1.18 mmol). The product was a pale yellow powder, and the yield was 0.44 g (73%). Anal. Calc. for [ZnC₂₃H₁₉O₆N₅ FW 526.84]: C, 52.38 H, 3.60; N, 13.28 Found: C, 53.54; H, 2.99; N, 14.01%. Selected FTIR data (KBr, cm⁻¹): 2795 (m, C-H), 1621 (s, C=N), 1257 (s, C-O), 545 (w, Zn-O).

3.6 Preparation of H₂L5 and its metal complexes

3.6.1 H₂L5

The method is the same as for H₂L1, using 4-hydroxysalicylaldehyde (2 g, 14.48 mmol) and 4 phenyl-1,3,5-triazine-2,6-diamine (1.35 g, 7.21 mmol). The product was an orange powder and the yield was 2.45 g (78%). Anal. Calc. for [C₂₃H₁₇O₄N₅; FW 427.45]: C, 64.56 H 3.97; N16.37. Found: C, 65.21; H, 3.49; N, 16.92%. Selected FT-IR data (KBr, cm⁻¹): 3401 (m, -OH), 2368 (m, C-H), 1628 (s, C=N), 1260 (s, C-O).

3.6.2 NiL5

The method is the same as for NiL1, using H₂L5 (0.50 g, 1.16 mmol) and Ni(II) acetate tetrahydrate (0.29 g, 1.16 mmol). The product was a light green powder, and the yield was 0.43 g (76%). Anal. Calc. for [NiC₂₃H₁₉O₆N₅; FW 520.14]: C, 53.06 H, 3.65; N, 13.45. Found: C, 54.26; H, 3.12; N, 14.02%. Selected FTIR data (KBr, cm⁻¹): 2238 (m, C-H), 1622 (s, C=N), 1269(s, C-O), 568 (w, Ni-O).

3.6.3 CuL5

The method is the same as for NiL1, using H₂L5 (0.50 g, 1.16 mmol) and Cu(II) acetate monohydrate (0.23 g, 1.15 mmol). The product was a light green powder and the yield was 0.39 g (71%). Anal. Calc. for [CuC₂₃H₁₇O₅N₅; FW 506.99]: C, 54.43 H, 3.35; N, 13.80. Found: C, 53.10 H, 3.05; N, 14.78%. Selected FTIR data (KBr, cm⁻¹): 2344 (m, C-H), 1626 (s, C=N), 1264 (s, C-O), 596 (w, Cu-O).

3.6.4 ZnL5

The method is the same as for NiL1, using H₂L5 (0.50 g, 1.16 mmol) and Zn(II) acetate dihydrate (0.26 g, 1.18 mmol). The product was a light yellow powder, and the yield was 0.45 g (79%). Anal. Calc. for [ZnC₂₃H₁₉O₆N₅ FW 526.84]: C, 52.38 H, 3.60; N, 13.28 Found: C, 53.15; H, 3.02; N, 13.82%. Selected FTIR data (KBr, cm⁻¹): 2358 (m, C-H), 1618 (s, C=N), 1258 (s, C-O), 511 (w, Zn-O).

3.7 Preparation of H₂L6 and its metal complexes

3.7.1 H₂L6

The method is the same as for H₂L1, using 3,5-di-*tert*-butylsalicylaldehyde (2 g, 8.53 mmol) and 4 phenyl-1,3,5-triazine-2,6-diamine (0.79 g, 4.21 mmol). The product was a pale yellow powder and the yield was 2.13 g (81%). Anal. Calc. for [C₃₉H₄₉O₂N₅; FW 619.89]: C, 75.49 H 7.90; N 11.29. Found: C, 76.35; H, 7.21; N, 11.87%. Selected FT-IR data (KBr, cm⁻¹): 3329 (m, -OH), 2923 (m, C-H), 1612 (s, C=N), 1273 (s, C-O).

3.7.2 NiL6

The method is the same as for NiL1, using H₂L6 (0.50 g, 0.81 mmol) and Ni(II) acetate tetrahydrate (0.20 g, 0.81 mmol). The product was a light green, and the yield was 0.41 g (75%). Anal. Calc. for [NiC₃₉H₅₁O₄N₅; FW 712.58]: C, 65.67 H, 7.15; N, 9.82. Found: C, 66.57; H, 6.46; N, 10.15%. Selected FTIR data (KBr, cm⁻¹): 3054 (m, C-H), 1606 (s, C=N), 1239(s, C-O), 550 (w, Ni-O).

3.7.3 CuL6

The method is the same as for NiL1, using H₂L6 (0.50 g, 0.81 mmol) and Cu(II) acetate monohydrate (0.16 g, 0.81 mmol). The product was a dark green powder and the yield was 0.43 g (78%). Anal. Calc. for [CuC₃₉H₄₉O₃N₅; FW 699.43]: C, 66.91 H, 7.01 N, 10.01. Found: C, 66.13 H, 6.34; N, 10.79%. Selected FTIR data (KBr, cm⁻¹): 2345 (m, C-H), 1605 (s, C=N), 1241 (s, C-O), 546 (w, Cu-O).

3.7.4 ZnL6

The method is the same as for NiL1, using H₂L1 (0.50 g, 0.81 mmol) and Zn(II) acetate dihydrate (0.18 g, 0.81 mmol). The product was a light yellow powder, and the yield was 0.39 g (75%). Anal. Calc. for [ZnC₃₉H₅₁O₄N₅ FW 719.28]: C, 65.06 H, 7.09; N, 9.73 Found: C, 66.14; H, 6.78; N, 10.03%. Selected FTIR data (KBr, cm⁻¹): 2532 (m, C-H), 1608 (s, C=N), 1240(s, C-O), 548 (w, Zn-O).

3.8 Anti-oxidant activities of the Schiff bases and complexes by DPPH method.

Free radical scavenging activity of the test compounds were determined by the 1,1-diphenyl picrylhydrazyl (DPPH) assay method. Each of the Schiff bases was dissolved in methanol to obtain a concentration of 1 mg/ml. These stock solutions were then diluted to 5, 10, 25, 50, and 100 µg / ml in methanol. Ascorbic acid was prepared in a similar way and used as a positive control. Then, 200 µl of each sample solution or positive control were combined with 50 µl of DPPH (0.3 mg/ml) in triplicate in a 96-well microtitre plate. Final concentrations of the Schiff bases were 4, 8, 20, 40, and 80 µg/ml. Microtitre plates were incubated for 30 min at room temperature.

3.9 Anti-oxidant activities of the Schiff bases and complexes by FRAP

method.

The FRAP was determined as previously described with modifications. This method is based on a redox reaction in which the antioxidants act as reductants, and an easily reduced oxidant (Fe^{3+}) is used in stoichiometric excess, resulting in a blue ferrous complex. The absorbance was then determined spectrophotometrically at 518 nm with the Infinite® 200 PRO plate reader (TECAN, Männedorf, Switzerland). For this a freshly prepared Fe^{3+} -TPTZ complex solution pre-incubated at 37 °C. This solution (FRAP reagent) was prepared by mixing acetate buffer (300 mmol L⁻¹, pH 3.6), TPTZ (10 mmol L⁻¹ in 1.0 mol L⁻¹ HCl), and FeCl_3 (20 mmol L⁻¹) at 10:1:1 (v/v/v). Then, 200 µl of FRAP reagent solution were combined with 50 µl of Schiff base or positive control solutions in triplicate in a 96-well microtitre plate. The mixtures were shaken and incubated at 37 °C for 30 min before absorbance reading at 593 nm. All treatments were run in triplicate. BHT and ascorbic acid (Vit.C) were used as positive controls.

3.10 Analysis

3.10.1 Elemental analyses

CHN analysis was carried out in PerkinElmer (Series II) CHNS/O analyzer 2400.

3.10.2 FTIR spectroscopy

FTIR spectra were recorded as KBr pellets in the range 4000-370 cm^{-1} on a Perkin-Elmer Spectrum 2000 FT-IR spectrophotometer.

3.10.3 NMR spectroscopy

The sample (about 20 mg) was dissolved in DMSO-d₆ (total volume about 1 cm^3). The spectrum was recorded at room temperature on a JEOL ECA-400 spectrometer, operating with a frequency of 400 MHz.

3.10.4 UV-vis spectroscopy

The UV-vis spectra were recorded for samples dissolved in DMSO (about 0.02 M) in 1-cm quartz cuvettes on a Varian 50 UV-visible spectrophotometer in the wavelength range 200-800 nm

3.10.5 Thermogravimetry

About 5mg of the sample was placed in a small pan and placed in the heating chamber of Perkin Elmer Precisely TGA 4000 thermogravimetric analyzer. The instrument was adjusted at a heating rate of 20°C/min. The heating was performed from 50-900 °C.

3.10.6 X-ray Crystallography

The crystal structures were recorded using ω -scan technique on an APEX-2 area detector diffractometer.

3.10.7 Free radical scavenging activity using DPPH method

The DPPH free radical scavenging assay, and the total reducing power method. . All assays were carried out in triplicate and the average value was obtained. All determinations were made spectrophotometrically using the Infinite® 200 PRO plate reader (TECAN, Männedorf, Switzerland).