

CHAPTER 3

EXPERIMENTAL

3.1 Chemicals

All solvents and reagents were commercially available and used as obtained without further purification.

3.2 Instruments

3.2.1 CHN Analysis

Carbon, hydrogen and nitrogen analyses were carried out at the Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

3.2.2 Melting Point Determination

Mp-1D Fargo melting point apparatus was used to determine the melting points of the compounds.

3.2.3 FTIR Spectroscopy

The IR spectra were recorded using Shimadzu 1600 spectrophotometer in KBr disks in the range 400-4000 cm^{-1} .

3.2.4 ^1H and ^{13}C NMR spectroscopy

The ^1H and ^{13}C NMR spectra were recorded on Lambda JEOL 400 MHz FT-NMR spectrometer. Deuterated DMSO was used as solvent.

3.2.5 UV-Visible Spectroscopy

The UV- Visible spectra were recorded on Perkin Elmer RX1 spectrophotometer in the region 200-1100 nm. DMSO was used as solvent.

3.2.6 Thermogravimetric analysis (TGA)

Thermogravimetric analysis were carried out using Perkin Elmer TGA 6 Thermogravimetric Analyzer over the temperature range of 30°C - 950°C. The TGA curves were obtained at a heating rate of 10°C / min in nitrogen atmosphere.

3.2.7 Single Crystal X- Ray Diffraction

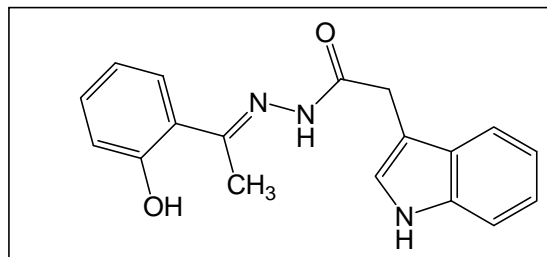
The crystal data was collected from a Bruker SMART APEX2 diffractometer Bruker (2007); cell refinement : SAINT Bruker (2007); data reduction : SAINT ; program (s) used to solve structure : SHELXS97 Sheldrick (2008); program(s) used to refine structure : SHELXL97 Sheldrick (2008); molecular graphics : XSEED (L.J. Barbour *et al.*, 2001) software used to prepare material for publication : publCIF (S.P Westrip, 2008). All the non-hydrogen atoms were refined anisotropically and all the hydrogen atoms were placed at calculated positions and refined isotropically.

3.3 Synthesis of Indole hydrazones

3.3.1 2- Hydroxyacetophenone Indolehydrazone (2- HapIH)

An ethanolic solution of 2-hydroxyacetophenone (0.22 g, 1.58 mmol) with indole-3-acetic acid hydrazide was refluxed for 2 hours. Yellow solid were formed, filtered and recrystallized from aceto-nitrile.

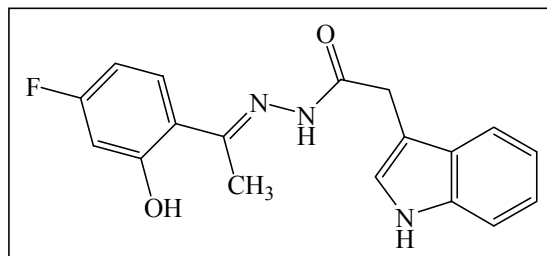
Structure 2- HapIH :



3.3.2 4-Fluoro-2-hydroxyacetophenone Indolehydrazone (4-F-2-HapIH)

An ethanolic solution of 4-fluoro-2-hydroxyacetophenone (0.25 g, 1.86 mmol) and indole-3-acetic acid hydrazide (0.3 g, 2.56 mmol) in 1: 1 mole ratio was refluxed for 2 hours. The white crystal formed was filtered and recrystallized from ethyl acetate.

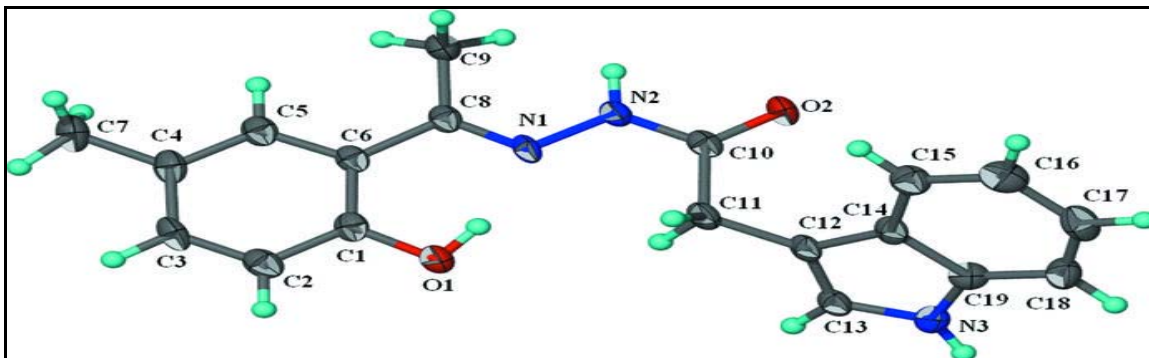
Structure 4-F- 2- HapIH :



3.3.3 5-Methyl-2- hydroxyacetophenone Indolehydrazone (5-CH₃-2-HapIH)

An ethanolic solution of 5-methyl-2- hydroxyacetophenone (0.24 g, 1.60 mmol) and indole-3-acid acetic (0.30 g, 2.38 mmol) in ethanolic solution was refluxed for 2 hours. Yellow solid was formed, filtered and recrystallized from aceto nitrile.

Structure 5-CH₃-2-HapIH :

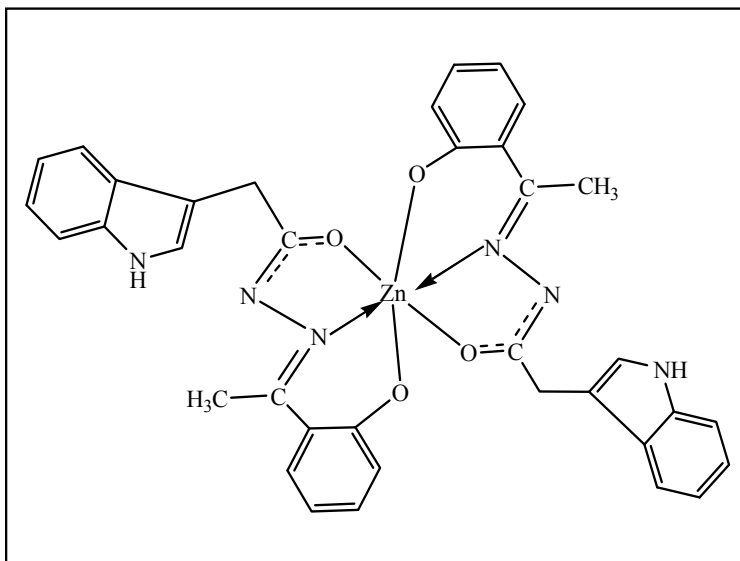


3.4 Synthesis of Metal Complexes of Indolehydrazones

3.4.1 Zn₂-HapIH

2- HapIH (3.00 g, 0.02 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Zinc (II) acetate (2.20 g, 0.01mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 50%).

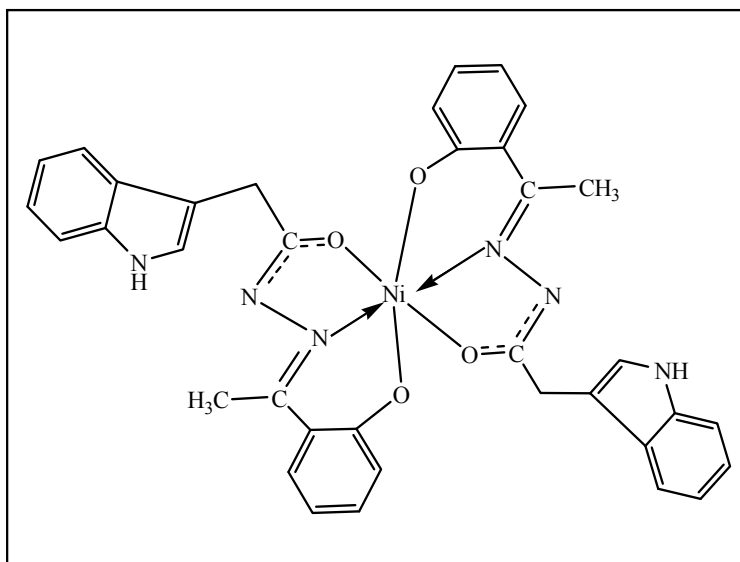
Structure Zn₂-HapIH :



3.4.2 Ni₂-HapIH

2- HapIH (3.00 g, 0.02 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Nickel (II) acetate (2.50 g, 0.01 mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 45%).

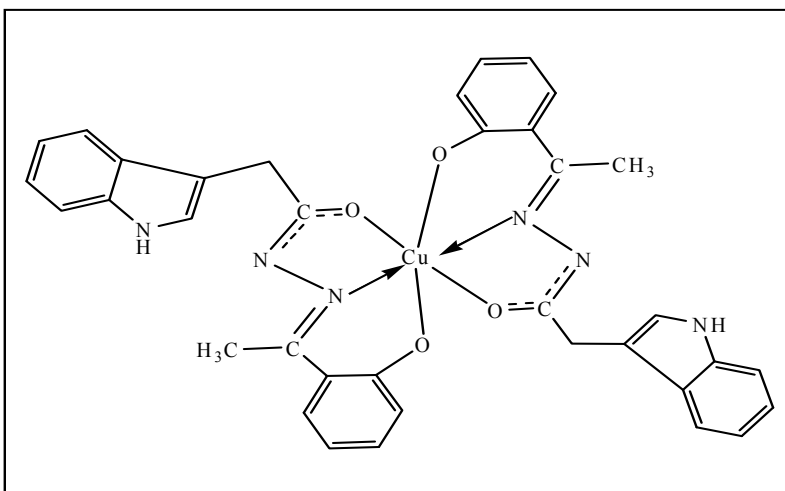
Structure Ni₂-HapIH :



3.4.3 Cu₂-HapIH

2- HapIH (3.00 g, 0.02 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Copper (II) acetate (1.99 g, 0.01 mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 40%).

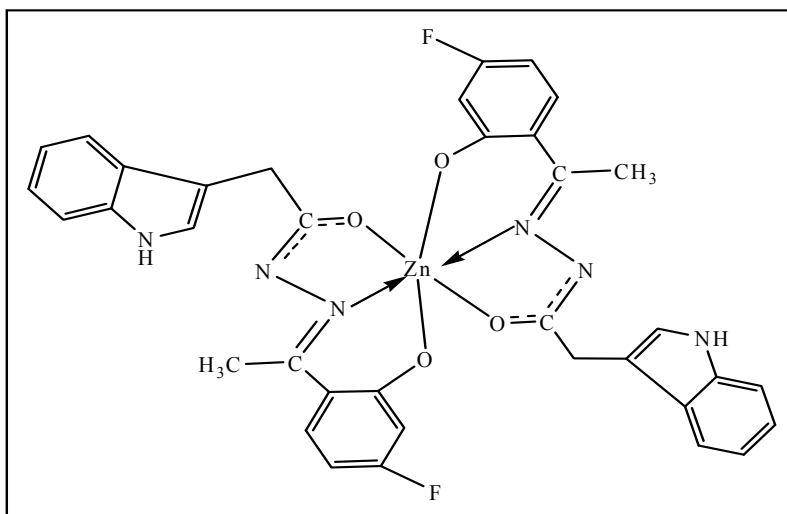
Structure $\text{Cu}_2\text{-HapIH}$:



3.4.4 $\text{Zn}_{4\text{-F-2-HapIH}}$

4-F-2-HapIH (3.00 g, 0.02 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Zinc (II) acetate (1.01 g, 0.01 mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 50%).

Structure $\text{Zn}_{4\text{-F-2-HapIH}}$:

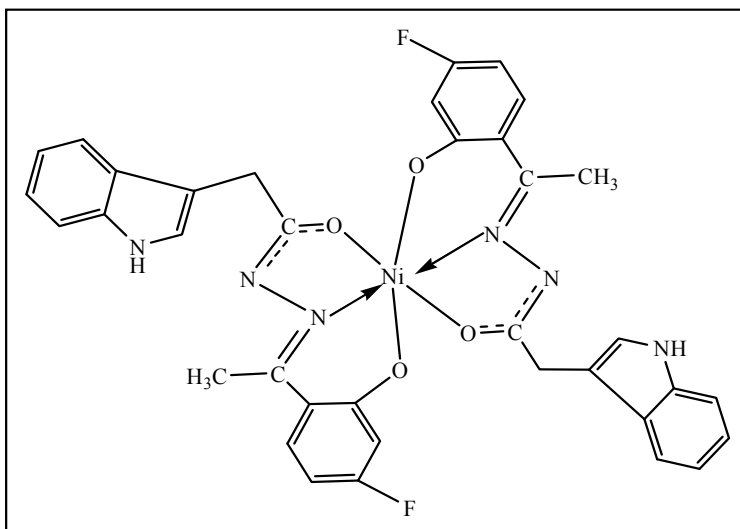


3.4.5 Ni_{4-F-2-HapIH}

4-F-2-HapIH (3.00 g, 0.02 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Nickel (II) acetate (2.49 g, 0.01 mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 35%).

Structure Ni_{4-F-2-HapIH} :

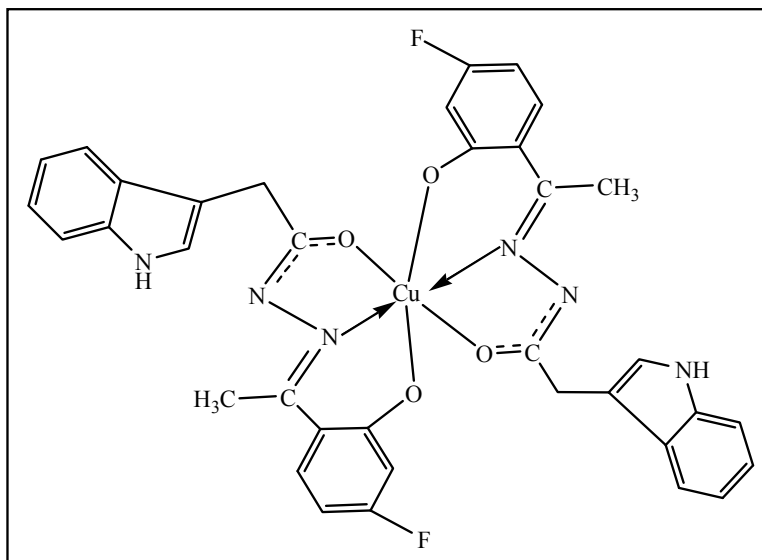
:



3.4.6 Cu_{4-F-2-HapIH}

4-F-2-HapIH (3.00 g, 0.01 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Copper (II) acetate (2.00 g, 0.005 mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 40%).

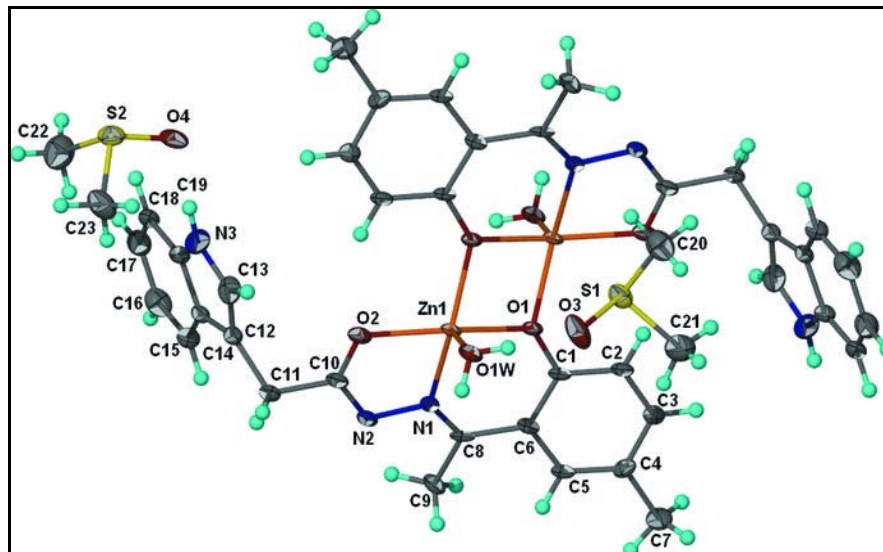
Structure $\text{Cu}_4\text{-F-2-HapIH}$:



3.4.7 $\text{Zn}_5\text{-CH}_3\text{-2-HapIH}$

5- $\text{CH}_3\text{-2-HapIH}$ (3.00 g, 0.01 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Zinc (II) acetate (1.10 g, 0.005 mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 50%).

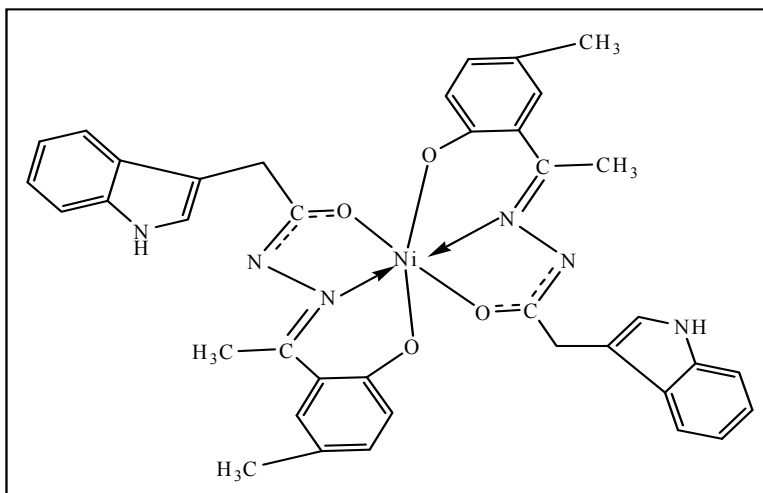
Structure Zn_5 -CH₃-2-HapIH :



3.4.8 Ni₅-CH₃-2-HapIH

5-CH₃-2-HapIH (3.00 g, 0.01 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Nickel (II) acetate (1.24 g, 0.005 mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 45%).

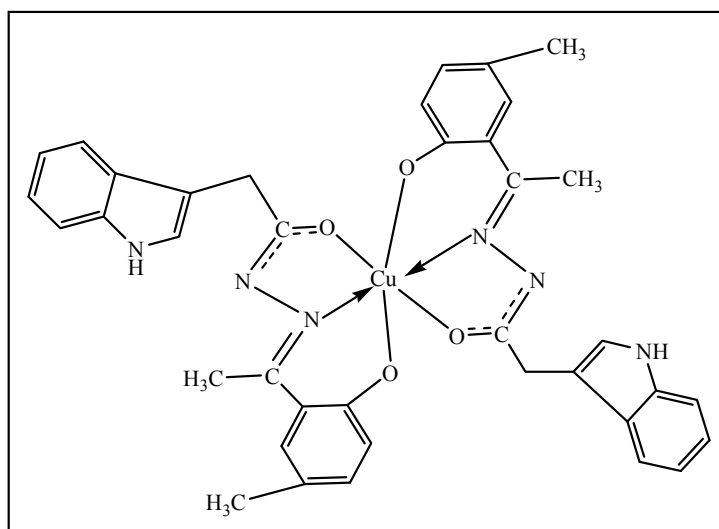
Structure Ni₅-CH₃-2-HapIH :



3.4.9 Cu₅-CH₃-2-HapIH

5-CH₃-2-HapIH (3.00 g, 0.01 mmol) was dissolved in ethanol (25 ml) and several drops of aqueous sodium hydroxide were added to raise the pH of the solution to about 8.5. Copper (II) acetate (1.00 g, 0.005 mmol) was then added and the mixture was refluxed for 5 hours. White solid was formed upon evaporation of the solution produced and was recrystallized from DMSO (Yield : 25%).

Structure Cu₅-CH₃-2-HapIH :



3.5 Cyclic Voltammetry

Cyclic voltammetry data was collected from Basic Autolab machine with potentiostat / galvanostat PGSTAT 30 using tetrabutylammonium tetraborofluoride $[\text{NBu}_4]\text{BF}_4$ as supporting electrolyte. Lead electrode with area of $19.625 \times 10^{-6} \text{ m}^2$ and titanium electrode with area of $3.14 \times 10^{-6} \text{ m}^2$ were the working electrode, a platinum wire was the counter electrode and the saturated calomel electrode (SCE) was used as the reference electrode. Both ligands and complexes were dissolved in ethanol. The sample was quantitatively dissolved in the supporting electrolyte 0.10 g dissolved in 25 ml DMSO. The nitrogen gas was bubbled for a few minutes prior to every measurement and all experiments were carried out at 25°C. The measurement was done from 25 mVs^{-1} to 400 mVs^{-1} .

3.6 Anti- Ulcerogenic Activity

This test was done at The Department of Pharmacology, Faculty of Medicine, University of Malaya. The rats were fasted 24 hours before the test but had free access to water. They were placed in individual cages to prevent coprophagy. The sample in a volume of 1ml was administered intra-gastrically using a metal orogastric tube. After 30 minutes, the rats were fed with 5 ml of absolute ethanol. One hour after ethanol administration, the rats were killed by diethyl ether in a jar. Then, the stomach was removed and opened along greater curvature. The area of gastric lesions were measured using microscope. The percentage of inhibition was calculated by the following formula (S.J. Konturek *et.*, *al* 1986) :

$$(\% \text{ Inhibition}) = [(UI_{\text{control}} - UI_{\text{treated}}) \div UI_{\text{control}}] \times 100$$

UI_{control} = Ulcer Index control

UI_{treated} = Ulcer Index treated