2.1 Chemicals

All chemicals and solvents were purchased from Sigma-Aldrich (M) Sdn. Bhd., Merck Sdn. Bhd. and Fisher (M) Sdn. Bhd. and were used without further purification. Biological apparatus were obtained from local suppliers. The standard drugs used as control, Tagamet^{TM} 200 mg (cimetidine) and glibenclamide were purchased from Caring Pharmacy and Sigma-Aldrich (M) Malaysia, Kuala Lumpur, Malaysia.

2.2 Instrumentation

2.2.1 Infra Red (IR)

The IR spectra were recorded by FTIR Perkin Elmer model using KBr. The samples were ground homogenously with KBr and were pressed to form a thin film.

2.2.2 Nuclear Magnetic Resonance (NMR)

All ligands were subjected to ^{1}H and ^{13}C NMR by using Lambda JOEL 400MHz and ECA JOEL 400MHz FT-NMR. The experiments were carried out in deuterated DMSO with TMS as a reference.

2.2.3 UV-Visible

The spectra were obtained from reflectance electronic technique by using UV-3600 Shimadzu UV-Vis-NIR Spectrophotometer and were scanned from 200 ï 1000 nm.

2.2.4 Elemental Analyses

Compositions of C, H and N were obtained by using Flash EA 1112 Series model.

2.2.5 Magnetic Susceptibility

Magnetic properties of the complexes were measured by using Sherwood Scientific magnetic susceptibility balance. The measurement were corrected by mercury(II) tetracyanatocobaltat, $Hg[Co(SCN)_4]$ ($\chi_g = 16.44 \times 10^{-6}$) as a reference.

2.2.6 Melting Point Determination

Melting points were determined by using Mel-Temp II and the heating rate used was 40°C per minute.

2.2.7 Measurement of pH

Measurement of pH was carried out by using a digital pH meter, WITEG (Model W-500) with a glass electrode.

2.2.8 X-ray Crystallographic Data Collection

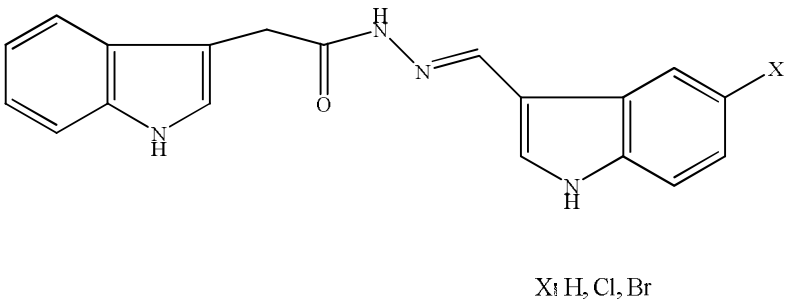
Single crystal X-ray diffraction data collection of selected complexes were performed on a Bruker Apex II CCD diffractometer at 100 K employing graphite-monochromated Mo KŬ radiation (ə = 0.71073A ę). The intensities were collected using the ɤ -2ɗ scan mode, in the range 2.4 < ɗ <27.0^{o} All structures were solved by direct method by using SHELXS-97 and refined by full matrix least-square methods on F^2 with the use of the SHELXL-97 program package (semi-empirical absorption corrections were applied using SADABS program).

2.3 Animals

Albino Sprague-Dawley rats and ICR mice (female and male) were purchased from the Animal House, Faculty of Medicine, University of Malaya. All experiments were performed at the Animal Experimental Block at the same faculty.

2.4 Syntheses

2.4.1 Synthesis of Ligands (L1, L2 and L3)



X: H, Cl, Br

Figure 5: Halogenated indole Schiff base compounds.

3.0g (15.9mmol) of ethanolic solution of indole-3-acetic hydrazide was added to a separate ethanolic solution of 2.3g (15.9mmol) of indole-3-carboxaldehyde, 2.8g (15.9mmol) of 5-chloroindole-3-carboxaldehyde and 3.6g (15.9mmol) of 5-bromoindole-3-carboxaldehyde to yield L1, L2 and L3. Each mixture was heated to reflux for 2 hours and then reduced to give light brown precipitate. Each precipitate was purified in hot ethanol and gave light brown powder of 2ô-[(1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazide (L1) and 2ô-[(5-bromo-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazide (L3) while 2ô-[(5-chloro-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazide (L2) was obtained as yellowish brown powder. Crystal growth was carried out in ethanol or ethyl acetate for X-ray crystallography determination.

2.4.2 Synthesis of Nickel(II) And Copper(II) Complexes

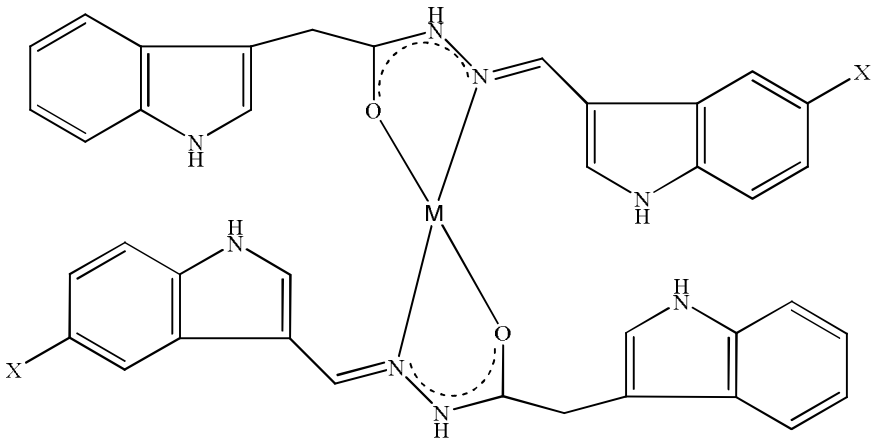


Figure 6: Copper(II) and nickel(II) complexes of halogenated indole Schiff base compounds.

3.0g (9.5mmol) of L1, 3.0g (8.6mmol) of L2 and 3.0g (7.6mmol) of L3 were dissolved in ethanol before the appropriate amount of copper(II) acetate monohydrate and nickel(II) acetate tetrahydrate were added into each of the ligands prepared in ratio ligand : metal ion 2 : 1. The dark and apple green mixtures were heated to reflux for 5 hours. Dark green precipitates were obtained as bis{2ô-[(1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido-ə^{2}N,O}cuprate (CuL1), bis{2ô-[(5-chloro-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido- ə^{2}N,O}cuprate (CuL2) and bis{2ô-[(5-bromo-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido-ə^{2}N,O}cuprate (CuL3). On the other hand, the red precipitates were of bis{2ô-[(1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido-ə^{2}N,O}nickelate (NiL1), bis{2ô-[(5-chloro-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido- ə^{2}N,O}nickelate (NiL2) and bis{2ô-[(5-bromo-1*H*-indol-3-yl)methylene]-2-(1*H*-indol-3-yl)acetohydrazido-ə^{2}N,O}nickelate (NiL3). These complexes were filtered off and were washed with ethanol and acetone to remove excess metal salt. Crystal growth was performed in DMSO or DMF to obtain single crystal for X-ray crystallography determination.

2.5 Biological Activity Studies

2.5.1 Neuroprotective Screening (*in vitro*)

2.5.1.1 Culture of NG108-15 cells

NG108-15 cells will be cultured in DMEM (Dulbeccoôs Minimum Essential Medium) supplemented with 10% FBS (Fetal Bovine Serum), 2% penicillin/streptomycin (100x),

and 1% amphotericin B (250µg/ml) solutions as a complete medium. The cells were cultured in 25cm^2 tissue culture flasks with 6 ml complete medium and incubated at 37 ºC with 5% CO_2 were sub-cultured every 3 ï 4 days (Weecharangsan *et al*., 2006).

2.5.1.2 Cell viability assay

Cell viability was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) assay. Metabolically active mitochondrial dehydrogenases convert the tetrazolium salt into insoluble purple formazan crystals at a rate that is proportional to cell viability. The cultured NG108-15 cells will be rinsed twice with Phosphate Buffered Saline (PBS), harvested with accutase and replated in 96-well plate at a concentration of $5x10^3$ cells per well. 5mg/ml of MTT solution will be added to each well of the 96-well plates after the treatment and H_2O_2 induction and then incubated for 4 h at 37 ºC with 5% CO_2. The medium was removed and 200µl of DMSO was then added to each well and vigorously mixed to dissolve the formazan crystals. The eluted samples were measured directly in a microplate reader at 570 nm (with a reference wavelength of 650 nm). The percentage of cell viability was calculated according to Equation 2 (Weecharangsan *et al*., 2006).

$$\% \text{ of cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100 \qquad \text{[Equation 2]}$$

2.5.1.3 Neuroprotective activity studies

NG108-15 cells were raised to confluence, rinsed twice with PBS, harvested by accutase and plated at a concentration of $5x10^3$ cells / well in a 96-well plate. Cells were left to

incubate at 37 °C with 5% CO_2 for 48 h. After 48-hours seeding, the cells were pre-incubated with various concentrations of the synthesized compounds at 37 °C with 5% CO_2 for 2 hours. 2mM of H_2O_2 was added into the well right after the pretreatment and the cells were incubated again at 37 °C with 5% CO_2 for 10 hours. After 10 hours of incubation, cell viability will be determined using the MTT assay. Tested compounds were dissolved in DMSO (final concentration in culture is approximately 0.5%) (Weecharangsan *et al*., 2006).

2.5.2 Acute Toxicity Test (*in vivo*)

ICR mice were grouped into separate gender and were deprived from food but were allowed access to water. Each gender was then separated according to groups of different doses where each group consists of 6 mice. The mice received high (5g/kg) and low (2g/kg) doses of compounds which were freshly prepared in suspension in 10% Tween 20 solution. Any mortality or abnormalities in behaviour were observed after 30, 60, 120, 180 minutes of sample administration and observation had been continued on the next day for 24 hours observation (Jamal *et al*., 2006).

2.5.3 Anti-Ulcerogenic Screening on Ethanol-Induced Gastric Ulcer (*in vivo*)

20 groups of albino Sprague-Dawley rats weighed 180 ï 230g were deprived of food for 48 hours but were allowed access to water. Each rat was caged individually to prevent caprophagy. 2 groups were for negative (10% Tween 20) and positive (70mg/kg cimetidine) controls while the other 18 groups were for the compounds that were tested on high (50mg/kg) and low (25mg/kg) doses. Samples were given orally in suspension in 10%

Tween 20 with an orogastric syringe. After 30 minutes, absolute ethanol (5ml/kg) was administered to induce ulceration and the rats were sacrificed under anesthesia with diethyl ether after 30 minutes. The esophagus and duodenum were tied up before removing the stomach. Mucus and gastric juice were carefully collected and centrifuged while the stomachs were cleaned under pipe water for gross gastric lesion measurement. Mucus that had been collected was weighed and pH of gastric juice was measured. All data were expressed in standard error mean (S.E.M.) using one-way analysis of varians (ANOVA) (Jamal *et al*., 2006).

2.5.4 Glucose Tolerance Test (*in vivo*)

ICR mice were deprived from food for 18 hours. Their body weight was determined and a drop of blood was withdrawn from the tip of their tails using a needle. The blood was placed carefully on the strips and fasting blood glucose level was measured by using an Active Accu-chek glucometer. The mice were separated into several groups which consist of 6 mice per group. Two groups would be the control groups; negative and positive control which were given 5% Tween 80 and 1mg/kg glibenclamide while each mouse of the other group were given 100, 30 and 10mg/kg of sample before the subcutaneous administration (modified from Orhan *et al*., 2006) of 1g/kg glucose 30 minutes later. The concentration of sample and glucose were given according to their body weight. Blood glucose level was measured after 90 minutes of glucose administration (Orhan *et al*., 2006).