*Malaysian Journal of Science* **25** (1): 99 – 105 (2006)

# Synthesis and Biological Activities of Nickel(II) and Cadmium(II) Complexes of Chlorohydroxyacetophenone-nitrobenzoylhydrazone: Mechanism for Formation of the Nickel(II) complex

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Received 1st June 2005, accepted in revised form 27th March 2006.

**ABSTRACT** The new Nickel(II) and Cadmium(II) complexes have been prepared in ethanol by template condensation of 4-nitrobenzhydrazide, 5-chloro-2-hydroxyacetophenone and metal acetate in the presence of triethylamine. The IR and UV spectra of the ligand and complexes indicate coordination of ligands to the metal centers. The initial product of the Nickel(II) complex is a square planar compound and upon recryctallization with pyridine, the complex has changed to octahedral geometry with coordination of the solvent molecules. The Schiff base ligand, $H_2$5-Clhap-4-$NO_2$bh was more sensitive towards the MCF-7 cells (human breast cancer cells) with $IC_{50}$ values of 4.5 μg $ml^{-1}$ than the unsubstituted ligand, $H_2$hapbh. However upon coordination to nickel, the activity has been reduced to the same level as the positive control drug, tamoxifen. The antioxidant properties of the Schiff base ligand (using the FTC method) exhibited higher activity than vitamin E or quercetine. However the activity is lower than the unsubstituted Schiff base ligand or the commercial antioxidant agent, BHT (butylated hydroxytoluene).

**ABSTRAK** Kompleks baru Nickel(II) dan Kadmium(II) disediakan dalam etanol secara kondensasi templat 4-nitrobenzhidrazida, 5-kloro-2-hidroksiasetofenon dan logam asetat dengan mencampurkan sedikit trietilamina. Spektra IR dan UV bagi ligan dan kompleks menunjukkan pengkoordinatan ligan kepada logam. Hasil permulaan kompleks Nikel(II) adalah merupakan sebatian sesatah empat segi. Setelah penghabluran semula dengan piridina, kompleks ini bertukar kepada kompleks bergeometri oktahedron dengan pengkoordinatan molekul pelarut. Bes Schiff, $H_2$5-Clhap-4-$NO_2$bh lebih sensitif terhadap sel MCF-7 (sel kenser payu dara) dengan nilai $IC_{50}$ 4.5 μg $ml^{-1}$ daripada ligan, $H_2$hapbh. Walau bagaimana pun, pengkoordinatan ligan ini kepada Nikel(II) mengurangkan ketoksikan ini ketahap yang sama dengan drug, Tamoksifen. Ciri pengoksidaan ligan bes Schiff (mengggunakan kaedah FTC) menunjukkan aktiviti yang lebih tinggi daripada vitamin E atau Kuersetin. Walau bagaimana pun aktiviti ini lebih rendah daripada ligan bes Shiff tak tertukarganti atau dengan agen pengoksidaan komersial BHT (butilated hydroksitoluena).

(Schiff base, metal complexes, mechanism, antioxidant and cytotoxicity properties)

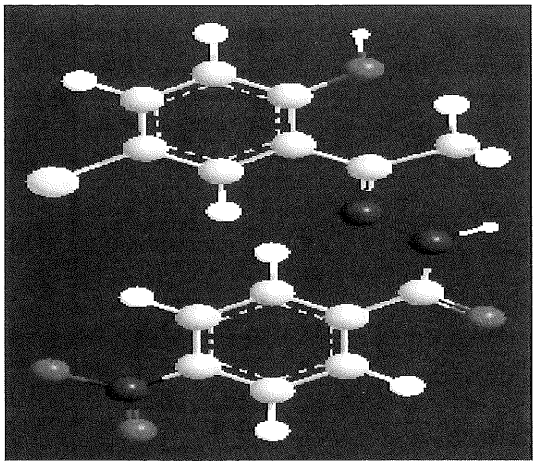
## INTRODUCTION

Schiff base ligands based on hydrazones and their complexes have received considerable attention because of their pharmacological properties such as antibacterial and anticancer agents [1]. In previous work, we had isolated some Zinc(II) complexes of substituted benzoylhydrazones, the x-ray structures, cytotoxicity and antioxidant properties of the complexes had been investigated [2 - 5].

This work has been extended to coordination of benzoylhydrazone ligands to other metals namely cadmium (same group as zinc) and nickel (a transition metal). Previous work on Nickel(II) hydrazones, [Ni(bhac)(Hdmpz) and [Ni(bhac)(Himdz)] was reported when nickel salt was

reacted with acetylacetone benzoylhydrazone and dimethylpyrazole (Hdmpz) or imidazole (Himdz) [6]. The x-ray structure of these Nickel(II) complexes indicated that nickel ions are in square planar with $N_2O_2$ geometry assembled via enolate-O, imine-N and amide-O. The Cadmium(II) complex of hydrazone is rare and only a few examples were reported.

With the aim of exploring this research field further, we have synthesized $H_2$5-Clhap-4-$NO_2$bh ligands and have determined their binding sites to the metal atoms. The effect of substituents on the cototoxicity and antioxidant activity of the ligands and the complexes were also investigated.



$H_2$5-Clhap-4-$NO_2$bh

## EXPERIMENTAL SECTION

All reagents were commercially available from Fluka or Aldrich and were used without further purification.

### Ligand

The Schiff base ligand was prepared by condensation of ethanolic solution of 4-nitro-benzhydrazide with equimolar amount of 5-chloro-2-hydroxyacetophenone.

The complexes were synthesized by stirring and refluxing the Schiff base ligand with metal acetate in ethanol in the presence of triethylamine for 5 hours. The resultant dark red solid was filtered and recrystallized from pyridine.

### Bioactivity

Cytotoxicity activity was evaluated at the National University of Malaysia, Bangi, Selangor.

## CULTURE OF CELLS AND CYTOTOXIC ASSAY

### Cytotoxicity Assay

The technique used was described by Freshney [7] with some modification. MCF-7 (human breast cancer cells) were seeded into 96 well plates at an initial cell density of approximately 5 X $10^5$ cells $cm^{-3}$. After 24 hours incubation for cell attachment and growth, the medium was removed and replaced with fresh medium containing varying concentrations of organic compounds. The compounds added were first dissolved in DMSO at the required concentration. Subsequent 6 desirable concentrations was prepared using growth medium. Control wells received only DMSO. Each concentration of the compound under study was assayed in six replicates. The assay was terminated after 24 hours incubation period. Again, the medium was removed and the cell viability was determined after further 4 hours incubation with 5 mg $cm^{-3}$ MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium] bromide; also named thiazol blue. DMSO was then added per well and the dissolving formazan precipitate was read by using elisa plate reader, Dynatech MR5000 at 570 nm. For comparison studies, tamoxifen.was used as a positive control.

$IC_{50}$ value is taken from the plotted graph of percentage live cells compared to control (%) versus tested concentration of compounds (μg $cm^{-3}$). The $IC_{50}$ value is the concentration required for 50% growth inhibition. It represents the growth inhibitory (cytostatic) effects on cell viability of the compounds tested [8].

## ANTIOXIDANT ASSAY

### Ferric thiocyanate (FTC) method

This assay was carried out as described in the modified method of Kikuzaki and Nakatani [9]. A mixture of 2 mg of the test sample in 4 ml of 99.5% ethanol, 4.1 ml of 2.51% linoleic acid in 99.5% ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water contained in screw-cap vial (diameter 38 mm, height 75 mm) was placed in an oven at 40°C in the dark. To measure the extent of antioxidant activity, 0.1 ml of the reaction mixture was transferred to a test tube (diameter 13 mm, height 150 mm) and followed by addition of 9.7 ml of 75% (v/v) aqueous ethanol, 0.1 ml of 30% aqueous ammonium thiocyanate and 0.1 ml of

0.02 M ferrous chloride in 3.5% hydrochloric acid. Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance of red colour (conversion to $Fe^{3+}$) was measured at 500 nm. The measurement was taken every 24 hour interval one day until absorbance of the control reached its maximum value.

## RESULTS AND DISCUSSIONS

The initial product of Nickel(II) complex was not very stable in air. Upon recrystallization with pyridine, the Nickel(II) and Cadmium(II) complexes are stable in air, red-brown in colour and non-hygroscopic. They are insoluble or sparingly soluble in common organic solvents and quite soluble in DMSO and pyridine. All the complexes melt over 250°C.

The IR spectra of the complexes when compared to that of free ligand shows that the ν(N-H) disappears in the spectra of the complexes supporting deprotonation of the ligand upon complex formation (Table 1). The ν(C=O) bands of the ligand also disappears in the spectra of the complexes indicating destruction of the keto group presumably *via* enolisation of the ligand and facilitates the formation of metal complexes. The ligand also showed strong band at 1602 $cm^{-1}$ which is assigned to the ν(C=N) band.

In the metal complexes, this stretching band has shifted to lower frequencies, due to the lowering of the ν(C=N) bond order as a result of metal-nitrogen bond formation. The shift of the ν(N-N) to higher wave numbers further supports coordination of the ligand *via* the azomethine nitrogen atom. The ν(C-OH) band of the phenol in the Cadmium(II) complex has disappeared indicating coordination of phenolic OH to the metal center. The new bands at *ca.* 590 and 430 $cm^{-1}$ are tentatively assigned to ν(M-O) and ν(M-N ) bands respectively.

### UV-vis of ligands and complexes

UV-vis spectra of all studied compounds were taken and assigned in DMSO. To help with the assignments, UV spectra of all the studied compounds were calculated by employing PM3 level configuration interaction calculation (single excitation). Assignments are given in Table 2.

The ligand absorption bands have been shifted to the red region upon complexation. In the spectrum of $H_2$5-Clhap-4-$NO_2$bh ligand, absorption band at 340nm is assigned to n – π* transition based on the lone pairs of the carbonyl oxygen and the bridging nitrogen atoms with a small character of a π – π* transition. This absorption band is red shifted (452 nm) upon complexation. The second absorption for the Cd (II) complex is a π – π* transition involving only the –$NO_2$ substituted phenyl ring.

The Ni (II) complex exhibits two π – π* transition. The first absorption (439nm) involves only the π orbitals of the phenyl ring with $NO_2$ substituent. The second absorption is a more generalized π – π* transition involving the pyridine ligands.

The differences are observed in the visible spectra of the complexes compared to that of the corresponding ligand. The π→ π* transition due to the C=N chromophores has undergone bathchromic shift upon coordination ca. 400 nm. The π→ π* transition of the aromatic ring undergoes a shift to higher energy and lower wavenumbers.

### IR Spectra

**Table 1.** Selected IR bands for ligands and complexes in $cm^{-1}$.

| COMPOUNDS | OH OF H2O | OH OF PHOH | N-H | C=O | C=N | C-OH | N-N | M-O | M-N |
|---|---|---|---|---|---|---|---|---|---|
| $H_2$5-Clhap-4-$NO_2$bh | 3404 | - | 3233 | 1655 | 1602 | 1290 | 1051 | - | - |
| [Ni(5-Clhap-4-$NO_2$bh)(py)$_3$] | 3431 | - | - | - | 1590 | - | 1082 | 590 | 437 |
| [Cd(5-Clhap-4-$NO_2$bh)(py)$_2$]$_2$ | 3432 | - | - | - | 1527 | - | 1071 | 588 | 525 |

**Table 2.** Assignments of UVvis spectra

| COMPOUNDS | ABSORPTION ($\Lambda_{MAX}$) | ASSIGNMENT |
|---|---|---|
| $H_2$5-Clhap-4-$NO_2$bh | 340 | $n - \pi^*$ and $\pi - \pi^*$ |
| [Ni(5-Clhap-4-$NO_2$bh)(py)$_3$] | 439 | $\pi - \pi^*$ |
| | 304 | $\pi - \pi^*$ |
| [Cd(5-Clhap-4-$NO_2$bh)(py)$_2$] $_2$ | 452 | $\pi - \pi^*$ with characters of $n - \pi^*$ |
| | 311 | $\pi - \pi^*$ |

**Mechanism for formation of the Ni (II) complex**

The mechanism for formation of the Nickel(II) complex has been confirmed by x-ray structures of the intermediate and the final product.

The initial product indicates formation of square planar Nickel(II) complex with coordination of a $H_2$5-Clhap-4-$NO_2$bh ligand and one molecule of triethylamine. This is a rare observation as triethylamine which is normally used to basify the reaction medium, now acts as a ligand to the metal complex in the present study [10]. This could be due to the electron withdrawing groups on both aromatic rings has reduced electron richness in the complex and effect the stability of the molecule. Coordination of triethylamine has reduced this effect and hence stabilized the complex.

Upon recrystallization from pyridine (the more basic molecule), the triethylamine was feasibly replaced by three molecules of pyridine to give the final product, an octahedral geometry of Nickel(II) complex (Figure 1) [11].

**Structure of [Cd(5-Clhap-4-$NO_2$bh)(py)$_2$] $_2$**

The structure of Cadmium (II) complex is confirmed by x-ray study (Figure 2) [11].

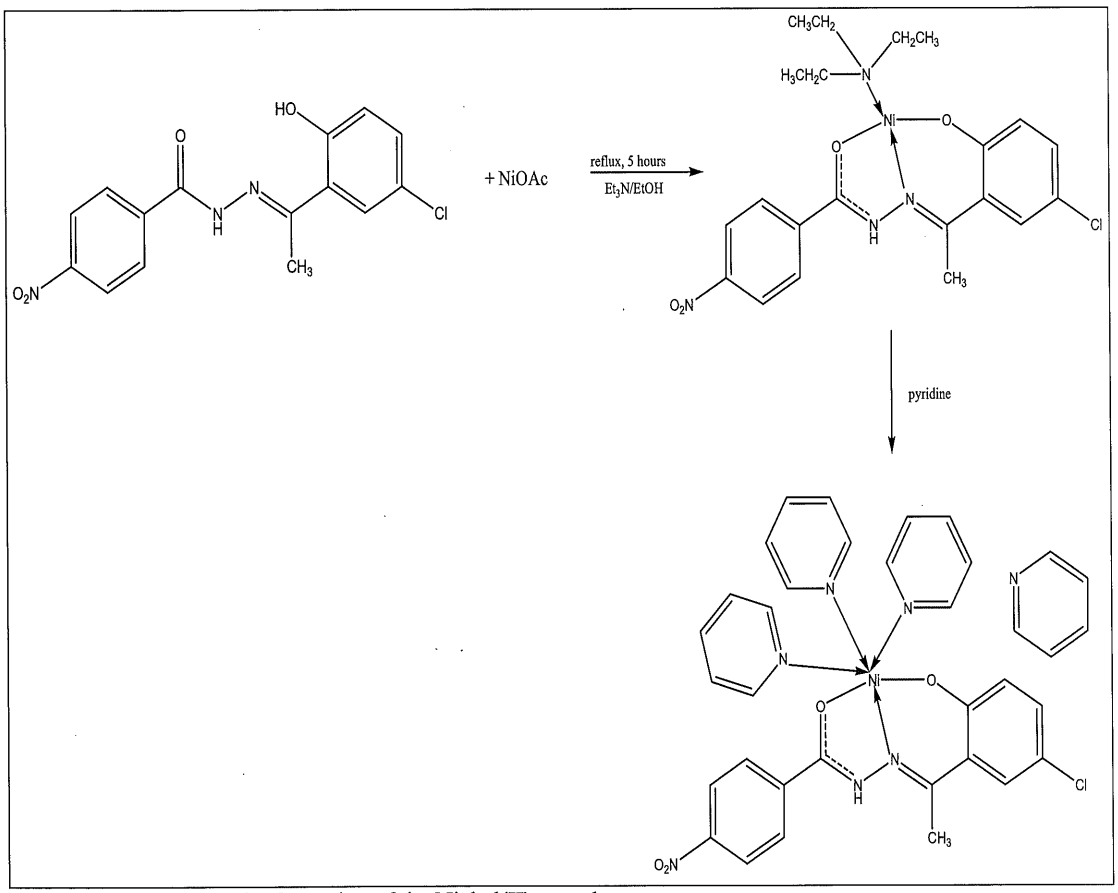



**Figure 1.** Mechanism for formation of the Nickel(II) complex

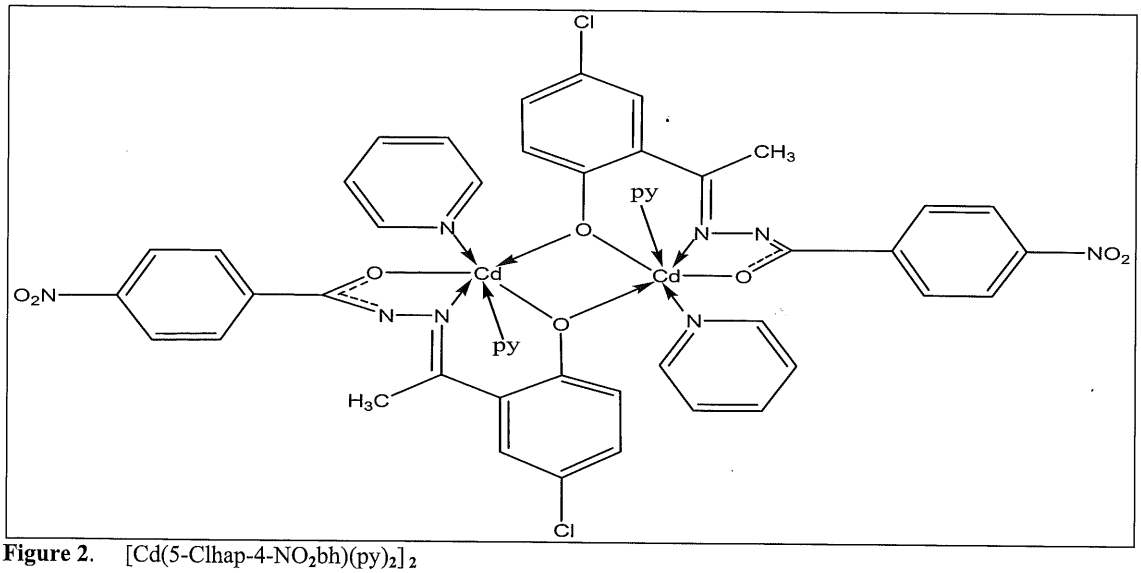



**Figure 2.** $[Cd(5\text{-}Clhap\text{-}4\text{-}NO_2bh)(py)_2]_2$

## BIOACTIVITY

### Cytotoxicity

The results of cytotoxicity testing are presented in Table 3. According to the United States National Cancer Institute, if the initial cytotoxicity test on cancer cell lines gives $IC_{50}$ value less than 20 $\mu g ml^{-1}$, this compound can be categorized as having potential anticancer activity.

**Table 3.** Cytotoxic activities of ligands and complexes.

| COMPOUNDS | $IC_{50}$ $\mu g$ $ml^{-1}$ |
|---|---|
| $H_2$hapbh | 10.0 |
| $H_2$5-Clhap-4-$NO_2$bh | 4.5 |
| [Ni(5-Clhap-4-$NO_2$bh)$(py)_3$] | 18.2 |
| $[Cd(5\text{-}Clhap\text{-}4\text{-}NO_2bh)(py)_2]_2$ | 20.0 |
| Tamoxifen (+ve control) | 18.2 |

The ligand, $H_2$5-Clhap-4-$NO_2$bh is more sensitive towards the MCF-7 cells (human breast cancer cells) with $IC_{50}$ values of 4.5 $\mu g$ $ml^{-1}$ than the unsubstituted ligand, $H_2$hapbh [12]. The nitro and chloro-substituents might have contributed to the marked increase in the cytotoxicity of the ligand, where these substituents could have interacted with the biological molecules *via* intermolecular hydrogen bonding.

However, upon complexation to either Nickel(II) or Cadmium(II), the activity has been reduced to the same level as the positive control drug, tamoxifen. The size of the metal molecules and lack of interaction between the complexes and the biological molecules could be the main factors for the higher $IC_{50}$ values.

### Antioxidant Property

The antioxidant property of the Schiff bases and the metal complexes are shown in the Table 4. The substituted ligand is less reactive as antioxidant agent compared to the unsubstituted ligand, $H_2$hapbh. This could be due to the effect of the electron withdrawing substituents, Cl and $NO_2$. However the antioxidant activity of $H_2$5-Clhap-4-$NO_2$bh ligand is higher than quercetine or vitamin E. Coordination of $H_2$5-Clhap-4-$NO_2$bh ligand to Nickel(II) or Cadmium(II) has little influence on the antioxidant property of the complexes.

**Table 4.** Absorbance values of ligands and complexes.

| COMPOUNDS | ABSORBANCE AT 500 nm (ANTIOXIDANT ACTIVITY) |
|---|---|
| $H_2$hapbh | 0.0214 |
| $H_2$5-Clhap-4-$NO_2$bh | 0.0698 |
| [Ni(5-Clhap-4-$NO_2$bh)$(py)_3$] | 1.847 |
| $[Cd(5\text{-}Clhap\text{-}4\text{-}NO_2bh)(py)_2]_2$ | 0.4989 |
| Vitamin E | 0.6055 |
| Quercetine | 0.4546 |
| BHT | 0.0028 |

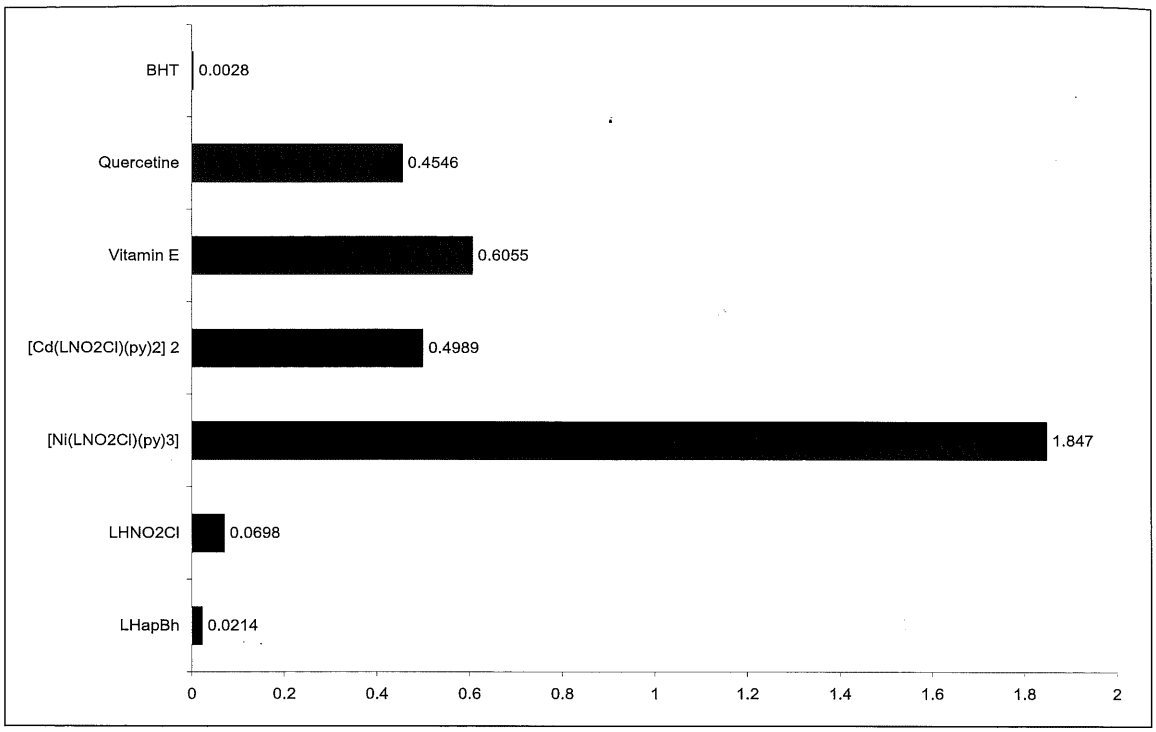



**Figure 3.** Anti-oxidant property of the ligand and the metal complexes.

## CONCLUSIONS

The initial product of Nickel(II) complex indicates formation of a square planar compound with coordination of a $H_2$5-Clhap-4-$NO_2$bh ligand and a molecule of triethylamine. Upon recrystallization from pyridine, the triethylamine was replaced to give the final product of Nickel (II) complex.

The $H_2$5-Clhap-4-$NO_2$bh ligand is more sensitive towards the MCF-7 cells (human breast cancer cells) with $IC_{50}$ values of 4.5 μg $ml^{-1}$ than the unsubstituted ligand, $H_2$hapbh. The nitro- and chloro-substituents might have contributed to the marked increase in the cytotoxicity of the ligand. However upon coordination to nickel or cadmium, the activity has been reduced to the same level as the control drug, tamoxifen.

The electron withdrawing substituents, (Cl and $NO_2$) could have caused the $H_2$5-Clhap-4-$NO_2$bh molecule to be less active as antioxidant agent as compared to the unsubstituted $H_2$hapbh ligand. The former is still stronger as antioxidant agent than quercetine or vitamin E. Nickel and Cadmium metals have little influence on the antioxidant property of the complexes.

**Acknowledgements** The authors would like to thank University of Malaya and the Academy of Sciences Malaysia for the research funds Vote-F and SAGA Grant (66-02-03-0046/Oracle 8150046).

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