

SYNTHESIS AND CHARACTERIZATION OF BENZOHYDROXAMIC ACID METAL COMPLEXES AND THEIR CYTOTOXICITY STUDY

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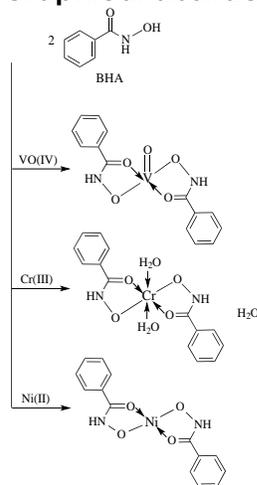
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Graphical abstract



Abstract

Hydroxamic acids (RCONHOH) are versatile compounds that constitute a significant biological importance. The syntheses, physico-chemical and characterization of benzohydroxamic acid (BHA) and its metal complexes (VO(IV), Cr(III) and Ni(II)) are reported herein. The metal complexes were synthesized via condensation reaction of BHA and metal salts in 2:1 molar ratio using ethanol as reaction medium. The compounds were characterized by elemental analysis, spectral (infrared, UV-Vis, ¹H and ¹³C NMR), TGA, magnetic susceptibility as well as molar conductance. The spectral study analysis reveals that all complexes coordinated to the metal via oxygen atoms (O,O) in bidentate manner to form octahedral for [Cr(BHA)₂·2H₂O]·H₂O, tetrahedral for [Ni(BHA)₂] and square pyramidal geometry for [VO(BHA)₂]. The molar conductance values suggested that all complexes were non-electrolytes. A cytotoxicity study against HCT116 displayed that VO(IV) has better IC₅₀ values than the parent ligand, BHA but not considered as potent anticancer agents.

Keywords: Benzohydroxamic acid, vanadium(IV), chromium(III), nickel(II), cytotoxicity

Abstrak

Asid hidrosamik (RCONHOH) adalah sebatian serba-boleh yang mempunyai kepentingan biologi. Sintesis, ciri-ciri fizikokimia dan karakter asid benzohidrosamik (BHA) dan logam kompleksnya ((VO(IV), Cr(III) dan Ni(II)) telah dilaporkan di sini. Logam kompleks telah disintesis melalui kondensasi antara BHA dengan garam logam dalam nisbah molar 2:1. Semua sebatian yang dihasilkan telah dicirikan menggunakan analisis asas, spektroskopi (inframerah, UV-Vis, ¹H dan ¹³C NMR), TGA, kerentanan magnet dan kekonduksian molar. Setiap kompleks logam berkoordinasi dengan logam melalui atom oksigen (O,O) dengan cara bidentat untuk menghasilkan geometri beroktahedron untuk [Cr(BHA)₂·2H₂O]·H₂O, tetrahedral untuk [Ni(BHA)₂] dan geometri piramid segiempat sama untuk [VO(BHA)₂]. Semua kompleks logam adalah bukan elektrolit berdasarkan nilai kekonduksian molar. Kajian sitotoksik terhadap HCT116 menunjukkan bahawa vanadium kompleks mempunyai nilai IC₅₀ yang lebih baik berbanding dengan ligan induk, BHA tetapi kompleks vanadium tidak boleh dikatakan sebagai agen antikanser yang berpotensi.

Kata kunci: Asid benzohidrosamik, vanadium(IV), kromium(III), nikel(II), sitotoksik

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1.0 INTRODUCTION

Hydroxamic acid known as flexible reagents for organic and inorganic analyses [1]. Their easiness to deprotonate and forming the hydroxamate ions showing that the structures flexibility due to frailer structure compared to structurally related carboxylic acid. Recently, hydroxamic acids have received attentions due to their importance in fulfilling a variety of roles in biology and medicine [2] such as drugs, inhibitors for sperm mobility and anti-amoebic agents [3]. They also have hypotensive, anti-cancer, anti-malarial, anti-tuberculosis and anti-fungal properties [2]. Their ability to form stable metal chelates and have possible NO-releasing properties made up their versatility in biological activity [2, 4].

Hydroxamate molecules, one of the major classes of naturally occurring metal complexing agents [5] show a diversity of coordination behavior that results in a rich array of metal complex structures [2]. The powerful metal-chelating ability of hydroxamic acids has also been utilized to construct a diverse host of fascinating metal complexes including hydroxamates, hydroximates and examples in supramolecular chemistry such as metallocrowns, coordination polymers and tetrahedral cluster complexes [4].

There is less development of new drugs that can treat the cancer cells without harming the hosts for past 30 years. The cancer cells evolved and become a drug-resistant makes the present drugs are less efficient. Thus, the development of potential anticancer drugs that orally active and stable are needed.

As part of our on-going interest in the synthesis of complexes, we prepared benzohydroxamic acid (BHA) and its complexes which are Vanadium(IV) benzohydroxamic acid $[\text{VO}(\text{BHA})_2]$, Chromium(III) benzohydroxamic acid $[\text{Cr}(\text{BHA})_2 \cdot 2\text{H}_2\text{O}] \cdot \text{H}_2\text{O}$ and Nickel(II) benzohydroxamic acid $[\text{Ni}(\text{BHA})_2]$. The toxicity abilities of the synthesized compounds towards the cancer cells was investigated.

2.0 METHODOLOGY

2.1 Materials and Methods

All the chemicals and reagents were purchased from Sigma Aldrich and Merck and used without further purification. BHA was commercially available. The percentage composition of the elements (CHN) for the synthesized compounds were determined by using Thermo Scientific Flash 2000 Elemental Analyzer with methionine as a standard. Melting points were determined in evacuated capillaries using Stuart SMP10 and were uncorrected. The infrared spectra (IR) were recorded using a Perkin-Elmer Model 1750X FTIR spectrophotometer in the range of 4,000-400 cm^{-1} using KBr disc. The UV-Vis spectra for the synthesized complexes were obtained in absolute

ethanol in the 200-900 nm range using Perkin Elmer UV-Vis Lambda 35 spectrophotometer at room temperature. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Varian-600MHz using TMS as an internal standard in DMSO. The thermal decomposition behavior of the metal complexes was recorded using NETZSCH TG 209 F3 under nitrogen atmosphere at heating rate of 10 $^\circ\text{C min}^{-1}$ from room temperature to 900 $^\circ\text{C}$. Magnetic moments for the synthesized complexes were characterized using the Guoy method with water as calibrant on Sherwood Auto Magnetic Susceptibility Balance. Molar conductivity measurements of the complexes were determined in dimethylsulphoxide (DMSO) and absolute ethanol ($\sim 10^{-3}$ M) at room temperature by using a Metler Toledo Inlab 730 conductivity meter.

2.2 Synthesis of Metal Complexes

2.2.1 Synthesis of $[\text{VO}(\text{BHA})_2]$

BHA (10 mmol, 1.5314 g) was dissolved in deionized water and nitrogen gas (N_2) was bubbled through the solution for 10 minutes. Aqueous solution of vanadium(IV) oxide sulfate hydrate, $\text{VOSO}_4 \cdot x\text{H}_2\text{O}$ (5 mmol, 0.815 g) was added dropwise. The mixture was stirred for 30 minutes under nitrogen at room temperature. A black-purple precipitate was appeared. The resulting precipitate was collected by filtration and carefully washed with deionized water. The solid obtained was dried in vacuum at room temperature for 24 h or longer and then stored in a desiccator until used. Yield 22.51%.

2.2.2 Synthesis of $[\text{Cr}(\text{BHA})_2 \cdot 2\text{H}_2\text{O}] \cdot \text{H}_2\text{O}$

Chromium(III) chloride hexahydrate, $\text{CrCl}_2 \cdot 6\text{H}_2\text{O}$ (1.63 mmol, 0.4343 g) was dissolved in a hot aqueous solution (10 mL) of BHA (3.26 mmol, 0.4471 g). The pH of the resulting solution was raised to 5.5 using 0.1 M NaOH solution where upon a greyish precipitate was appeared. The solid obtained was filtered, washed with distilled water and dried over P_2O_5 in vacuum after being left standing at room temperature for several hours. The yield was 27.54%.

2.2.3 Synthesis of $[\text{Ni}(\text{BHA})_2]$

Nickel(II) acetate tetrahydrate, $\text{Ni}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ (5 mmol, 1.2443 g) was dissolved in a hot aqueous solution (10 mL) of solid BHA (5 mmol, 0.8941 g). The pH of the resulting solution was raised to 5.5 using 0.1 M NaOH solution where upon a light green precipitate was appeared. The solid obtained was filtered, washed with distilled water and dried over P_2O_5 in vacuum after being left standing at room temperature for several hours. The yield was 48.26%.

2.3 Anticancer Screening

2.3.1 Cell Culture

The human colorectal carcinoma cell line, HCT116 (ATCC® CCL-247™), was cultured in the Roswell Park Memorial Institute RPMI 1640 Medium w/ 25 mM HEPES & L-Glutamine, Biowest, supplemented with 10% heat inactivated fetal bovine serum (FBS) (PAA Laboratories) and 1% penicillin/streptomycin, Sigma Aldrich, (St Louis, US). Cultures were maintained in a humidified incubator at 37 °C in an atmosphere of 5% CO₂.

2.3.2 MTT Assay

HCT116 cells were plated at 7,000 cells per well and allowed to incubate at 37 °C for 24 h. BHA and their metal complexes were subjected to serial dilutions before being added to each well. The cells were treated with the compounds at concentrations ranging between 0.01–100 μM and incubated at 37 °C for 72 h. The cytotoxicity of the compounds was assessed using the MTT method utilizing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT or formazan) with minor modifications [14]. Briefly, 50 μL of 0.06 mol/L MTT solution was added to each well and plates were incubated at 37 °C for 4 h. All solutions were aspirated and the formazan crystals were dissolved in DMSO. The plate was read at 450 nm. Data generated were used to plot a dose-response curve when the concentration of compounds required to kill 50% of cell population (IC₅₀) was determined.

3.0 RESULTS AND DISCUSSION

3.1 Physical Data

The results of the elemental analysis and physical characteristics of the synthesized compounds are listed in Table 1. The complexes are air-stable and soluble in most of organic solvents. The reaction between metal ions with BHA at 1:2 molar ratio produced the bidentate complexes. The analytical data are in a good agreement with the proposed stoichiometry of the complexes. For Cr(III) and Ni(II) complexes, the melting point is not an absolute number like the ligands because the solid was decomposed during the heating process. The purity of the complexes was confirmed based on the CHN analysis and IR spectrum.

3.2 Infrared Spectroscopy (IR)

The infrared spectra of BHA and their metal complexes are quite different. The differences of the BHA and their metal complexes spectra are being illustrated Figure 1. Hence, by comparing the ligand and the metal complexes spectrum obtained, the donor sites of BHA towards the metal ions can be identified from Figure 3.

From the infrared data shown on Table 2, the principal infrared absorption bands of ligand, BHA are due to $\nu(\text{C}=\text{O})$, $\nu(\text{O}-\text{H})$, $\nu(\text{N}-\text{O})$ and $\nu(\text{M}-\text{O})$ stretching vibrations of the hydroxamate group. BHA shows a strong band at 1647 cm⁻¹ due to $\nu(\text{C}=\text{O})$ and 3297 cm⁻¹ due to $\nu(\text{O}-\text{H})$. In general, the $\nu(\text{C}-\text{N})$ and $\nu(\text{N}-\text{O})$ bands occur as a sharp peak in the ranges 1436, 1022 cm⁻¹ respectively [8].

The shifting of $\nu(\text{C}=\text{O})$ to a lower wavenumber and appeared at 1602-1605 cm⁻¹ indicating the involvement of C=O during complexation [15]. The involvement of C=O during complexation supported by the appearance of new band M-O at 479–489 cm⁻¹ [7]. In addition, the vanadyl complex displays a band at 926 cm⁻¹ assignable to V=O mode [12]. Band at 3297 cm⁻¹ assigned to $\nu(\text{O}-\text{H})$ in BHA disappeared on the metal complexes bands suggesting that -OH are involved during the complexation [20].

The medium band at 1024, 1025 and 1018 cm⁻¹ in VO(BHA)₂·[Cr(BHA)₂·2H₂O]H₂O and [Ni(BHA)₂], ascribed to $\nu(\text{N}-\text{O})$ did not undergo any change from the band of BHA, suggesting that -NO is retained and not coordinated [17]. The difference $\Delta\nu$ is less than 200 cm⁻¹ indicating that the ligand acts as bidentate in all compounds [9]. The shifting of $\nu(\text{C}=\text{O})$ to lower wavenumber and disappearance of $\nu(\text{O}-\text{H})$ in the metal complexes suggesting that the bonding of BHA to the metal salts via oxygen atoms of carbonyl and hydroxyl group [10, 11].

Table 1 Physical data for BHA and their metal complexes

COMPOUNDS	Molecular Formula	Molecular Weight (g/mol)	Colour	Melting Point (°C)	Found (Calculated)%			Molar conductivity ($\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$)
					C	H	N	
VO(BHA) ₂	C ₁₄ H ₁₂ N ₂ O ₅ V	339.20	Black-purplish	140-142	49.57 (49.77)	3.57 (3.65)	8.26 (9.47)	1.00
[Cr(BHA) ₂ ·2H ₂ O]H ₂ O	C ₁₄ H ₁₈ N ₂ O ₇ Cr	378.30	Muddy green	>280 to decomposed at 280	44.45 (44.27)	4.80 (4.10)	7.41 (7.96)	3.37
Ni(BHA) ₂	C ₁₄ H ₁₂ N ₂ O ₁ Ni	330.95	Light green	>240 to decomposed at 240	50.81 (49.56)	3.65 (3.75)	8.46 (8.35)	6.82

Table 2 Infrared spectral data for BHA and its complexes

Ligand/Complex	Frequency (cm ⁻¹)				
	$\nu(\text{C}=\text{O})$	$\nu(\text{O}-\text{H})$	$\nu(\text{V}=\text{O})$	$\nu(\text{N}-\text{O})$	$\nu(\text{M}-\text{O})$
BHA	1647 (s)	3297 (s)	-	1022 (m)	-
VO(BHA) ₂	1605 (s)	-	926 (s)	1024 (m)	484 (w)
[Cr(BHA) ₂ ·2H ₂ O]H ₂ O	1602 (s)	-	-	1025 (m)	489 (w)
[Ni(BHA) ₂]	1606 (s)	-	-	1018 (m)	479 (w)

s=strong; m=medium;w=weak

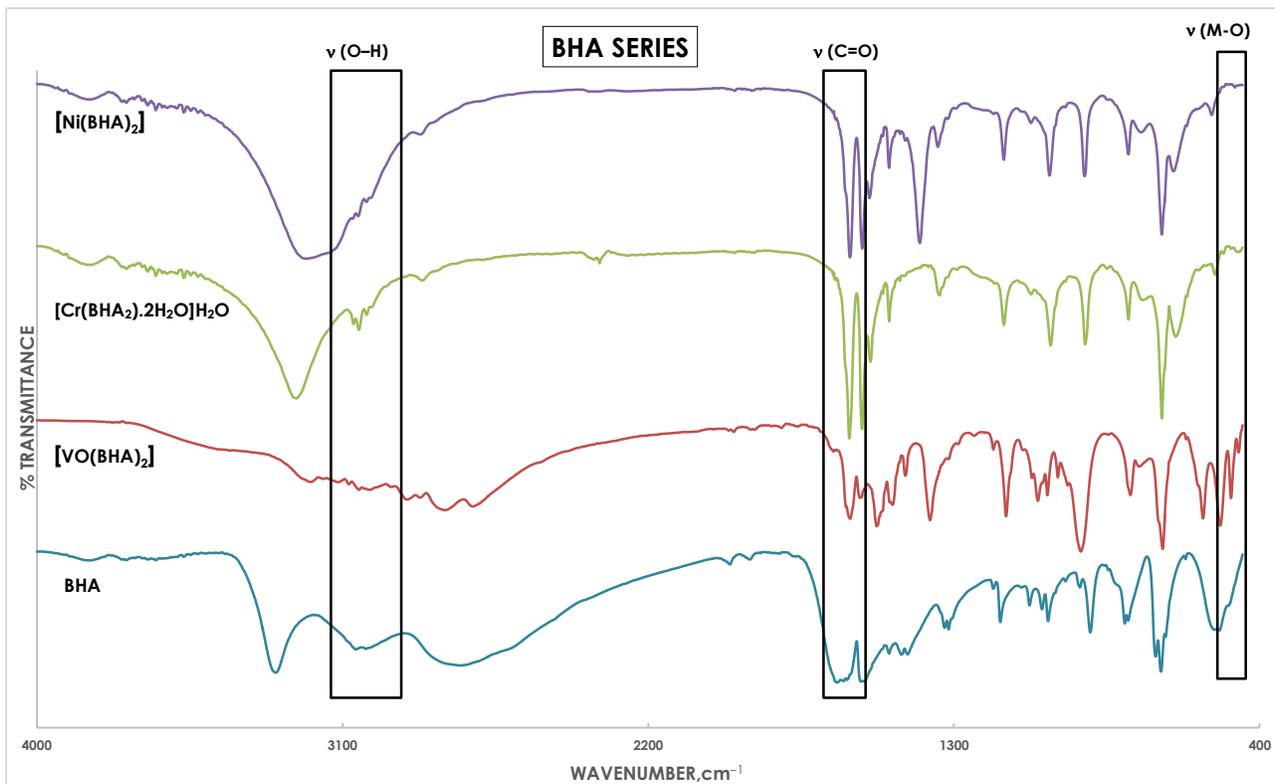


Figure 1 IR spectra of BHA and their metal complex

3.3 Nuclear Magnetic Spectroscopy (NMR)

The ^1H NMR spectra for the ligand was recorded in *d*-MeOH using tetramethylsilane (TMS) as the internal standard. Ligand (BHA) gives a single resonance near δ 9.35 ppm attributable to N-H proton. Multiple resonance was observed near δ 6.84–7.69 ppm indicating to aromatic protons.

The ^{13}C NMR spectra of the ligands showing there are two types of carbon which is carbonyl and aromatic carbon. The C=O resonance was observed at very downfield region at δ 167.36 ppm. For aromatic carbons, the peaks were observed at the range of δ 114.38 - 133.80 ppm [8].

Unfortunately, the paramagnetic behavior of the complexes make the complexes cannot be analysed using NMR. Thus, there are no proton and carbon NMR spectra for complexes.

3.4 UV-Vis Spectroscopy (UV-Vis)

The UV-Vis spectra of BHA and their metal complexes were measured in the range of 200-900 nm with concentration of 10^{-4} M in ethanol (Figure 2). The peaks observed at the range of 200 -207 nm on each spectrum were solvent peaks (ethanol). The electronic transition on π to π^* of aromatic ring of BHA was observed at 224 nm. Meanwhile, for $[\text{VO}(\text{BHA})_2]$ and $[\text{Cr}(\text{BHA})_2] \cdot 2\text{H}_2\text{O} \cdot \text{H}_2\text{O}$, these transitions were observed at 227 nm showing that these transitions were undergo a red shift. These shifts indicates that there are donations of lone pair of electrons by the oxygen of the phenoxy group to the metal atoms [18-19].

The electronic transition of n to π^* within C=O only observed for $[\text{Cr}(\text{BHA})_2] \cdot 2\text{H}_2\text{O} \cdot \text{H}_2\text{O}$ at 273 nm. This transitions involving the molecular orbitals of C=O chromophore and the benzene ring. These transitions were not observed on $[\text{VO}(\text{BHA})_2]$ and BHA spectrum. For $[\text{VO}(\text{BHA})_2]$, the shoulder about 463 nm can be assigned to the ligand-to-metal charge transfer (LMCT) transition [14, 16].

3.5 Thermogravimetric Analysis (TGA)

Table 3 presents the summary of the behavior of Cr(III) complexes upon thermal decomposition through thermogravimetric analysis. Cr(III) complexes clearly indicates the loss of one molar equivalent of water molecules at the temperature of 100-150 °C and another two at a higher temperature 250-300 °C. These results corroborate the suggestion that there are 2 coordinated water molecules in Cr(III) complexes and one other uncoordinated water molecules in the solid packing [13]. For Ni(II) complexes, no water loss indicates that there are no water molecules exists in the complexes.

Table 3 Thermal behavior indicating the loss of H_2O molecules from Cr(III) complexes

COMPOUND	TEMPERATURE RANGE (°C)	WEIGHT LOSS (%)	LOST SPECIES
		Found (Calculated)	
$[\text{Cr}(\text{BHA})_2] \cdot 2\text{H}_2\text{O} \cdot \text{H}_2\text{O}$	100-150	4.76 (5.00)	1 H_2O
	250-300	9.52 (10)	2 H_2O
$\text{Ni}(\text{BHA})_2$	100-200	0.00 (0.00)	-

3.6 Magnetic Moment

Since the VO(IV), Cr(III) and Ni(II) complexes are paramagnetic, their ^1H NMR spectra could not be obtained. The magnetic susceptibility value of VO(IV), Cr(III) and Ni(II) complexes are 1.57, 3.05 and 3.24 B.M., respectively. Octahedral geometry was proposed for $[\text{Cr}(\text{BHA})_2] \cdot 2\text{H}_2\text{O} \cdot \text{H}_2\text{O}$ and tetrahedral for $[\text{Ni}(\text{BHA})_2]$. Square pyramidal geometry was proposed for $[\text{VO}(\text{BHA})_2]$ referred to the value obtained from the magnetic susceptibility balance [12].

3.7 Molar Conductivity

From Table 1, the molar conductivity (Λ_m) values for the BHA complexes at the concentration of 10^{-3} M in dimethyl sulphoxide (DMSO) solvent, in the range of (1.00-6.82) $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ suggesting the non-electrolytic nature of the complexes in solution [12]. Conductivity measurement usually used on determining structural of metal chelates (mode of coordination) within the limits of their solubility. The high value of molar conductivity indicates the presence of anions that may be present inside the coordination sphere or absent or inside the coordination sphere. The molar conductivity values for the metal complexes indicating of the non-existence of free ions acting as electrolytes in the DMSO solution of the complex [11]. Figure 3 shows the proposed structure of the complexes based on the spectral evidence.

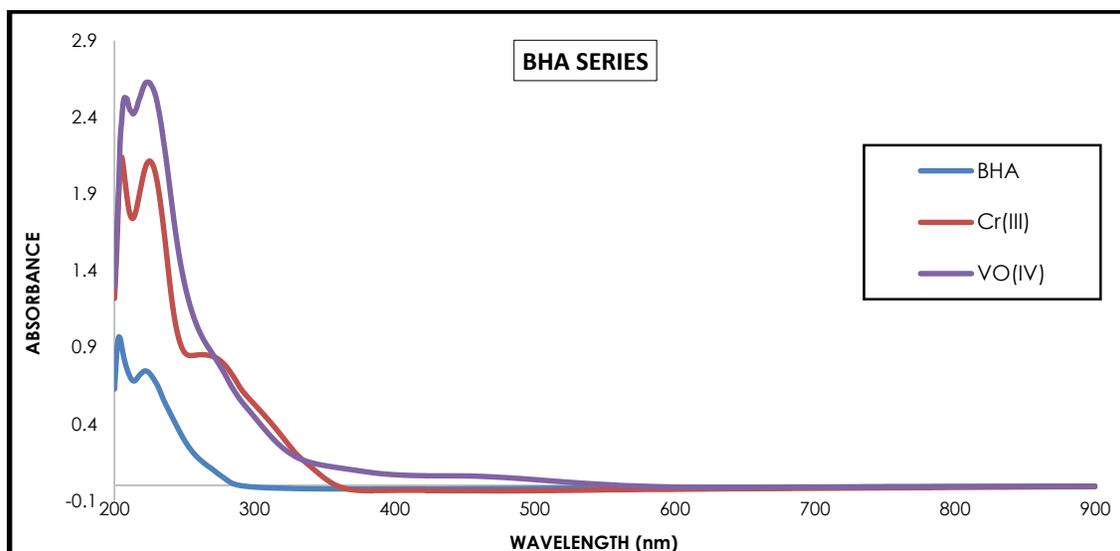


Figure 2 UV-Vis spectra of BHA and their metal complexes

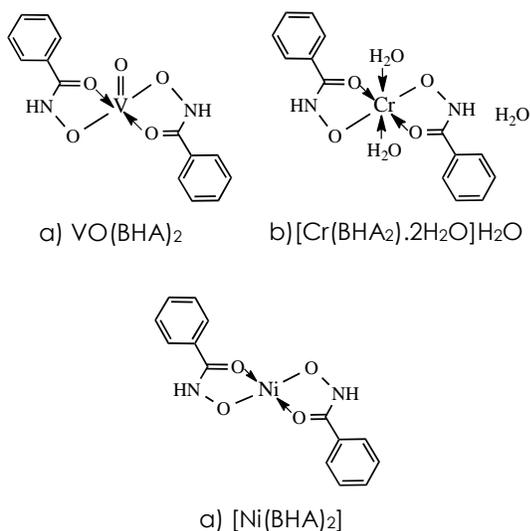


Figure 3 Proposed structures of BHA complexes

3.8 Cytotoxicity Assay

The synthesized BHA metal complexes were evaluated for the biological activity, specifically cytotoxicity against HCT116 colorectal carcinoma cell line. All the tested compounds induced a concentration-dependent anti-proliferative effect towards HCT116 cells upon treatment for 24 h [4]. All compounds are soluble in DMSO. $[\text{VO}(\text{BHA})_2]$ has the highest cytotoxicity effect with IC_{50} value of $42.00 \mu\text{M}$, followed by $[\text{Ni}(\text{BHA})_2]$ and $[\text{Cr}(\text{BHA})_2 \cdot 2\text{H}_2\text{O}]\text{H}_2\text{O}$ and the parent ligand, BHA as shown in Table 4. BHA and chromium complexes have no cytotoxic effect on HCT116 cells up to $100 \mu\text{M}$ treatment for 24 h.

From the results, IC_{50} value of complexes are better than the parent ligands. This observation can be explained by Tweedy's chelation theory. According to Tweedy, the polarity of the metal ion will be lowered on complexation because its positive charge will be shared with donor groups. Moreover, it will increase the delocalization of π -electrons over the entire chelate ring. Consequently, this enhances the lipophilicity of the complexes. Overtone's concept of cell permeability states that entry of any molecule into a cell is governed by its lipophilicity because the lipid membrane surrounding the cell favors the passage of materials that are soluble in lipids. Thus, the increased lipophilicity upon complexation enhances the penetration of the complexes into cells and blocks the metal binding sites of receptors. That is why complexes are showing a better cytotoxicity results than their parent ligands [21].

Therefore, [VO(BHA)₂] has better IC₅₀ value than [Ni(BHA)₂], [Cr(BHA)₂.2H₂O].H₂O and the parent ligand, BHA. The IC₅₀ value of [VO(BHA)₂] is still higher if compared with the standard drug used. Thus, [VO(BHA)₂] cannot be considered as a potent anticancer agent.

Table 4 IC₅₀ values of BHA and its complexes on HCT116

COMPOUNDS	IC ₅₀ VALUES (μ M)
BHA	>100
VO(BHA) ₂	42.00 \pm 2.44
[Cr(BHA) ₂ .3H ₂ O]	>100
[Ni(BHA) ₂]	60.00 \pm 4.03
5-FLUOROURACIL	13.07 \pm 0.00

4.0 CONCLUSION

BHA and its metal complexes ((VO(IV), Cr(III) and Ni(II)) were successfully synthesized and characterized by elemental analysis, infrared, UV-Vis, ¹H and ¹³C NMR, melting point, molar conductivity, TGA analysis and magnetic susceptibility measurements. The spectroscopy results of metal complexes suggesting that the ligands were coordinated to the metal center through oxygen atom (O,O) in bidentate manner. [VO(BHA)₂] gives the lowest IC₅₀ value against HCT116 cells than its ligand, BHA. From the metal complexes values, they support the fact that the presence of metal inside the compounds assisting on improving the effectiveness of compounds towards the targeted cells. But, they are still not considered as potent anticancer agent due to higher IC₅₀ values compared to the standard drug, 5-fluorouracil.

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