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RESEARCH ARTICLE

Cytotoxicity and α-glucosidase inhibition studies of Cu(II) and Ni(II) salicylhydroxamic acid complexes

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Abstract

Hydroxamic acids (RCONHOH) are weak acids that can exist naturally or synthetically. These acids are easy to deprotonate and produce hydroxamate ions. The syntheses, physico-chemical and characterization of salicylhydroxamic acid (SHA) and its copper(II) and nickel(II) complexes were reported herein. The metal complexes were synthesized by condensation reaction of SHA with metal salts in 2:1 molar ratio. SHA and its metal complexes were characterized by elemental analysis, infrared spectroscopy, ^{1}H and ^{13}C NMR, UV-Vis, TGA, magnetic susceptibility and molar conductance. From IR and magnetic susceptibility, each complexes coordinated to the metal via oxygen atoms (*O,O*) in a bidentate manner to form octahedral geometries. The molar conductance values suggested that all complexes were non-electrolytes. A cytotoxicity study against HCT116 displayed that Cu(II) and Ni(II) complexes have better anticancer than their parent ligands, SHA but not categorized as potent anticancer agents. Both complexes have better inhibition towards α-glucosidase compared to acarbose, suggesting that Cu(II) and Ni(II) complexes have potential as antidiabetic agents.

Keywords: salicylhydroxamic acid, copper(II), nickel(II), cytotoxicity, α-glucosidase

INTRODUCTION

Hydroxamic acids are known as multifunctional biological agents such as anti-cancer, anti-tuberculosis and anti-malarial (Srivastava *et al*., 2009). Anions of hydroxamic acid known as hydroxamate ions are capable on chelating with metal ions and formed stable complexes. Their versatility in biological activities come from their ability on forming the stable metal complexes and *NO*-releasing properties (Chauhan *et al*., 2016; Sharma *et al.,* 2010). These anions flexibility have received a lot of attentions due to their importance in fulfilling variety of roles in biology and medicine (Chauhan *et al*., 2016).

Salicylhydroxamic acid is identified as developed anti-cancer agent. The development of drug resistance towards cancer with lesser side effects leads to discovery of new metal complexes that can overcome this problem (Desoize, 2002; Ndagi *et al*., 2017). The patients around the world suffered with Type 2 Diabetes Mellitus nowadays have increased. This disease becomes a serious medical concern worldwide which triggered the exploring of a new therapeutic agent that can overcome this problem. Transition metals have been used as a treatment of diabetes since 1899. The orally active metal complexes containing oxovanadium ion have widely used since 1990. A wide class of transition metals has been tested on experimental animals and found to exhibit potential on treating diabetes. An α-glucosidase is responsible on converting polysaccharides to monosaccharides and increasing the glucose level in the blood. The effective metal complexes that can inhibit the α-glucosidase enzyme can be considered as potential antidiabetic agents (Tripathi *et al*., 2017).

This paper reported the synthesis and characterization of SHA and its Cu(II) and Ni(II) complexes. The preliminary cytotoxicity screening against HCT116 and inhibition activities of the synthesized compounds towards α-glucosidase were carried out and the results were reported herein.

EXPERIMENTAL

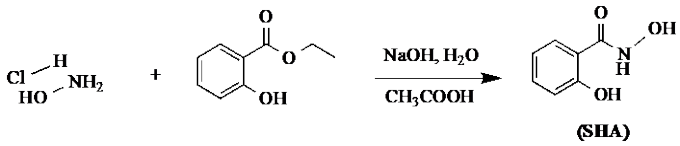
Materials and methods

All the chemicals and reagents were purchased from Sigma Aldrich (United States) and Merck (United States) and used without further purification. Elemental analyses (C, H, and N) of SHA and its metal complexes were carried out 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 in the range of 4,000–400 cm^{-1} as KBr disc by using a Perkin-Elmer Model 1750X FTIR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were determined by Bruker Varian-600MHz, using TMS as an internal standard in deuterated dimethylsulphoxide (DMSO-d_6). The UV-Vis spectra were obtained in ethanol in the 200-900 nm range using Perkin Elmer UV-Vis Lambda 35 spectrophotometer at room temperature. The thermogram of Cu(II) and Ni(II) complexes were recorded using NETZSCH TG 209 F3 under nitrogen (N_2) atmosphere at heating rate of 10 °C/min from room temperature to 900 °C. The magnetic moments for complexes were characterized using the Guoy method with water as calibrant on Sherwood Auto Magnetic Susceptibility Balance. Molar conductivity measurement of Cu(II) and

Ni(II) complexes in ethanol (~10^{-3} M) was measured using a Mettler Toledo Inlab 730 conductivity meter at room temperature.

Synthesis of SHA

The synthesis of SHA is represented in Scheme 1.

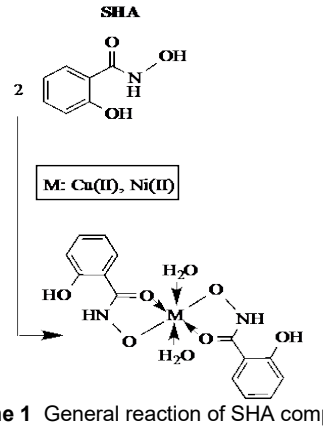


Scheme 1 The synthesis reaction of SHA.

A 200 mL of 12% sodium hydroxide solution was added to hydroxylamine hydrochloride (200 mmol, 14 g). The white precipitate was formed at the bottom of the flask when the mixture was cooled off. Then, the precipitate was filtered off and the filtrate was added with a small portion of ethyl salicylate (100 mmol, 15.2 g). The filtrate was vigorously shaken after each addition to ensure a complete dissolution of the precipitate. The mixture was left aside at room temperature for 2 days until the straw brown solution was formed. Then, the solution was acidified to pH 5.5, washed and recrystallized from water containing little acetic acid. The pinkish-white precipitate was collected (Yield: 100%).

Synthesis of SHA complexes

The overall reaction of metal salts with SHA in 1:2 molar ratio is represented in Scheme 1.



Scheme 1 General reaction of SHA complexes

Synthesis of copper salicylhydroxamic acid, $[Cu(SHA)_2(H_2O)_2]$

A hot aqueous solution (10 mL) of SHA (5 mmol, 0.7861 g) was added with copper(II) acetate tetrahydrate, $[Cu(CH_3COO).4H_2O]$ (5 mmol, 1.2443 g). Then, pH 5.5 of the resulting solution was achieved by adding 0.1 M NaOH solution. The light blue precipitate was formed, filtered and washed with warm water before stored in a desiccator (Yield: 72.79%).

Synthesis of nickel salicylhydroxamic acid, $[Ni(SHA)_2(H_2O)_2].H_2O$

Nickel(II) acetate tetrahydrate, $[Ni(CH_3COO).4H_2O]$ (5 mmol, 1.2443 g) was dissolved in a hot aqueous solution (10 mL) of SHA (5 mmol, 0.7861 g). The green precipitate was formed when the pH of the mixture was adjusted to 5.5 using 0.1 M NaOH solution. Then, the green precipitate was filtered and washed with the warm water before being stored in desiccator for the next usage (Yield: 79.25%).

Biological activities screening

MTT assay

The human colorectal carcinoma cell line, HCT116 (ATCC® CCL-247™), was cultured in the Roswell Park Memorial Institute (RPMI-1640) medium with 25 mM HEPES and L-Glutamine (Biowest) supplemented with 10% heat inactivated fetal bovine serum (FBS) (PAA Laboratories) and 1% penicillin/streptomycin (Sigma Aldrich). The cell culture was put in a humidified incubator at 37 °C in an atmosphere of 5% CO_2 . 7×10^4 of HCT116 cells were cultured per well before being incubated at 37 °C for 24 h. Then, SHA and its metal complexes were diluted into two-fold dilution gradient ranging between 0.01–100 μM before adding into the wells and were incubated at 37 °C for another 72 h. Then, each well was topped up with 50 μL of 0.06 mol/L MTT solution. The plates were put into the incubator at 37 °C for 4 h. After 4 h of incubation, 200 μL of the solutions on each well was removed using micropipette before adding with 100 μL of DMSO. The formazan crystals were dissolved in DMSO that giving out the purple solutions. The plates were read using a microplate reader at wavelength of 450 nm. Thus, the data obtained was used to determine at which concentration of compounds can kill 50% of cell population (IC_{50}) (Kumar *et al*., 2015).

α-Glucosidase inhibitory assay

The effects of SHA and its Cu(II) and Ni(II) complexes on α-glucosidase activity were based on methods reported by Elya *et al* (2012) using α-glucosidase from *Saccharomyces cerevisiae*. The substrate, *p*-nitrophenyl glucopyranoside (pNPG) (1 mM) was prepared in 30 mM of potassium phosphate buffer (pH 6.8). 10 μL of SHA and its Cu(II) and Ni(II) complexes with different concentrations ranging from 7.8 to 625 μM was pre-incubated with 10 μL of α-glucosidase (0.02 U/mL) at 37 °C for 10 min. Then, the reaction was started after pre-incubation by adding 50 μL of 1 mM pNPG to the mixture on the well. The plates were incubated at 37 °C for another 30 min. Acarbose was used as a positive control. The quantity of yellow colored para-nitrophenol that had been released from pNPG was measured by using spectrophotometer at 405 nm indicating α-glucosidase inhibitory activity. The concentration of tested compounds that can inhibit 50% of the α-glucosidase activity was defined as IC_{50} values that calculated and tabulated by using Prism 7.0.

RESULTS AND DISCUSSION

Physical properties and elemental analysis

Physical characteristics and microanalysis data of SHA and its Cu(II) and Ni(II) complexes are tabulated in Table 1. The calculated values of each elements (C, H, N) were in a good agreement with the measured ones. Cu(II) and Ni(II) complexes are stable at room temperature and soluble in most of the organic solvents such as DMSO and EtOH. The molar ratio of SHA and metal ions found in the complexes was 2:1, indicating the formation of bidentate complexes which supported by the infrared spectra. Both of the complexes were decomposed at 230 °C.

Infrared spectroscopy

The nature of the functional groups that existed in the molecule can be determined by using the valuable information provided by the infrared. The main infrared bands of SHA and its Cu(II) and Ni(II) complexes with their assignments are listed in Table 2. Figure 1 shows the comparison spectra between SHA, Cu(II) and Ni(II) complexes. There were observable differences of the spectra between SHA and its metal complexes, showing that the complexation between metal ions and SHA was occurred.

The characteristic bands of SHA and its metal complexes are ν(C=O), ν(N–H), ν(N–O) and ν(OH). The ν(C=O) stretch of the hydroxamate group of SHA was observed to reach a strong peak at 1620 cm^{-1}. These bands for Cu(II) and Ni(II) complexes were shifted to lower wavenumber, 1602–1605 cm^{-1}, due to the withdrawal of electron density in oxygen indicating that carbonyl (C=O) groups were involved during complexation via oxygen atoms (Haratake *et al*., 2005). The O-H band at 2885 cm^{-1} was disappeared in the metal complexes spectra due to the deprotonation upon complexation through the oxygen atoms.

Table 1 Physical and elemental analysis data of SHA and its metal complexes.

Ligand/Complex	Molecular Formula	Molecular Weight (g/mol)	Colour	Calculated (**Found**)%			Melting point (°C)
				C	H	N	
SHA	$C_7H_7NO_3$	153.10	Pinkish white	54.90 **(55.73)**	4.61 **(4.63)**	9.15 **(9.92)**	175–176
$[Cu(SHA)_2(H_2O)_2]$	$C_{14}H_{16}N_2O_8Cu$	403.80	Light blue	41.64 **(40.90)**	3.99 **(3.36)**	6.94 **(7.05)**	Decomposed at 230
$[Ni(SHA)_2(H_2O)_2].H_2O$	$C_{14}H_{18}N_2O_9Ni$	417.00	Light green	40.33 **(40.90)**	4.35 **(3.36)**	6.72 **(7.05)**	Decomposed at 230

The phenolic oxygen bands were identified in Cu(II) and Ni(II) spectra at 1256 and 1249 cm^{-1} respectively (Shotor *et al*., 2010). It showed that the phenolic oxygen did not involve in complexation due to no shifting of these bands on the complexes. The involvement of C=O during complexation was supported by the appearance of new bands, *ν*(M-O) at 433 and 477 cm^{-1} for SHA complexes (Fazary, 2014).

The ν(N–H) of SHA appeared as a strong and sharp peak at 3289 cm^{-1}. This band shown in Cu(II) and Ni(II) complexes at 3304 and 3336 cm^{-1} respectively, indicating that the N atoms did not take part in complexation. A similar trend for ν(N–O) in Cu(II) and Ni(II) spectra, suggesting that –NO was retained and not coordinated. The bidentate coordination mode of SHA to the metal ions was confirmed by the Δν values (Δν values = ν(C=O)asy – ν(C=O)sym) and the differences of Δν were~~is~~ less than 200 cm^{-1} indicated the ligands were chelated to the metal ions in a bidentate manner (Graisa *et al*., 2008). Therefore, the suggested structures of the metal complexes are shown in Scheme 2.

Nuclear magnetic spectroscopy (NMR)

The ^1H NMR spectrum for SHA was recorded in DMSO-d_6 using tetramethylsilane (TMS) as the internal standard. There are three types of protons that existed in SHA. The protons are N–H, O–H and aromatic protons. All of these protons were observed in the spectrum, confirming the structure of SHA (Scheme 1). N–H proton was observed as a singlet peak at 2.0 ppm. The phenolic O–H was observed as a single resonance at 9.35 ppm. The multiplet resonance was observed near 7.47–7.75 ppm, indicating the aromatic protons.

For ^{13}C NMR, there are two types of carbon existed in SHA, carbonyl (C=O) and aromatic carbon. The C=O peak was appeared at the very downfield region, 166.80 ppm due to the deshielded effects by oxygen. Then, the peaks of aromatic carbon were observed in the range of 114.51–159.38 ppm. Unfortunately, due to the paramagnetic behaviour of Cu(II) and Ni(II) complexes, ^1H and ^{13}C NMR for these complexes could not be obtained (Sharma and Sharma, 2013).

UV-visible spectroscopy (UV–Vis)

The spectral data of the SHA and its Ni(II) complex in EtOH is listed in Table 3.

Table 3 UV-Vis data for SHA and Ni(II) complex.

Ligand/Complex	π → π* transition (nm)	n → π* transition (nm)
SHA	234	302
$[Ni(SHA)_2(H_2O)_2].H_2O$	238	306

The UV-Vis spectra of SHA and Ni(II) complex were measured in the range of 200-900 nm with a concentration of 10^{-3} M in EtOH. Unfortunately, UV-Vis spectra of Cu(II) complex could not be provided due to solubility problem. The π → π* transitions appeared at 234 and 238 nm for SHA and Ni(II) complex. This transition indicated the electron excitation that occurred in the molecular orbital located in the phenolic chromophore. Thus, there was donation of lone pair of electrons from deprotonated OH to the metal atom indicated by the slightly shifting towards the higher wavelength. The n → π* transitions of SHA and Ni(II) complex appeared at 302 and 306 nm respectively. This transition involved~~s~~ the molecular orbital of C=O chromophore and the benzene ring. The complexes bands undergone a shifting towards the higher wavelength, indicating the oxygen from carbonyl (C=O) coordinated to the metal ion.

Thermogravimetric analysis (TGA)

The summary of the behavior of Cu(II) and Ni(II) complexes upon thermal decomposition through thermogravimetric analysis is presented in Table 4.

Table 4 Thermal behaviour indicating the loss of H_2O molecules from Cu(II) and Ni(II) complexes.

Compound	Temperature Range (°C)	Weight Loss (%) Found (Calculated)	Lost Species
$[Cu(SHA)_2(H_2O)_2]$	167–250	**10.77** (8.53)	$2H_2O$
$[Ni(SHA)_2(H_2O)_2].H_2O$	45–100	**6.35** (4.32)	$1H_2O$
	210–250	**16.00** (8.63)	$2\ H_2O$

$[Cu(SHA)_2(H_2O)_2]$ and $[Ni(SHA)_2(H_2O)_2].H_2O$ clearly indicate~~s~~d that there was loss of two molar equivalent of water molecules at higher temperature, 167–250 °C. For $[Ni(SHA)_2(H_2O)_2].H_2O$, at lower temperature, 45–100 ºC, there was loss of one molar equivalent of water molecules. These results supported the suggestion that there were two coordinated water molecules in Cu(II) and Ni(II) complex and one uncoordinated water molecules in the solid packing (Sharma and Sharma, 2012).

Table 2 Infrared spectral data for SHA and its metal complexes, Cu(II) and Ni(II).

Ligand/Complex	Frequencies,ν (cm^{-1})					
	C=O	N-H	N-O	M-O	OH	Phenolic (OH)
SHA	1619 (*s*)	3289 (*s*)	1033 (*m*)	-	2885 (*br*)	1248 (*s*)
$[Cu(SHA)_2(H_2O)_2]$	1606 (*s*)	3304 (*s*)	1039 (*m*)	477 (*w*)	-	1256 (*s*)
$[Ni(SHA)_2(H_2O)_2].H_2O$	1608 (*s*)	3336 (*s*)	1027 (*m*)	433 (*w*)	-	1249 (*s*)

Note: *s=strong; m=medium; w= weak; br= broad*

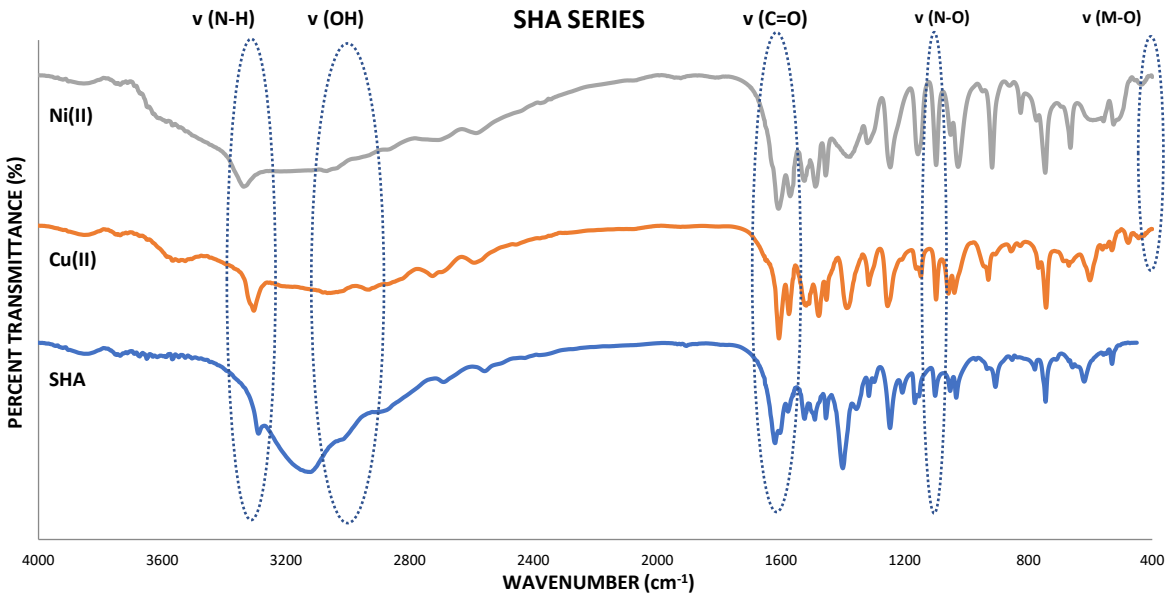


Fig. 1 IR spectra for SHA series.

Magnetic susceptibility and molar conductivity

The magnetic susceptibility and molar conductivity data for complexes is tabulated in Table 5. Magnetic moments for the complexes were measured at room temperature.

Table 5 Magnetic susceptibility and molar conductance data for Cu(II) and Ni(II) complexes.

Complexes	μ_{eff} B.M Found (Calculated)	Molar conductance (Ω^{-1} cm^2 mol^{-1})
$[Cu(SHA)_2(H_2O)_2]$	3.07 **(1.73)**	10.85
$[Ni(SHA)_2(H_2O)_2].H_2O$	3.24 **(2.83)**	15.04

The magnetic susceptibility values obtained for $[Cu(SHA)_2(H_2O)_2]$ and $[Ni(SHA)_2(H_2O)_2].H_2O$ suggested that the complexes are having octahedral geometry (Marmion *et al*, 2004). For $[Cu(SHA)_2].3H_2O$, the value obtained was higher than calculated due to orbital contribution (Pakchung *et al*., 2011).

The coordination modes of metal chelates were determined from the measurement of conductance within the limits of their solubility. Cu(II) and Ni(II) complexes were found to be non-electrolytic in nature because of their molar conductance values were in the range of 10.85-15.04 Ω^{-1} cm^2 mol^{-1} (Temel and Sekerci, 2001). This conductance data also indicated that there was no free ions that act as electrolytes in the solution (Chauhan *et al*., 2016).

Cytotoxicity assay

The cytotoxicity activities of SHA and its Cu(II) and Ni(II) complexes were evaluated against the colorectal carcinoma cell line, HCT116. SHA and its Cu(II) and Ni(II) complexes induced a concentration-dependent anti-proliferative effect towards HCT116 cells upon treatment for 24 hours (Sharma *et al*., 2012). All compounds were soluble in DMSO. IC_{50} values of SHA series on HCT116 cells are shown in Table 6.

The parent ligand, SHA gave a lower cytotoxicity compared to its Cu(II) and Ni(II) complexes. The behavior of the metal complexes can be explained through the chelation theory by Tweedy and cell permeability by Overtone. Tweedy's stated that the complexation makes the polarity of the metal ion decreases due to the sharing of the positive charge with the donor groups. Then, the delocalization of π-electrons over the entire chelate ring will be increased. Consequently, this enhances the lipophilicity of the complexes. Meanwhile, Overtone's concept of cell permeability stated that the 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 (Tripathi *et al*., 2017). That ~~is~~ explained why Cu(II) and Ni(II) complexes gave better cytotoxicity values compared to SHA, the parent ligand.

Table 6 IC_{50} values of SHA and Cu(II) and Ni(II) complexes on HCT116 cells.

Ligand/Complex	IC_{50} ± SD values (μM)
SHA	>100
$[Cu(SHA)_2(H_2O)_2]$	46.00 ± 2.20
$[Ni(SHA)_2(H_2O)_2].H_2O$	36.00 ± 3.60
5-FLUOROURACIL	13.07 ± 0.00

Both of the metals are capable of generating reactive oxygen species (ROS). ROS is a great part on the metals that contributes on their carcinogenicity and aptitude on treating the cancers. Both complexes showed better cytotoxicity results compared to their ligand, SHA due to the presence of metals. By comparing these two metal salts, Ni(II) showed the best results than Cu(II). This observation was due to increasing production of ROS by Ni(II) compared to Cu(II). The molecular mechanism on how these complexes transformed the cells was not clear (Desoize, 2002). Unfortunately, all the synthesized compounds were categorized as non-potent anticancer agents due to the IC_{50} value obtained was far higher compared to the standard, 5- fluorouracil.

α-Glucosidase inhibitory activity

SHA and its Cu(II) and Ni(II) complexes were tested on their α–glucosidase inhibitory activity against *Saccharomyces cerevisiae.* The results are listed in Table 7**.**

From the results, SHA was inactive at 100 μM while the complexes, Cu(II) and Ni(II) showed better activity than the parent ligand and positive control, acarbose. From IC_{50} values, Cu(II) complex exhibited a better and more potential inhibitor than Ni(II) complex. This displayed that Cu(II) complex has better viability on

blocking the active sites of α-glucosidase at a lower concentration, 26.42 μM compared to Ni(II) complexes at 173.6 μM.

Table 7 α-glucosidase Inhibitory Activity against SHA and its complexes.

Ligand/Complex	IC_{50} ± SD values (μM)
ACARBOSE (STANDARD)	418 ± 0.55
SHA	Not active
$[Cu(SHA)_2(H_2O)_2]$	26.42 ± 0.52
$[Ni(SHA)_2(H_2O)_2].H_2O$	173.6 ± 0.46

CONCLUSION

SHA and its metal complexes**,** Cu(II) and Ni(II) had successfully synthesized and characterized by melting point, elemental analysis, infrared (IR) spectroscopy, ^{1}H and ^{13}C NMR, UV–Vis, TGA analysis, magnetic susceptibility measurement as well as molar conductivity. SHA was coordinated to the metal center through deprotonated OH and C=O in a bidentate manner. The presence of two ligands per one metal ion was confirmed from the IR spectral studies. On the basis of chelation theory, Cu(II) and Ni(II) had shown the best toxicity than free ligand, SHA against HCT116 but not considered as a potent anticancer agents due to higher IC_{50} values than 5-fluorouracil. Cu(II) complex is the potent α-glucosidase inhibitor compared to Ni(II) complexes, SHA and acarbose.

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REFERENCES

Chauhan, S., Deepak, Garg, S., Verma, K. K. 2016. Study on 4-Methoxybenzohydroxamate complexes of Aryltellurium (IV) and Diaryltellurium (IV). *Int. J. Chem. Sci. 14*(1), 269–282.

Desoize, B. 2002. Cancer and metals and metal compounds: Part 1 – Carcinogenesis. *Crit Rev. Oncol. Hematol.*, *42*(1), 1–3.

Fazary, A. E. 2014. Metal complexes of salicylhydroxamic acid and 1,10-phenanthroline; equilibrium and antimicrobial activity studies. *B. Chem. Soc. Ethiopia*, *28*(3), 393–402.

Graisa, A., Farina, Y., Yousif, E., Kassem, M. 2008. Synthesis and characterization of Some Diorganotin (IV) complexes of n-tolyl-m-nitrobenzohydroxamic acid. *ARPN J. Eng. Appl. Sci., 3*(6), 47–50.

Haratake, M., Fukunaga, M., Ono, M., Nakayama, M. 2005. Synthesis of vanadium (IV,V) hydroxamic acid complexes and in vivo assessment of their insulin-like activity. *J. Biol. Inorg. Chem., 10*(3), 250–258.

Kumar, P., Narasimhan, B., Ramasamy, K., Mani, V., Mishra, R., Majeed, A. 2015. Synthesis, antimicrobial, anticancer evaluation and QSAR studies of 3/4-bromo benzohydrazide derivatives. *Curr. Top. Med. Chem., 15*(11), 1050–1064.

Marmion, C. J., Griffith, D., Nolan, K. B. 2004. Hydroxamic acids-an intriguing family of enzyme inhibitors and biomedical ligands. *Eur. J. Inorg. Chem.* 3003–3016.

Ndagi, U., Mhlongo, N. N., Soliman, M. E. 2017. Metal complexes in cancer therapy – An update from drug design perspective. *Drug. Des. Devel. Ther*., *11*, 599–616.

Pakchung, A. A. H., Soe, C. Z., Lifa, T., Codd, R. 2011. Complexes formed in solution between vanadium (IV)/(V) and the cyclic dihydroxamic acid putrebactin or linear suberodihydroxamic acid. *Inorg. Chem., 50*(13), 5978–5989.

Sharma, N., Kumari, M., Kumar, V., Chaudhry, S. C., Kanwar, S. S. 2010. Synthesis, characterization, and antibacterial activity of vanadium(IV) complexes of hydroxamic acids. *J. Coord. Chem., 63*(1), 176–184.

Sharma, N., Kumari, M., Sharma, R. 2012. Thermoanalytical studies of oxovanadium (IV) hydroxamate complexes. *J. Therm. Anal. Calorim., 107*(1), 225–229.

Sharma, R., Sharma, N. 2012. Thermal studies of some biologically active oxovanadium (IV) complexes containing 8-hydroxyquinolinate and hydroxamate ligands. *J. Therm. Anal. Calorim., 110*(2), 539–543.

Sharma, S., Sharma, N. 2013. Biological activity of bis(nicotinohydroxamato) oxidovanadium(IV) complex. *Der Chemica Sinica*, *4*(4), 87–96.

Shotor, S. N. M., Kassim, K., Bahron, H., Tajuddin, A. M., Yusof, F. Z. 2010. In-vitro cytotoxicity studies of vanadyl complexes with hydroxamic acid series. *In 2010 International Conference on Science and Social Research (CSSR 2010), Kuala Lumpur, Malaysia*. 1283–1286.

Srivastava, K. P., Singh, B., Akbar, A. 2009. Synthesis and characterisation of Co^{II} complexes of dibasic tetradentate (OO'NO donor) ligand. *Int. J. Chemtech Res., 1*(1), 71–79.

Temel, H., Sekerci, M. 2001. Novel complexes of manganese(III), cobalt(II), copper(II) and zinc(II) with Schiff base derived from 1,2-bis(p-amino-phenoxy)ethane and salicylaldehyde. *Synth. React. Inorg. M., 31*(5), 849–857.

Tripathi, I. P., Dwivedi, A., Mishra, M. 2017. Metal-based α-glucosidase inhibitors : Synthesis, characterization and α-glucosidase inhibition activity of transition metal complexes. *Asian Med. Health*, *2*(3), 1–14.