Annals of the University of Craiova
The Chemistry Series
Volume XLIX, No. 1 (2023) 86-93
homepage: chimie.ucv.ro/annals/
10.52846/AUCCHEM.2023.1.09

Cytostatic activity of some binuclear complexes with Schiff base derived from an aromatic dialdehyde and 2-aminobenzoic acid

Research article

Florina Ciolan

University of Craiova, Faculty of Sciences, Department of Chemistry, Calea București 107i, Craiova, Romania
E-mail: florina_ciolan@yahoo.com

Received: 20. 07. 2023 / Accepted: 20. 08. 2023 / Published: 01.09.2023

Abstract
The binuclear complex compounds of Cu(II), Ni(II), Co(II) and Zn(II) with the Schiff bases derived from an aromatic dialdehyde and 2-aminobenzoic acid were tested for their antitumor activity. The results of the biological tests indicated that the complexes of Zn(II) and Cu(II) are the most active cytostatic compounds.

Keywords: cytostatic activity, HeLa cells, binuclear complexes, Schiff bases, 2-aminobenzoic acid

1. INTRODUCTION

Schiff base metal complexes have a versatile biological activity towards various kinds of pathogens and tumors and present

biochemical, clinical and pharmacological properties. The main reason for the biological properties is the presence of the imine group in this compounds [1]. Amino acids, a significant class of organic compounds, contain potential donor sites such as COOH and/or NH_2 which have good ability to coordinate with the metal ions [2]. The Schiff bases derived from amino acids, when compared with the classical Schiff bases, have a more stable and higher solubility in organic solvents and new compounds can be derived in easier coordination form owing to the conformational flexibility of their backbones [3].

Many metal complexes with amino acid derived Schiff base have been synthesized and tested for their biological activity [4, 5]. For example, the Schiff base, obtained from 2-thiophene carboxaldehyde and 2-aminobenzoic acid and its metal complexes, show antibacterial activity [6]. Salicylidene anthranilic acid possesses antiulcer activity and the complexation with copper shows an increase in antiulcer activity [7]. Especially, the Zn(II) complexes show higher activity than other metal complexes.

The synthesis and characterization of Cu(II), Ni(II), Co(II) and Zn(II) binuclear complexes with the Schiff bases derived from the aromatic dialdehyde, 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde and 2-aminobenzoic acid, were presented in two previous studies [8, 9].

In this paper we present the results of the cytotoxic tests for these binuclear complexes.

2. MATERIALS AND METHODS

2.1. Materials

The binuclear complexes of Cu(II), Ni(II), Co(II) and Zn(II) with the new Schiff base ligand derived from 2-aminobenzoic acid and 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde were prepared using two methods described in previous studies [8, 9]. So, according to the first method, all the mentioned complexes were obtained by template condensation of 2-aminobenzoic acid, aromatic dialdehyde and the corresponding metal salt in alcoholic medium [8, 9]. The second

method, also used to obtain all these complexes, consists in refluxing of the ethanolic solutions of the isolated Schiff base (obtained by a method also described in the same previous study) sodium salt with the metal salts, in a 1:2 molar ratio [8, 9].

The prepared complexes are of $[M_2L'(OH)_2(H_2O)_4]$ type, where M= Cu(II), Ni(II), Co(II) or Zn(II) and L= $C_{31}H_{24}O_6N_2$.

Analytical grade reagents from Sigma and Merck were used in all experiments.

2.2. Method for the cytotoxic tests

Evaluation of the cytotoxicity of these compounds was performed on HeLa cells, human cancer cells, using the MTT assay, at 24h and 48h incubation times and at different concentrations of the complex combinations (10, 50, 100, 200 $\mu g \cdot mL^{-1}$).

HeLa cells are the oldest human cancer cells most commonly used in scientific research [10]. These cells were first harvested on February 8, 1951 from a patient – Henrietta Lacks, who died of cervical cancer on October 4, 1951.

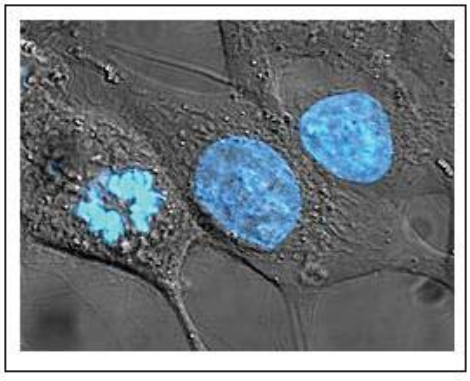

Figure 1. HeLa tumor cells

The viability assay most commonly used throughout the world is the MTT assay, first described by Tim Mosmann in 1983 [11]. This colorimetric assay uses reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT) to

measure cellular metabolic activity as a proxy for cell viability. Viable cells contain NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT reagent to formazan, an insoluble crystalline product with a deep purple color. Formazan crystals are then dissolved using a solubilizing solution and absorbance is measured at 500-600 nm using a plate-reader (Absorbance Reader Tecan). Below is a brief description of the protocol steps:

1. Place 1000-100000 cells per well in a 96-well plate and incubate with the appropriate stimulus for the desired time (24, 48 hours).
2. Remove incubation medium and wash cells with PBS.
3. Add MTT in the incubation medium and made up to a final concentration of 0.5 $mg \cdot mL^{-1}$.
4. Incubate for 30 minutes to 4 hours at 37°C, until intracellular purple formazan crystals are visible under microscope.
5. Remove MTT and add solubilizing solution (DMSO, acidified isopropanol or SDS) and triturate.
6. Incubate at room temperature or 37°C for 30 minutes to 2 hours, until cells have been lysed and purple crystals have been dissolved.
7. Measure absorbance at 570 nm.

The absorbance reading of the blank (well containing incubation medium only) must be subtracted from those of all samples. Absorbance readings of test samples must then be divided by those of the control (untreated cells) and multiplied by 100 to give percentage of cell viability or proliferation.

Absorbance values greater than the control indicate cell proliferation, while lower values suggest cell death or inhibition of proliferation.

3. RESULTS AND DISCUSSION

The spectroscopic data (IR and UV-Vis electronic spectra), as well as elemental analysis, molar conductivity measurements and thermal

analysis results, presented in two previous studies [8, 9], support the proposed general structures of the studied compounds (Figure 2).

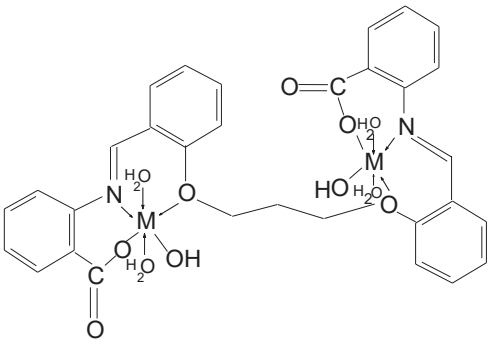

Figure 2. The general structures for the metal complexes with the new Schiff base ligand (where M = Cu(II), Ni(II), Co(II) or Zn(II))

3.1. Antitumor activity

Relative cell viability, expressed as a percentage of untreated cells, was calculated for each concentration. Concentration-response curves were plotted for each experiment (Figure 3 and Figure 4). Each of the obtained data represents the average of three independently obtained values.

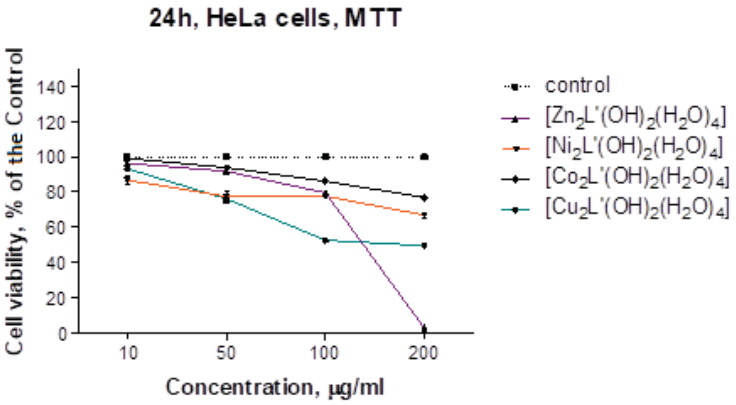

Figure 3. Viability of tumor cells in the presence of different concentrations of

the tested compounds and at an incubation time of 24 h

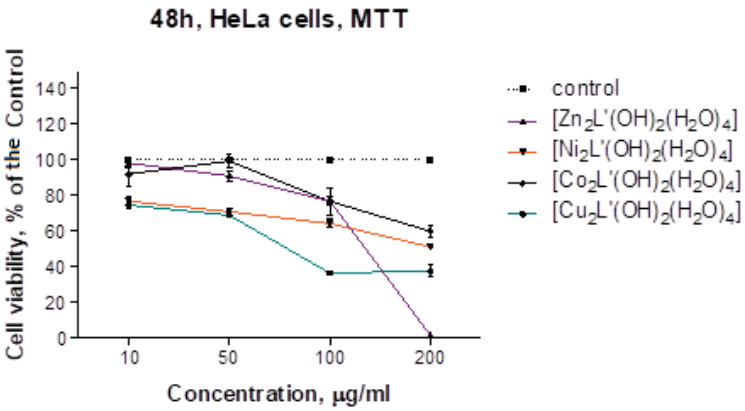

Figure 4. Viability of tumor cells in the presence of different concentrations of the tested compounds and at an incubation time of 48 h

The cytotoxicity test results for the studied compounds, using the MTT assay [11], are presented in the Table 1.

The analysis of these results showed that the $[ZnL'(OH)_2(H_2O)_4]$ complex and the $[CuL'(OH)_2(H_2O)_4]$ complex have the highest cytostatic activity and that the antitumor activity of the tested complexes followed the order: $[ZnL'(OH)_2(H_2O)_4]$ >$[CuL'(OH)_2(H_2O)_4]$ >$[NiL'(OH)_2(H_2O)_4]$ >$[CoL'(OH)_2(H_2O)_4]$.

The binuclear complexes of Zn(II) and Cu(II) with the Schiff bases derived from 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde and 2-aminobenzoic acid also exhibited the most pronounced antimicrobial activity [12, 13].

It must be noticed that viability of HeLa cells in the presence of $[ZnL'(OH)_2(H_2O)_4]$ complex at a concentration of 200 $\mu g \cdot mL^{-1}$ and 24 hours incubation time was 2.64+/-0.04, while at 48 hours incubation time attained 1.57+/- 0.01.

These results indicate that the $[ZnL'(OH)_2(H_2O)_4]$ complex has the highest cytostatic activity.

Table 1. The results of the cytotoxicity assessment of the tested compounds

Compound	Concentration/ $\mu g \cdot mL^{-1}$	Cell viability/ % of the control	
		24 h	48 h
$[ZnL'(OH)_2(H_2O)_4]$	10	96.33 ± 0.96	98.36 ± 2.73
	50	91.80 ± 1.82	91.22 ± 2.87
	100	79.55 ± 0.65	76.90 ± 7.68
	200	**2.64 ± 0.04**	**1.57 ± 0.01**
$[NiL'(OH)_2(H_2O)_4]$	10	86.85 ± 2.76	76.98 ± 2.16
	50	77.55 ± 2.49	71.05 ± 1.68
	100	77.73 ± 1.40	64.45 ± 2.29
	200	66.96 ± 1.51	51.36 ± 1.25
$[CoL'(OH)_2(H_2O)_4]$	10	99.15 ± 1.42	92.18 ± 6.58
	50	93.97 ± 0.72	99.48 ± 3.63
	100	86.23 ± 0.99	76.93 ± 2.26
	200	76.96 ± 0.90	60.03 ± 3.58
$[CuL'(OH)_2(H_2O)_4]$	10	93.31 ± 1.59	74.78 ± 2.18
	50	76.15 ± 2.65	69.00 ± 0.97
	100	52.57 ± 0.96	36.69 ± 0.60
	200	49.55 ± 0.57	37.85 ± 3.36

4. CONCLUSION

In this study, the Cu(II), Ni(II), Co(II) and Zn(II) binuclear complexes with a new Schiff base derived from 2-aminobenzoic acid and the aromatic dialdehyde, 2,2'-(propane-1,3-diyldioxy)dibenzaldehyde, were tested for their antitumor activity on HeLa cells, human cancer cells, using the MTT assay.

The results of the biological evaluation showed that the viability of HeLa cells in the presence of the binuclear complex of Zn(II) exhibited the lowest values and that the cytostatic activity of the tested compounds followed the order: $[ZnL'(OH)_2(H_2O)_4]$ >$[CuL'(OH)_2(H_2O)_4]$

>[NiL′$(OH)_2(H_2O)_4$] >[CoL′$(OH)_2(H_2O)_4$].

These results indicate that the [ZnL′$(OH)_2(H_2O)_4$] complex has the highest cytostatic activity.

REFERENCES

[1] P. Ghanghas, A. Choudhary, D. Kumar, K. Poonia, *Inorg. Chem. Commun.*, 130 (**2021**) 108710.
[2] G. Frauscher, E. Karnaukhova, A. Muehl, H. Hoeger, B. Lubec, *Life Sci.*, 57 **(1995)** 813.
[3] F. Sevgi, U. Bagkesici, A. N. Kursunlu, E. Guler, *J. Mol.Struct.*, 1154 (**2018**) 256-260.
[4] Z. H. Chohan, M. Arif, M. Sarfraz, *Appl. Organometal. Chem.*, 21 **(2007)** 294.
[5] M. M. Omar, G. G. Mohamed, A. M. M. Hindy, *J. Therm. Anal. Cal.*, 86(2) **(2006)** 315.
[6] G. G. Mohamed, M. M. Omar, A. M. M. Hindy, *Spectrochim. Acta*, 62 (**2005**) 1140.
[7] R. K. Parashar, R. C. Sharma, G. Mohan, *Biol. Trace Elem. Res.*, 23 (**1989**) 145.
[8] F. Ciolan, L. Patron, G. Marinescu, N. Cioatera, M. Mureseanu, *Rev. Chim. (Bucharest)*,
a. 62(12) **(2011)** 1145.
[9] F. Ciolan, L. Patron, M. Mureșeanu, P. Rotaru, I. Georgescu, *Rev. Chim. (Bucharest)*, 63 (**2012**) 34.
[10] R. Rahbari, T. Sheahan, V. Modes et al., *Biotechniques*, 46(4) **(2009)** 277-284.
[11] T. Mosmann, *J. Immunol. Methods*, 65(1-2) **(1983)** 55-63.
[12] F. Ciolan, A. Gănescu, *Annals of the University of Craiova-The Chemistry Series*, XLVII(1) (**2021**) 5.
[13] F. Ciolan, *Annals of the University of Craiova-The Chemistry Series*, XLVIII(2) (**2022**) 22.