Journal of Applied Chemistry **JAC** http://chemistry.journals.semnan.ac.ir

Synthesis, characterization and comparative study of cytotoxic effect of copper(II) and zinc *β*-diketonate complexes

Rahimeh Eshaghi Malekshah[a], Mehdi Salehi[a,]* and Ali Khaleghian[b]

[a]*Department of Chemistry, Semnan University*

[b]*Biochemistry Department, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran*

Article history:
Received: 20/Jan/2016
Received in revised form: 15/Apr/2016
Accepted: 13/May/2016

Abstract

Two mononuclear complexes [Zn^{II}(MAA)(phen)(Cl)] (**A**) and [$Cu^{II}(TTA)_2$] (**B**) (TTA = 4,4,4-Trifluoro-1-(2-furyl)-1,3-butanedione), (MAA = methyl acetoacetate and phen = 1,10-phenanthroline) were synthesized and fully characterized by UV–Vis and FTIR spectroscopy. In this study, the MKN-45 cell line used for MTT assay. Two complexes exhibited lethal effects against MKN-45 cell lines compared to the untreated control. The IC_{50} is observed about 1 μg/mL for both complexes. Besides, the migration studies revealed that complex **(B)** is more active than complex **(A)** against the MKN-45 cancer cell lines. In morphology assay, it was also found that many of the cells showed cytoplasmic shrinkage and loss of normal nuclear architecture.

Keywords: *β*-diketone, 1, 10-phenanthroline, Cytotoxicity effect, Migration assay.

1. Introduction

Platinum agents (cisplatin, carboplatin, and oxaliplatin) are a class of chemotherapy agents for the treatment of a wide spectrum of anticancer, however, these drugs show several side effects [1]. In recent years, a lot of new non-platinum anticancer complexes have been developed [2,3]. This means that a systematic development of inorganic and organometallic compounds as copper(II) and zinc(II) complexes have possible medical uses in the treatment of many diseases including cancer [4-6].

Palaniandavar et. all have reported that copper (II) complexes are the best alternatives to cisplatin because copper plays many significant parts in biological systems and is a biocompatible metal, because of the strong interactions of copper complexes with DNA through surface associations or intercalation [7]. Also, Chalkidou et el. have shown that metal–(N-N) complexes [Cu(flmq)(bipyam)Cl], [Cu(flmq)(bipy)Cl] and [Cu(flmq)(phen)Cl] inhibit DNA replication [8]. Rink et. al. reported that zinc(II) complexes seems to have potential activity for achieving lower side effects in anticancer therapeutics [9].

*.**Corresponding author:** *Associate Professor, Department of Chemistry, Semnan University E-mail address:* msalehi@semnan.ac.ir

In general, fluorinated compounds have been focused much in modern medicinal chemistry and ideal choise for use in drug design because of good biological activity and low toxicity [10]. Trifluoromethyl-containing compounds show high biological activity [11, 12]. 2-Thenoyltrifluoroacetone (HTTA) showed biological activity [13]. HTTA is a classical inhibitor of the mitochondrial electron flux [14, 15], and mitochondria appear to play a major role in the mechanisms of cytotoxicity and antitumor activity of complexes [16]. For this reason, the aim of this work is synthesis, characterization and biological activities of the new Cu(II) and Zn(II) complexes with 4,4,4-Trifluoro-1-(2-furyl)-1,3-butanedione (TTA) and 1,10-phenanthroline (phen) ligands (Scheme 1).

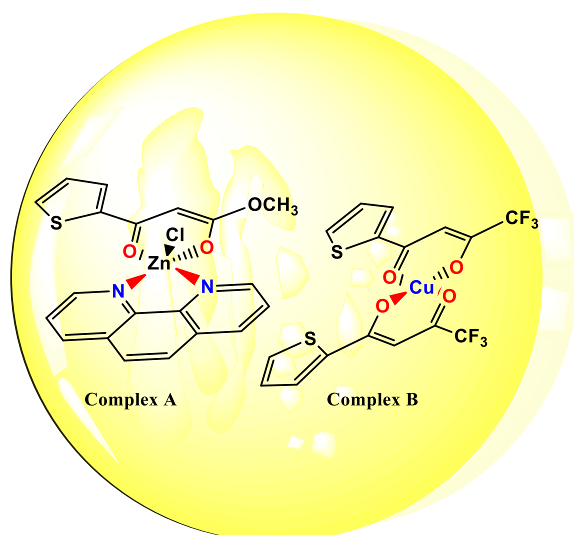

Figure 1. Chemical structures of compounds **(A)** and **(B)**.

2. Experimental procedure

2. 1. Materials and Methods

All other chemicals were purchased from Aldrich and Merck. UV-Vis spectra were recorded on a SHIMADZU UV-1650PC spectrophotometer. Infrared spectra (KBr pellets) were obtained on a FT-IR SHIMADZU spectrophotometer.

The cell culture medium (RPMI 1640), fetal bovina serum (FBS) and penicillin-streptomycin were purchased from Gibco BRL (Life technologies, Paisley, Scotland). The culture plates were obtained from Nunc (Roskilde, Denmark). Cell lines were obtained from Pasteur Institute of Iran (Tehran). MTT assay [3- (4, 5'-dimethyltiazol-2-yl)- 2, 5-diphenyltetrazolium bromide], 5-fluorosalicyl aldehyde (97%) and actinomycine D were purchased from Sigma. Chem. Co. (Munich, Germany). Treated cells were collected by trypsinization and employed in the experiments. 5-FU was treated in vitro as anticancer standard.

2.2. Synthesis of monodentate complexes

2.2.1. [Zn(MAA)(phen)(Cl)] (A)

A methanolic solution (15 mL) containing (MAA) (0.5 mmol, 0.058 mg) and KOH (0.5 mmol, 0.028 mg) was added dropwise into a methanol solution (5 mL) of $ZnCl_2$ (0.5 mmol, 0.068 mg). After refluxing for 24 h, phen (0.5mM, 0.099 mg) in 10 cm^{-3} MeOH was added with stirring for 24 h. The obtained precipitates were filtered off, washed with ethanol and then dried in air. Yield: 90%. FT-IR: ν_{max} cm^{-1} (KBr): 1623 (C=O), 1582 (C=C), 420 (Cu-N). UV–Vis: λ_{max} (nm) (ε, M^{-1} cm^{-1})(CH_3CN): 222 (30000), 254 (25000), 343 (4200).

2.2.2. [Cu(TTA)₂](B)

A methanolic solution (15 mL) containing HTTA (0.5 mmol, 0.11 mg) was added dropwise into a methanol solution (5 mL) of $Cu(ClO_4)_2.6H_2O$ (0.5 mmol, 0.185 mg). The mixture was allowed to stir for 24 h and then filtered; yielding a fine green solid that was washed with ethanol and then dried in air. Yield: 70%. FT-IR: ν_{max} cm^{-1} (KBr): 1595 (C=O), 1582 (C=C). UV–Vis: λ_{max} (nm) (ε, M^{-1} cm^{-1})(CH_3CN): 269 (17000), 277 (17200), 326 (8800), 424 (5000), 673 (14).

2.3. Biological assays

2.3.1. Cell lines and culture conditions

The human gastric cancer cell line, was cultured at a density of 5000 cell per well in RPMI 1640 medium (Invitrogen, Auckland, New Zealand) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Biosera, Ringmer, East Sussex, UK). Cell cultures were kept in a humidified incubator with 5% CO_2, atmosphere containing 95% air at 37°C.

2.3.2. Complexes

Stock solutions were diluted with sterile serum-free culture medium to the desired concentration immediately before each experiment. Various concentrations of complexes **(A)** and **(B)** (1, 5 and 10 μM) were added to each well.

2.3.3 Cytotoxicity assay

MKN-45 cancer cell line was plated in 96-well micro plate with 100 µl medium/well and allowed to adhere overnight. After 24 h cells were treated with complexes at 1, 5 and 10 µM concentrations of complex **(A)** and **(B)** and cultured up to, at the following one time point 48 h.

Chemosensitivity was assessed using 3-(4, 5-dimethylthiazol-2-yl)- 2, 5-diphenyl tetrazolium bromide (MTT) assay [17]. After 48 h, 10 mL of MTT (10 mg/mL) was added. After incubation for 3 h at 37 °C, the purple formazan crystals (a reduced form of MTT) was generated from viable cells that was dissolved by adding 100 mL DMSO in each well. Cytotoxicity effect was revealed as the percentage of treated cells relative to untreated cells at 570 nm. The value of IC_{50} was applied to express the sensitivity of MKN-45 cells to the drug treatment [18].

2.5.3. Migration Assay

The inhibition of the endogenous migrating capacity of MKN-45 was assessed through an in vitro scratch assay. For this assay, the cells were seeded in 35 mm cell culture dishes in RPMI 1640. Cells were allowed to grow in the culture medium in the presence of a final concentration of 1 µg/ml of the Zn(II) and Cu(II) complexes. 48 h after the scratch had been made, images were captured at three distinct time points.

2. Results and discussion

3.1. Synthesis

Complexes (**A**) and (**B**) have been prepared by the direct reaction of the ligands, Phen and HTTA, with $ZnCl_2$ and $Cu(ClO_4)_2.6H_2O$. All complexes are stable at room temperature in the solid state and soluble in common organic solvents (DMF, CH_2Cl_2 and CH_3CN).

3.2. Infrared Spectra

The IR spectra of complexes show that peaks had been shifted to lower frequencies, which can be attributed to the coordination of the *β*-diketone oxygen to the metal center. Infrared spectra show two strong bands, 1623 and 1582 cm^{-1}, for complex **(A)**, 1595 and 1582 cm^{-1}, for complex **(B)**, that can be assigned to (C=O) and (C=C), respectively. The Cu–N stretching vibration in the 425 cm^{-1} region, for complex **(A)** is assigned to the diimines [19-22].

3.3. UV-vis spectra of complexes

Electronic spectra of complexes **(A)** and **(B)** was recorded in acetonitrile. Spectra exhibit the d–d transition as a broad band centered at around 590 nm for **(B)**. The electronic absorption in **(A)** show bounds at 243, 299 and 302 nm, and in **(B)** at 226, 269 and 292 nm, that they assigned to the π-π* and n-π* transitions of aromatic ring and C=O [19, 20].

3. Biological assays

4.1. In vitro growth inhibition assay (MTT) and Cell viability

The results of cytotoxic activity in vitro are expressed as IC_{50} (the concentration of compound (in M) that inhibits a proliferation rate of the tumor cells by 50%) as compared to control untreated cells. The complexes **(A)** and **(B)** were tested for their anti-proliferative activity in vitro against the gastric cancer cells.

As shown in Fig. 2 and 3, compounds inhibited the growth of MKN-45 cells in a dose-dependent manner. The IC_{50} value of complex (**A**) and (**B**) was calculated 1 µg/mL at 48 h. The cytotoxic evaluation by MTT assay indicated that cell viability decreased after 48 h compared to the untreated control.

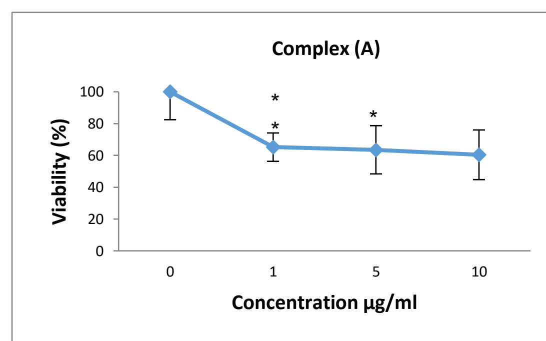

Figure 2. The IC_{50} values by MTT assay in MKN-45 cells. Data are presented as mean ± SD of three independent experiments. * $p< 0.05$ compared with the untreated control.

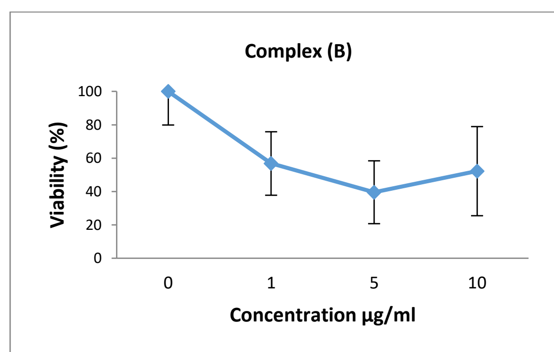

Figure 3. The IC_{50} values by MTT assay in MK-45 cells. Data are presented as mean ± SD of three independent experiments. * $p < 0.05$ compared with the untreated control.

4.2. Migration Assay

To evaluate the cell invasion effect of the complexes on MKN-45 cells, a scratch assay was performed (Figure 4). Cells were treated with IC_{50} concentration of complexes at 48 h. The images at the beginning and the end of each incubation period were captured, which showed a significant decrease in migration of MKN-45 cells as compared to control cells. The results indicate that the complexes can effectively pronounce reduction of cell migration **(B)** > **(A).**

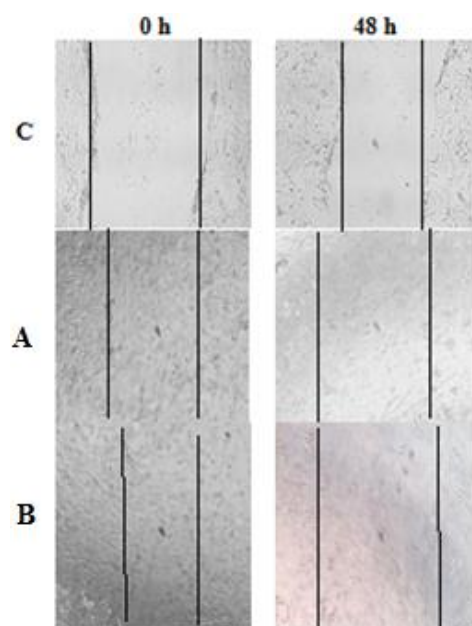

Figure 4. Temporal progress of the wound healing in MKN-45 cells (**c**) and treated with complexes (1 µg/mL).

4.3. Morphological Analysis

MKN-45 cells were seeded in 96-well plates with different concentrations of complexes **(A)** and **(B)** (1, 5 and 10 µM) at 1 × 10^5 cells/well. As shown in Figure 5 many of the cells showed cytoplasmic shrinkage and loss of normal nuclear architecture. As result shows, the number of cell death increased with complexes with the high inhibitory effect on cell proliferation than the control cells.

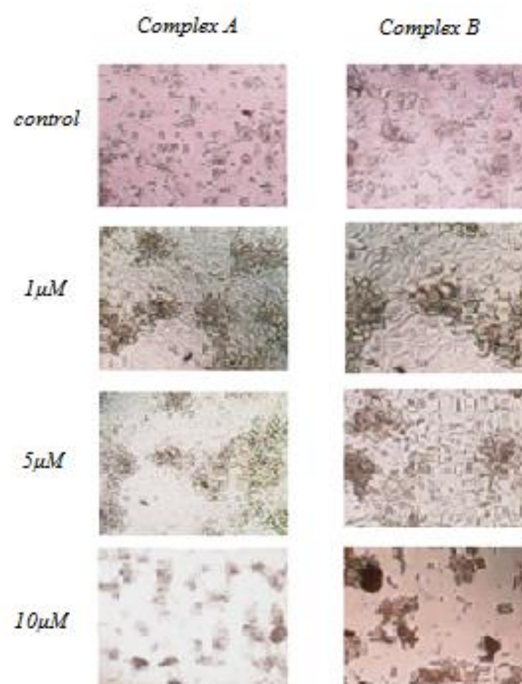

Figure 5. Morphological changes of cells after treatment with different concentration of complexes (**A**) and (**B**)

5. Conclusion

In summary, we presented the synthesized of two complexes and study for anticancer activity against human cancer cell lines using an MTT assay. Two compounds exhibited better anti-cancer activity against cancer cell lines compared to standard drug. The present study reveals that the human cancer cell lines are more sensitive to **(A)** complexes than **(B)** complexes. A major reason, importance of l,l0-phenanthroline in chemistry is its ability to adopt coordination with metals and chelators, also act as potential antitumor agents [22]. Migration studies revealed that Cu(II) complex are more active than Zn(II) complex.

Acknowledgments

We thank University for supporting this study.

References

[1] Sh. Dasari, P. B. Tchounwou, *Eur J Pharmacol.* (2015) 364.

[2] S. Bhattacharyya, A. Sarkar, S. Kr. Dey, A. Mukherjee, *J. Inorg. Biochem.* **140** (2014) 131.

[3] E. R. T. Tiekink, *Inflammopharmacol.*, **16** (2008) 138.

[4] R. Zhao, R. P. Planalp, R. Ma, Bryan T. Greene, B. T. Jones, M. W. Brechbiel, F. M. Torti, S. V. Torti, *Biochemical Pharma.*, **67(**2004) 1677.

[5] Chun-Y.Gao, X. Qiao, Zhong-Y. Ma, Zhi-G.Wang, J. Lu, Jin-L.Tian, Jing-Y.Xu, Shi-P. Yan, *Dalton Trans.,* **41** (2012)12220.

[6] J. L. Garća-Giménez, M. González-Álvarez, M. Liu-González, B. MacÍas, J. Borrás, G. Alzuet, *J. InorgBiochem.,***103** (2009) 923.

[7] S. Ramakrishnan, D. Shakthipriya, E. Suresh, V.S. Periasamy, M.A. Akbarsha, M. Palaniandavar, *Inorg. Chem.,* **50** (2011) 6458.

[8] E. Chalkidou, F. Perdih, I. Turel, D. P. Kessissoglou, G. Psomas, *J. Inorg. Biochem.*, **113** (2012) 55.

[9] L. Rink, P.Gabriel, *Proc. Nutr. Soc.*, 59 (2000) 541.

[10] B.A. Song, S. Yang, H.M. Zhong, *J. Fluorine Chem.*, **126** (2005) 87.

[11] G. Magueur, B. Crousse, S. Charneau. *J. Med. Chem.*, **47** (2004) 2694.

[12] S.A. Gamage, A.J. Spicer, G.W. Rewcastle, G.W, *J. Med. Chem.,* **45** (2002) 740.

[13] Y.B. Acheam Pong, A.A. Adimado, K.S. Patel, *Indian J. Pharm. Sci.,* **46** (1984) 207.

[14] Y. Dong, S.J. Berners-Price, D.R. Thorburn, *Biochem. Pharmacol.*, **53** (1997) 1673.

[15] M.P. Rigobello, L. Messori, Marcon. *J. Inorg. Biochem.*, **10** (2004) 1634.

[16] M.J. McKeage, L. Maharaj, S.J. Berners-Price, *Coord. Chem. Rev.,* **232** (2002) 127.

[17] A. Ghadersohi, D. Pan, Z. Fayazi, D.G. Hicks, J.S. Winston, F.Z. Li, *Breast Cancer Res.Treat.,***102** (2007) 19.

[18] A. Khaleghian, S. H. Ghaffari, Sh.Ahmadian, K. Alimoghaddam, A. Ghavamzadeh, *J. Cellular Biochemis.,* **115** *(*2014)1729.

[19] Y. Lv, J. Zhang, W. Cao, J. Ch. Juan, F. Zhang, Zh. Xu, *J. Photochem. Photobiology A: Chem.*, **188** (2007) 155.

[20] P.S. Lopes, D.A. Paixão, F.C.S. de Paula, A.M.D.C. Ferreira, J. Ellena, S. Guilardi, E.C. Pereira-Maia, W. Guerra, J. Molecul Struc., **1034** (2013) 84.

[21] N. Shahabadi, M. Falsafi, N. H. Moghadam, *J. Photochem. Photobiol. B Biol.,* **122** (2013) 45.

[22] A. Altomare, G. Cascarano, C. Giacovazzo and A. Gualardi, *J. Appl. Crystallogr.*, **26** (1993) 343.