

Progress in the synthesis and antitumor activity of copper(II)-based complexes

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Abstract: Copper is kind of life element, which plays an important role in the development of tumor and is used to predict the prognosis and treatment of tumor. Copper ions play a vital role in living organisms after forming copper(II)-based complexes with corresponding ligands. Copper(II)-based complexes are considered as an alternative to cisplatin-based antitumor drugs due to with the characteristics of low toxicity and low drug resistance. In this paper, the synthesis of Copper(II)-based complexes and their antitumor activities by inducing apoptosis, inhibiting inflammation and tumor angiogenesis in vivo and in vitro studies are reviewed, which is important for the development of highly efficient and low-toxicity metal-complexed antitumor drugs.

Keywords: Copper(II)-based complexes, Synthesis, Antitumor activity

1. Introduction

It is estimated that nearly 10 million people will die of cancer worldwide by 2020 [1]. Chemotherapy, radiation and surgical removal are the current treatment for cancer, although cisplatin is the most widely used antitumor drug, due to its toxic side effects, thus, we are need to develop highly effective and low-toxicity antitumor drugs [2]. Metallodrugs are a potential class of antitumor drugs in the treatment of tumors because of their unique drug effects, which are closely related to the selection of metal ions, oxidation states and the number of ligands [3]. Copper is vital to life and can induce apoptosis and autophagy leading to cell death through mechanisms such as reactive oxygen species accumulation and anti-angiogenesis, making a hot research topic in the field of tumor therapy [4]. In recent years, a number of copper(II)-based complexes of ligands have been synthesized, after synthesis, they exhibit higher biological activity than ligands and exert important pharmacological effects through synergistic effects, and have good antitumor activity against various cancer cells [5].

Therefore, copper(II)-based complexes are considered as anticancer drugs with potential development value. This paper highlights the research progress in recent years in designing and synthesizing copper(II)-based complexes with antitumor activity.

2. Synthesis of copper(II)-based complexes

2.1. Synthesis of copper(II)-based complexes using phenanthroline analogs as ligands

It is well known that 1,10'-phenanthroline is a classical chelating bidentate ligand for metal ions with a planar structure and two nitrogen atoms containing lone pair of electrons, which can be coordinated with metal ions to form a more stable chelating ring structure, and it has been selected as a ligand for metal complexes by many researchers [6]. Pivetta T et al have synthesized copper(II)-based complexes from 1,10'-phenanthroline, hydroquinone and basic copper carbonate by solution synthesis method and characterized by determination of cytotoxicity and DNA binding properties, it has been shown that copper(II)-based complexes with strong DNA affinity due to the presence of 1,10'-phenanthroline interact with DNA mainly through groove binding or electrostatic interactions, and exhibit higher antitumor activity than DDP [7].

The copper(II)-based complexes synthesized by our predecessors have provided valuable information for the development of novel drugs with a wider range of effects, but there are also many shortcomings, such as the lack of in vivo studies on the antitumor activity of complexes with pharmacological effects,

and we should continue to study the synthesis of 1,10'-phenanthroline with metallic copper and other aspects of activity on the basis of our previous work.

2.2. Synthesis of copper(II)-based complexes using pyridine as ligands

Angel N R et al who synthesized a series of copper(II)-based complexes with alkyl substituted polypyridine ligands, there are more studies reported in the literature on the synthesis and bioactivity of penta-coordinated and hexa-coordinated copper(II)-based complexes with pyridinyl ligands, but there are very few reports showing that alkyl-substituted polypyridine ligands have been used to synthesize four-coordinated distorted tetrahedral copper(II)-based complexes [8]. Filho J C et al have investigated the synthesis of Cu and 2,2'-bipyridine with naringenin and found that the synthesis method has higher yield and purity and demonstrated that the synthesized copper(II)-based complex inhibited the colony formation, proliferation and migration of MDA-MB-231 cells more efficiently than naringenin, which opens a new field for the study of novel anticancer drugs [9].

2.3. Synthesis of copper(II)-based complexes using Schiff base as ligands

Schiff bases are one of the most convenient and effective complexing compounds, synthesized by the condensation reaction of different primary amino compounds with aldehydes or ketones [10, 11]. The Schiff base contains nitrogen and other heteroatoms in the molecule, which makes the ligand have good complexing ability, and can be complexed with different transition metal ions to form a series of transition metal complexes with unique structural features and excellent specific properties. Daravath S A et al synthesized new three Schiff base ligands and their complexes and screened them for their biological activities and found that the metal complexes have higher activity than free Schiff base [12]. Thus, the reaction of Schiff bases with copper salts to form stabilized copper complexes can result in a synergistic effect, resulting in enhanced bioactivity of copper(II)-based complexes.

Xiu-Ying Qin et al designed and synthesized two binuclear copper(II)-based complexes, Cu-1 and Cu-2, with various anticancer functions by choosing N-(2)-L-alanyl-L-glutamine and β -alanyl Schiff base as ligands, and found that Cu-1 and Cu-2 had a clear preference for HeLa and HUVECs cells by MTT screening. The two cells were selected to study the anti-angiogenic properties of the two copper(II) complexes [13]. It was found that Cu-2 significantly inhibited the expression of two important signaling molecules downstream of the VEGFR2 pathway, p-AKT and p-ERK1/2 proteins, in both types of cells, resulting in inhibition of proliferation, angiogenesis, and induction of apoptosis. The above studies have shown that copper coordination with Schiff base ligands can exhibit specific biological activities.

2.4. Synthesis of copper(II)-based complexes using sulfonamide derivatives as ligands

Sulfonamides have attracted widespread interest as an important class of compounds containing sulfonamide structures [14]. Sulfonamide derivatives have a wide range of pharmacological activities such as antimicrobial [15], anti-inflammatory [16], antiviral [17] and anticancer [18]. Considering the specific antitumor and anti-inflammatory activities of sulfonamides and the multi-targeted antitumor activities of metal ions, the development of novel sulfonamide metal complexes with better performance as anticancer drugs is an important area of research. The presence of N and O donor atoms in the structure of sulfonamides offer numerous coordination options for metal ions, especially for the transition metals Cu^{2+} . Heterocyclic sulfonamides can function as ligands for the coordination of transition metals through donor atoms in the sulfonamide group ($-\text{SO}_2\text{-NH}-$) and/or other functional groups in the molecule [19, 20]. Cu is mainly coordinated through aromatic amino groups. Sulfamethazine contains three potential donor sites, namely N atoms of amino and sulfonamide groups and N atoms of dimethylpyrimidine ring, and has good ability to form complexes with metal ions [21, 22].

A related study synthesized and characterized two Cu^{2+} complexes $[\text{Cu}(\text{L}1)_2(\text{H}_2\text{O})_3]$ (C1) (HL1= N-(5-(4-methylphenyl)-[1,3,4]-thiadiazole-2-yl)-naphthalenesulfonamide) and $[\text{Cu}(\text{L}2)_2(\text{py})_2(\text{H}_2\text{O})]$ (C2) (HL2= N-(5-ethyl-[1,3,4]-thiadiazole-2-yl)-naphthalenesulfonamide), with two new ligands were synthesized [23]. Both complexes C1 and C2 were cytotoxic on tumor cell lines (HeLa, WM35), with the C2 being more cytotoxic than cisplatin, while the free ligands HL1 and HL2 were not significantly cytotoxic. It is shown that cytotoxicity is produced by the complexes and that chelation of the ligands with copper ions is essential for the cytotoxicity of the complexes.

3. Antitumor activity of copper(II)-based complexes

In recent years, the antitumor activity of copper(II)-based complexes has been extensively studied by many researchers at home and abroad. These compounds have obvious inhibitory effects on a variety of tumor cells, among which they are effective against lung, liver, colon, stomach, breast and other cancers. The results of these experiments indicate that copper(II)-based complexes have good antitumor activity.

3.1. Antitumor activity of copper(II)-based complexes in vitro assays

The multifunctional copper(II)-based complexes reported in previous studies by Xiu-Ying Qin et al [24-28] showed significant antitumor effects on cells by inducing apoptosis and inhibiting angiogenesis, such as the synthesis of two chiral tetranuclear copper ($\text{Cu}_4(\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_4\text{S})_4$) and $\text{Cu}_4(\text{C}_{12}\text{H}_{13}\text{NO}_2\text{SClBr})_4 \cdot 4\text{C}_2\text{O}$ (abbreviated TNCu-1 and TNCu-2) by using the copper salts with L-methioninol-derived Schiff base ligands. Both TNCu-1 and TNCu-2 exhibited good cellular uptake and significantly induced apoptosis, inhibited MDA-MB-231 cell proliferation, inhibited migration and metastasis, and inhibited angiogenesis and growth in vitro. At the same time, these complexes exhibit anticancer and antiangiogenic functions by activating important protein molecules in the VEGF/VEGFR2 signaling pathway and disrupting mitochondrial membrane potential and reactive oxygen species homeostasis. However, its anti-tumor properties and mechanisms in vivo have not been studied. Caruso Bavisotto et al human reported that copper bis(pyridyl)oxadiazole complexes interfere with the anti-apoptotic effect of Hsp60 by decreasing Hsp60 levels and preventing the formation of the Hsp60/pC3 complex, leading to the death of NCI-H292 cancer cells [29].

3.2. Antitumor activity of copper(II)-based complexes in vivo experiments

Xiao-Xiao Hou et al human reported the antitumor efficacy of MDA-MB-231 cells and xenografts in nude mice, synthesized two novel chiral tetranuclear copper(II)-based complexes and analyzed cytotoxicity, apoptosis, and tube formation [30]. In nude mice MDA-MB-231 cell xenograft tumors, TNCu-A inhibited proliferation, significantly suppressed the expression of the anti-apoptotic protein Bcl-2, up-regulated the expression of pro-apoptotic proteins Caspase-9 and Bax, and significantly induced apoptosis of MDA-MB-231 cells, as well as inhibited tumor angiogenesis by decreasing the density of vascular endothelial cells, inhibiting migration, and even partially inducing apoptosis. The expression of Caspase-9 and Bax significantly induced apoptosis of MDA-MB-231 cells and inhibited tumor angiogenesis by reducing vascular endothelial cell density, inhibiting migration and even partially inducing apoptosis. In addition, anti-tumor activity was further investigated by HE staining and immunohistochemistry.

Chakraborty et al [31] who studied a family of Schiff base ligands including two pyridine and one imine donors and various monodentate ligands. After screening for cytotoxic activity [$\text{Cu}(\text{Pyimpy})\text{Cl}_2$] was selected for further analysis of the anticancer properties of MCF7 breast cancer cells, and copper(II)-based complex was further assayed on a rat mammary tumor model. The results showed a 2.5-fold decrease in tumor growth rate after 1 month of treatment compared to DDP. In addition, the complex did not show any significant systemic toxicity; histologic analysis of the kidneys and liver showed no signs of toxicity.

The most common step after an in vitro assay is to assess its effectiveness in vivo, as animal models are preferred over in vitro cell culture. In vivo studies support different mechanisms of action to explain the anticancer activity of copper(II)-based complexes, including ROS generation, GSH/GSSG imbalance, cell cycle blockade, proteasome inhibition, DNA damage and apoptosis [32].

4. Conclusions

With the development of science and technology as well as the deepening of the study of copper(II)-based complexes, new synthesizing methods keep appearing which greatly promote the development of the preparation technology of copper(II)-based complexes, but it still needs to be studied in depth. Therefore, the design of anticancer copper complexes targeting multiple key pathways will help to circumvent drug resistance and improve therapeutic efficacy, and the study of antitumor activity of copper(II)-based complexes will lead to new drug candidates, which will provide good guidance for the role of other metal complexes in anticancer and for the development of novel antitumor drugs.

References

- [1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A et al. *Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries*. *CA Cancer J Clin*. 2021;71(3):209-49. doi:10.3322/caac.21660.
- [2] Cao BB, Li D, Xing X, Zhao Y, Wu K, Jiang F et al. *Effect of cisplatin on the clock genes expression in the liver, heart and kidney*. *Biochem Biophys Res Commun*. 2018;501(2):593-7. doi:10.1016/j.bbrc.2018.05.056.
- [3] Barry NP, Sadler PJ. *Exploration of the medical periodic table: towards new targets*. *Chem Commun (Camb)*. 2013;49(45):5106-31. doi:10.1039/c3cc41143e.
- [4] Jiang Y, Huo Z, Qi X, Zuo T, Wu Z. *Copper-induced tumor cell death mechanisms and antitumor theragnostic applications of copper complexes*. *Nanomedicine (Lond)*. 2022;17(5):303-24. doi:10.2217/nmm-2021-0374.
- [5] Gul NS, Khan TM, Chen M, Huang KB, Hou C, Choudhary MI et al. *New copper complexes inducing bimodal death through apoptosis and autophagy in A549 cancer cells*. *J Inorg Biochem*. 2020;213:111260. doi:10.1016/j.jinorgbio.2020.111260.
- [6] Sun M, Xin H, Wang KZ, Zhang YA, Jin LP, Huang CH. *Bright and monochromic red light-emitting electroluminescence devices based on a new multifunctional europium ternary complex*. *Chem Commun (Camb)*. 2003(6):702-3. doi:10.1039/b212467j.
- [7] Pivetta T, Trudu F, Valletta E, Isaia F, Castellano C, Demartin F et al. *Novel copper(II) complexes as new promising antitumour agents. A crystal structure of [Cu(1,10-phenanthroline-5,6-dione)2(OH2)(OCIO3)](ClO4)*. *J Inorg Biochem*. 2014;141:103-13. doi:10.1016/j.jinorgbio.2014.08.011.
- [8] Angel NR, Khatib RM, Jenkins J, Smith M, Rubalcava JM, Le BK et al. *Copper (II) complexes possessing alkyl-substituted polypyridyl ligands: Structural characterization and in vitro antitumor activity*. *J Inorg Biochem*. 2017;166:12-25. doi:10.1016/j.jinorgbio.2016.09.012.
- [9] Filho JC, Sarria AL, Becceneri AB, Fuzer AM, Batalhão JR, da Silva CM et al. *Copper (II) and 2,2'-bipyridine complexation improves chemopreventive effects of naringenin against breast tumor cells*. *PLoS One*. 2014;9(9):e107058. doi:10.1371/journal.pone.0107058.
- [10] Liu X, Hamon J-R. *Recent developments in penta-, hexa- and heptadentate Schiff base ligands and their metal complexes*. *Coordination Chemistry Reviews*. 2019;389:94-118. doi:https://doi.org/10.1016/j.ccr.2019.03.010.
- [11] More MS, Joshi PG, Mishra YK, Khanna PK. *Metal complexes driven from Schiff bases and semicarbazones for biomedical and allied applications: a review*. *Mater Today Chem*. 2019;14:100195. doi:10.1016/j.mtchem.2019.100195.
- [12] Daravath S, Rambabu A, Ganji N, Ramesh G, Anantha Lakshmi PV, Shivaraj. *Spectroscopic, quantum chemical calculations, antioxidant, anticancer, antimicrobial, DNA binding and photo physical properties of bioactive Cu(II) complexes obtained from trifluoromethoxy aniline Schiff bases*. *Journal of Molecular Structure*. 2022;1249:131601. doi:https://doi.org/10.1016/j.molstruc.2021.131601.
- [13] Qin XY, Yang LC, Le FL, Yu QQ, Sun DD, Liu YN et al. *Structures and anti-cancer properties of two binuclear copper complexes*. *Dalton Trans*. 2013;42(41):14681-4. doi:10.1039/c3dt51548f.
- [14] Zhao C, Rakesh KP, Ravidar L, Fang WY, Qin HL. *Pharmaceutical and medicinal significance of sulfur (S(VI))-Containing motifs for drug discovery: A critical review*. *Eur J Med Chem*. 2019;162:679-734. doi:10.1016/j.ejmech.2018.11.017.
- [15] Chohan ZH, Youssoufi MH, Jarrahpour A, Ben Hadda T. *Identification of antibacterial and antifungal pharmacophore sites for potent bacteria and fungi inhibition: indolenyl sulfonamide derivatives*. *Eur J Med Chem*. 2010;45(3):1189-99. doi:10.1016/j.ejmech.2009.11.029.
- [16] Chen P, Yang J, Zhou Y, Li X, Zou Y, Zheng Z et al. *Design, synthesis, and bioactivity evaluation of novel amide/sulfonamide derivatives as potential anti-inflammatory agents against acute lung injury and ulcerative colitis*. *Eur J Med Chem*. 2023;259:115706. doi:10.1016/j.ejmech.2023.115706.
- [17] Mandal MK, Ghosh S, Naesens L, Bhat HR, Singh UP. *Facile synthesis, antimicrobial and antiviral evaluation of novel substituted phenyl 1,3-thiazolidin-4-one sulfonyl derivatives*. *Bioorg Chem*. 2021;114:105153. doi:10.1016/j.bioorg.2021.105153.
- [18] Refat MS, Sharshar T, Elsabawy KM, El-Sayed MY, Adam AMA. *Synthesis, physicochemical characterization and anticancer screening of sulfa drug ruthenium complexes as anticancer agent*. *Journal of Molecular Liquids*. 2016;222:334-49. doi:https://doi.org/10.1016/j.molliq.2016.07.006.
- [19] Hangan A, Borodi G, Filip X, Tripon C, Morari C, Oprean L et al. *Structure of N-(5-ethyl-[1,3,4]-thiadiazole-2-yl)toluenesulfonamide by combined X-ray powder diffraction, ¹³C solid-state NMR and molecular modelling*. *Acta Crystallogr B*. 2010;66(Pt 6):615-21. doi:10.1107/s0108768110039327.
- [20] Hangan A, Borrás J, Liu-Gonzalez M, Oprean L. *Synthesis, Crystal Structures and Properties of [Cu(L1)2(py)2(H2O)](H2O) [HL1 = N-(5-ethyl-[1,3,4]-thiadiazole-2-yl)-toluenesulfonamidate] and*

[Cu(L2)2(py)2(H2O)] [HL2 = *N*-(5-ethyl-[1,3,4]-thiadiazole-2-yl)-benzenesulfonamidate]. *Zeitschrift für anorganische und allgemeine Chemie.* 2007;633(11-12):1837-41. doi:<https://doi.org/10.1002/zaac.200700214>.

[21] Tommasino J-B, Renaud FNR, Luneau D, Pilet G. Multi-biofunctional complexes combining antiseptic copper(II) with antibiotic sulfonamide ligands: Structural, redox and antibacterial study. *Polyhedron.* 2011;30(10):1663-70. doi:<https://doi.org/10.1016/j.poly.2011.03.033>.

[22] Chebout O, Trifa C, Bouacida S, Boudraa M, Imane H, Merzougui M et al. Two new copper (II) complexes with sulfanilamide as ligand: Synthesis, structural, thermal analysis, electrochemical studies and antibacterial activity. *Journal of Molecular Structure.* 2022;1248:131446. doi:<https://doi.org/10.1016/j.molstruc.2021.131446>.

[23] Hangan AC, Turza A, Lucaciu RL, Sevastre B, Páll E, Oprean LS et al. New Cu(+2) Complexes with *N*-Sulfonamide Ligands: Potential Antitumor, Antibacterial, and Antioxidant Agents. *Molecules.* 2022;27(10). doi:10.3390/molecules27103338.

[24] Zhang PL, Hou XX, Liu MR, Huang FP, Qin XY. Two novel chiral tetranucleate copper-based complexes: crystal structures, nanoparticles, and inhibiting angiogenesis and the growth of human breast cancer by regulating the VEGF/VEGFR2 signal pathway in vitro. *Dalton Trans.* 2020;49(18):6043-55. doi:10.1039/d0dt00380h.

[25] Qin XY, Wang YN, Liu HF, Luo ZH, Zhang PL, Li-Fang H et al. Anti-cancer activities of metal-based complexes by regulating the VEGF/VEGFR2 signaling pathway and apoptosis-related factors Bcl-2, Bax, and caspase-9 to inhibit angiogenesis and induce apoptosis. *Metallomics.* 2020;12(1):92-103. doi:10.1039/c9mt00248k.

[26] Qin XY, Wang YN, Yang XP, Liang JJ, Liu JL, Luo ZH. Synthesis, characterization, and anticancer activity of two mixed ligand copper(ii) complexes by regulating the VEGF/VEGFR2 signaling pathway. *Dalton Trans.* 2017;46(47):16446-54. doi:10.1039/c7dt03242k.

[27] Qin XY, Liu YN, Yu QQ, Yang LC, Liu Y, Zhou YH et al. Mixed-ligand mononuclear copper(II) complex: crystal structure and anticancer activity. *ChemMedChem.* 2014;9(8):1665-71. doi:10.1002/cmdc.201402060.

[28] Fan R, Wei JC, Xu BB, Jin N, Gong XY, Qin XY. A novel chiral oxazoline copper(II)-based complex inhibits ovarian cancer growth in vitro and in vivo by regulating VEGF/VEGFR2 downstream signaling pathways and apoptosis factors. *Dalton Trans.* 2023;52(33):11427-40. doi:10.1039/d3dt01648j.

[29] Caruso Bavisotto C, Nikolic D, Marino Gammazza A, Barone R, Lo Cascio F, Mocciano E et al. The dissociation of the Hsp60/pro-Caspase-3 complex by bis(pyridyl)oxadiazole copper complex (CubipyOXA) leads to cell death in NCI-H292 cancer cells. *J Inorg Biochem.* 2017;170:8-16. doi:10.1016/j.jinorgbio.2017.02.004.

[30] Hou XX, Ren YP, Luo ZH, Jiang BL, Lu TT, Huang FP et al. Two novel chiral tetranucleate copper-based complexes: syntheses, crystal structures, inhibition of angiogenesis and the growth of human breast cancer in vitro and in vivo. *Dalton Trans.* 2021;50(41):14684-94. doi:10.1039/d1dt02033a.

[31] Chakraborty A, Kumar P, Ghosh K, Roy P. Evaluation of a Schiff base copper complex compound as potent anticancer molecule with multiple targets of action. *Eur J Pharmacol.* 2010;647(1-3):1-12. doi:10.1016/j.ejphar.2010.08.003.

[32] Balsa LM, Baran EJ, León IE. Copper Complexes as Antitumor Agents: In vitro and In vivo Evidence. *Curr Med Chem.* 2023;30(5):510-57. doi:10.2174/0929867328666211117094550.