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SYNTHESIS, STRUCTURAL CHARACTERIZATION AND ANTITUMOR ACTIVITY EVALUATIONS OF COPPER COMPLEX WITH TETRAAZAMACROCYCLIC LIGAND

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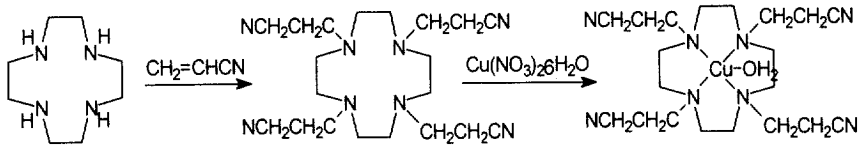
Abstract: Cu (II) complex with 1,4,7,10-tetrakis(2-cyanoethyl-)-1,4,7,10-tetraazacyclododecane was prepared and characterized by X-ray diffraction. Four nitrogen atoms of macrocyclic ligand and oxygen atom of water molecule defined a tetragonal pyramidal polyhedron surrounding the central copper atom. Preliminary pharmacological tests showed that it had antitumor activity against P388 and BEL-7404 cell lines *in vitro*. Also it exhibited perturbation effects to K562 tumor cell lines at G_0-G_1 stage and further studies showed that it can cleave supercoiled DNA(pBR 322) to nicked and linear DNA in aerobic condition.

Several complexes are biologically important species related to a wide aspects of life processes including oxygen transport[1], digestion[2] and gene transcription.[3] Effective anti solid tumor drug: cis-platine, has been found to make a covalent 1,2-intrastrand adducts at N7 of guanine base of DNA.[4] Screening new metal complexes with antitumor activity are of our research interests. It has been reported that the macrocyclic complexes with tetraazamacrocyclic ligand, such as Cyclen, Cyclam or bicyclam exhibit antitumor or anti-HIV virus activity[5], which stimulates researchers to do more exploitation on their derivatives. Recently new studies have been focused on the macrocyclic metal complexes with amine or carboxylates as chelators which have cleaving or binding interaction to DNA.[6] In this paper, we report the preparation and structural determination of copper complex with 1,4,7,10-tetrakis(2-cyanolethyl-)1,4,7,10-tetraazacyclododecane (L) and its antitumor activity. Also we evaluated the possible mechanism of its antitumor activity.

The copper complex with 1,4,7,10-tetrakis(2-cyanolethyl-)-1,4,7,10-tetraazacyclododecane (L) was synthesized in mild condition by Michael addition according to Scheme 1.[7,8]



Scheme 1 Synthetic route of Cu complex

PII: S0960-894X(99)00139-0

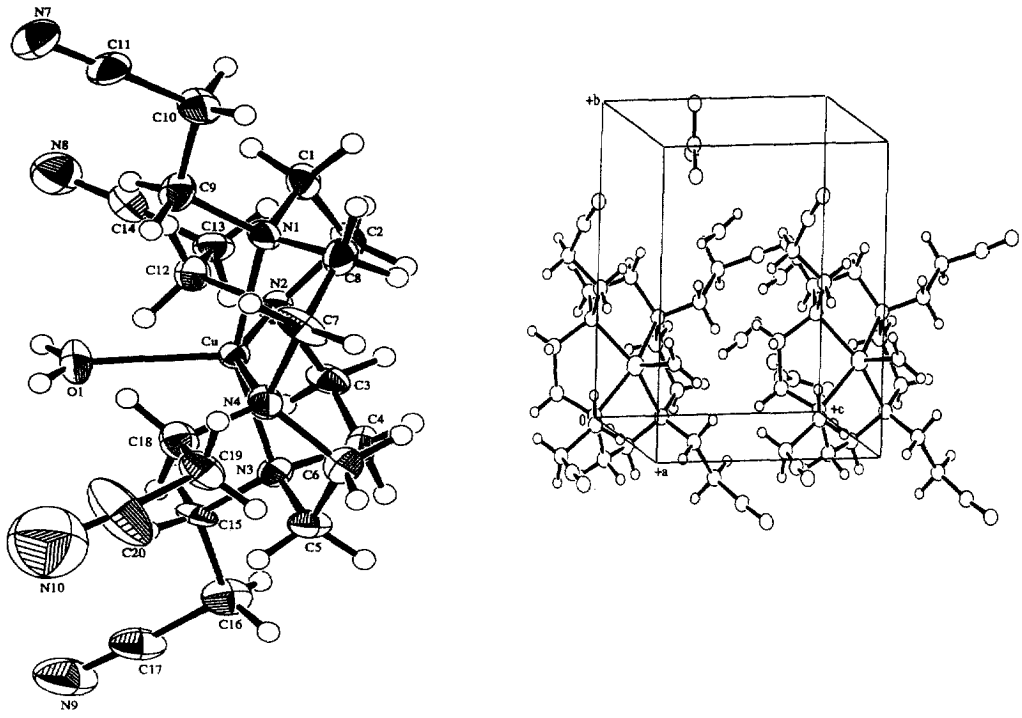


Figure 1. Structure of Cu complex (a) Molecular structure; (b) Unit cell packing.

The crystal structure of title complex was determined by X-ray diffraction technique, which supported that there was an ion pair existing in this complex. Cu atom coordinates to four nitrogen atoms of L and oxygen atom of water molecule, which defines a tetragonal pyramidal polyhedron. Four nitrogen atoms are coplanar with the mean deviation 0.0053 Å. Cu deviates from this plane 0.492 Å. The Cu-N bond lengths are almost equal in the range of 2.03(1)-2.08(1) Å. The bond length of Cu-O is 2.154(8) Å, which is slightly longer than Cu-N. Compared to the similar complex $Co(L)(NO_3)_2$,[8] one nitrate ion was introduced into the coordination sphere of Co atom as bidendate ligand to form six-coordinated octahedron. The reason may be that the ion radius of Co is larger than Cu ion, which makes Co atom coordination sphere more unsaturated and need higher coordination number. All four pendent groups at N residuals and a water molecule are on the same side. Fig. 1 gives the ORTEP drawing of the crystal structure.

Antitumor Activity of Cu complex against P388 and BEL-7404 cell lines ***in Vitro***

The antitumor activity of this complex was measured on P388 and BEL-7404 cell lines using MTT[9] and SRB[10] assay, respectively. The percentage inhibition was 100% and 96.4% at 10^{-4} and 10^{-5} $molL^{-1}$ to P388 cell lines, respectively. But when it was diluted to 10^{-6} $molL^{-1}$ the percentage inhibition decreased sharply to zero. On the contrary, the complex still kept its antitumor activity to BEL-7404 cell lines even at 10^{-8} $molL^{-1}$. The percentage inhibition of Cu complex to BEL-7404 was shown in Fig. 2. From Fig. 2 it can be observed that the inhibitory activity of Cu complex increased with the concentration of dilution in the range of 10^{-4}-10^{-6} $molL^{-1}$ and lost the correlation when the concentration of this complex exceeded 10^{-6} $molL^{-1}$. This may suggest that 10^{-6} $molL^{-1}$ is the optimal concentration against the BEL-7404 cell lines.

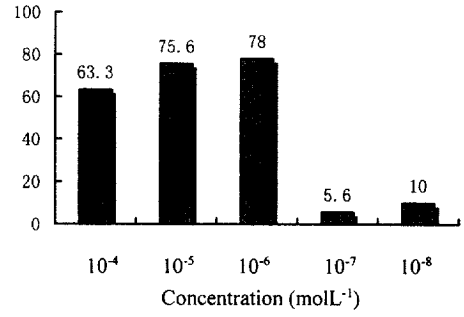


Figure 2. The percentage inhibition of BEL-7404 tumor cell lines

Perturbation of cell cycle progression

The percentage of K562 cells on each phase of cell cycle subsequent to treatment with 10 and 50 $\mu molL^{-1}$, respectively, of Cu complex for 24h were shown in Fig. 3. Cu complex caused an increase in the proportion of cells in G_1 phase (from 26.6% to 40.5%) accompanied by a decrease in S phase (from 54.4% to 46.9%), and slight changes in G_2-M phase.

The results showed that the complex apparently interacts with DNA and exhibits the perturbation of K562 tumor cell lines at G_0-G_1 phase thus would affect the DNA synthesis in cell cycle. This is more or less similar with the action of cis-platine complex. In our experiment no induction of tumor cell apoptosis was observed.

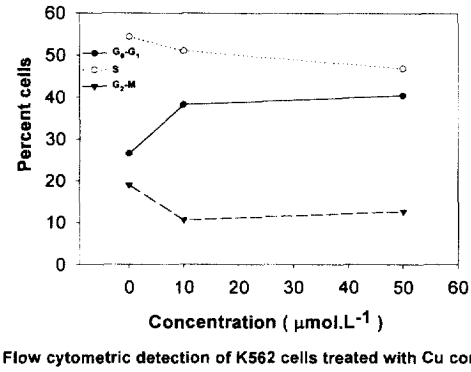


Figure 3 The perturbation of Cu complex to cell cycle

Measurements on the interaction to pBR 322 DNA

DNA cleavage was analyzed by monitoring the conversion of supercoiled DNA (Form I) to nicked DNA (Form II) and linear DNA (Form III) in aerobic conditions. Fig.5 showed the results performed under aerobic conditions in the presence of 2-mercaptopropionic acid (MPA) as a reducing agent. As showed in Fig. 4. the supercoiled pBR 322 DNA have turned into nicked DNA and linear DNA in line.

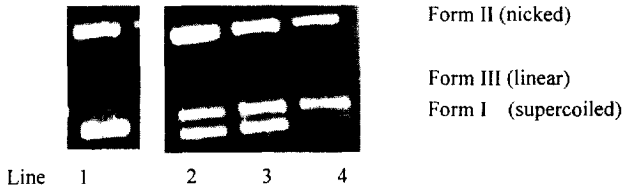


Figure 4. Cleavage of pBR 322 DNA to nicked and linear DNA

Line1: DNA alone; Line2: 10μmolL^{-1} Cu complex + DNA; Line3: 50μmolL^{-1} Cu complex + DNA; Line4: 100μmolL^{-1}Cu complex + DNA.

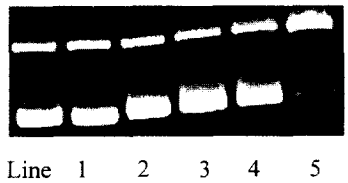


Figure 5. Cleavage of pBR 322 DNA to nicked DNA under MPA condition

Line1: DNA alone; Line2: MPA + DNA; Line3:10μmolL^{-1} Cu complex + DNA + MPA; Line4: 20μmolL^{-1} Cu complex + DNA + MPA; Line5: 40μmolL^{-1} Cu complex + DNA + MPA; Line6: 100μmolL^{-1} Cu

complex + DNA + MPA.

Interestingly, we have found that the Cu complex can cleave the supercoiled DNA to nicked and linear DNA at the same time. From Fig.4 it can be observed that the circular supecoiled DNA converted to nicked DNA via single-strand cleavage (lane 2 to 4). With the increase of complex concentration, the supercoilled DNA decreased and finally completely converted to nicked and linear DNA.

In order to clarify the possible mechanism of this cleavage, we repeated the experiment in the presence of 2-mercaptopropionic acid (MPA) as reducing agent. In this case linear DNA was not formed. No apparent change was observed in lines 2-5. But in Line 6 the supercoiled DNA almost completely converted to nicked DNA. This result is similar to the Cu-salen complex as chemical nuclease reported by Bailly et al.[11] They suggested the presumable reactive species existing in this cleavage were oxygen-based radicals. Further investigation on this cleaving mechanism is in progress.

Acknowledgements

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12. Crystallographic data for Cu complex $C_{20}H_{38}N_{10}O_9Cu$: space group P1, triclinic, Mr=636.13, a=9.156(4)Å, b=10.128(8)Å, c=8.006(5)Å, α=96.60(6)°, β=95.79(5)°, γ=70.90(5)°, V=695.3(8)Å^3. Z=1, R=0.063, Rw=0.067, GOF=1.70. The data were collected on a Rigaku AFC 7R diffractometer at 293K. The structure was solved by direct methods and expanded using Fourier techniques. The data were corrected for Lorenz and polarization effects. Atomic coordinates, bond angles, bond lengths and thermal parameters assisted to this complex have been deposited at the Cambridge Crystallographic Data Center in CIF file. CCDC number: 112691.