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基于对异丙基甲苯和二甲基双胍的对称双核钌(Ⅱ)配合物的合成及其体外抗癌活性

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摘要: 在碱性水溶液中合成了一种对称双核桥联配合物$(NH_4)_2[Ru(Cym)(L)]_2Cl_2\cdot 4H_2O$ (**1**)(Cym=对异丙基甲苯,H_2L=1,1-二甲基双胍)。采用红外光谱、核磁共振谱和X射线单晶衍射进行了结构表征,结晶水数目由热重分析法得出。采用MTT法测定了其对4种人癌细胞系HepG-2、A549、Hela、MCF-7的细胞毒性,以临床用药顺铂为对照,结果表明该配合物对HepG-2(肝细胞癌,HCC)的作用与顺铂相当。

关键词: 钌配合物; 晶体结构; 抗癌活性; 顺铂
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Symmetrical Diruthenium(Ⅱ) Complex Based on 1-Isopropyl-4-methylbenzene and Dimethylbiguanide: Synthesis and Anticancer Activity *in Vitro*

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Abstract: A novel symmetrical dinuclear bridging complex $(NH_4)_2[Ru(Cym)(L)]_2Cl_2\cdot 4H_2O$ (**1**) (Cym=*p*-cymene=1-isopropyl-4-methylbenzene, H_2L=1,1-dimethylbiguanide) was obtained by treatment of the precursor $[Ru(Cym)Cl_2]_2$ with metformin hydrochloride. In aqueous base solution, deprotonation of the proligand (1,1-dimethylbiguanide) occured and the corresponding neutral ruthenium complex **1** was obtained. The structure of complex **1** has been established by FT-IR and NMR spectroscopy and single-crystal X-ray diffraction analysis. The number of crystal water was obtained by thermogravimetric analysis. The inhibition of cell proliferation activity against four human cancer cell lines (HepG-2, A549, Hela, MCF-7) of complex **1** relative to cisplatin was measured by MTT method *in vitro*. Notably, the novel complex displayed comparable potency toward HepG-2 (hepatocellular carcinoma, HCC) compared to cisplatin. CCDC: 2058702.

Keywords: ruthenium complex; crystal structure; anticancer activity; cisplatin

0 Introduction

Today platinum-based complexes including cisplatin, carboplatin, and oxoplatin are potent antitumor agents, and they are precious class of antitumor metallotherapeutics commonly prescribed in the clinic. However, the platinum-based chemotherapeutics are far from ideal: they cause

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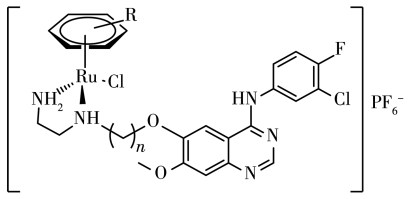
drug resistance and a range of side effects, by which their therapeutics value is curtailed. Serious adverse drug reactions in hospitalized patients rank as the 4th~6th leading cause of death, highlighting the desire need for safer and selective metallotherapeutics[1].

Ruthenium - based antineoplastic agents are among the most investigated non-platinum metallodrugs, and they are appealing candidates that have certain merits over platinum - based therapeutics. Ruthenium anticancer agents show high selectivity for tumor cell lines, and low cytotoxicity to normal cells. Furthermore, they have cytotoxicity against some cisplatin resistant cell lines. The reported mechanisms of action by which the ruthenium therapeutics work include acting as protein kinase inhibitors, DNA binding, protein binding, apoptosis, and so on[2]. However, the oncotherapeutic value of ruthenium based anticancer agents can be influenced by the coordination mode of ligands. Typically, the N,N-, S,O-, S,N-, C,N-bidentate donating ligands generally yield potent antineoplastic metallodrugs[3].

In more recent times, research groups have endeavored to tether bioactive ligands to ruthenium center. The strategy may obtain a new molecular entity (NMEs) having pharmacokinetic and therapeutic profiles distinct to the free ligands themselves. The metal center and bioactive ligand may exert synergistic effect, endowing the new molecular with multitargeted and reduced toxicity properties of which many chemotherapeutics lack. For example, Wang et al.[4] demonstrated that when the 4-anilinoquinazolines (4-AQs) epidermal growth factor receptor (EGFR) inhibitor ligands (**2~7**) (Fig. 1) were incorporated to the ruthenium center, their ability to induce early stage apoptosis was enhanced compared to the ligands alone, while also retaining the DNA binding capacity ascribed to the ruthenium(Ⅱ) center.

Metformin (1,1-dimethylbiguanide), a derivative of biguanide, has been prescribed for the treatment of type 2 diabetes for over 30 years. It is a



(n = 2), arene = benzene (**2**)
(n = 2), arene = *p*-cymene (**3**)
(n = 2), arene = bipheny (**4**)
(n = 3), arene = benzene (**5**)
(n = 3), arene = *p*-cymene (**6**)
(n = 3), arene = bipheny (**7**)

Fig.1 Chemical structures of organoruthenium(Ⅱ) complexes incorporating 4-AQs (**2~7**), analogues of the EGFR inhibitor gefitinib

magic molecule that exhibits antimalarial biological properties[5], and recently, it has been assessed for therapeutic treatment of pain, anxiety, and memory disorders. Furthermore, there are concrete evidences that metformin has the ability to reduce the incidence of overall cancer, liver cancer, pancreatic cancer, colorectal cancer and breast cancer as well as the mortality of overall cancer, liver cancer and breast cancer. Possible modes of action could be ascribed to anti-inflammatory effects, antioxidant effects and killing of cancer stem cells, suppressing tyrosine kinase receptors such as HER1 and HER2, inhibiting cancer cells by initiating the pivotal LKB1/AMPK/mTOR axis which regulates energy metabolism and protein synthesis of the cell[6].

In light of above, we want to incorporate the pharmacophore of metformin into ruthenium center to get a new ruthenium complex and test its cytotoxicity against cancer cell lines *in vitro*.

1 Experimental

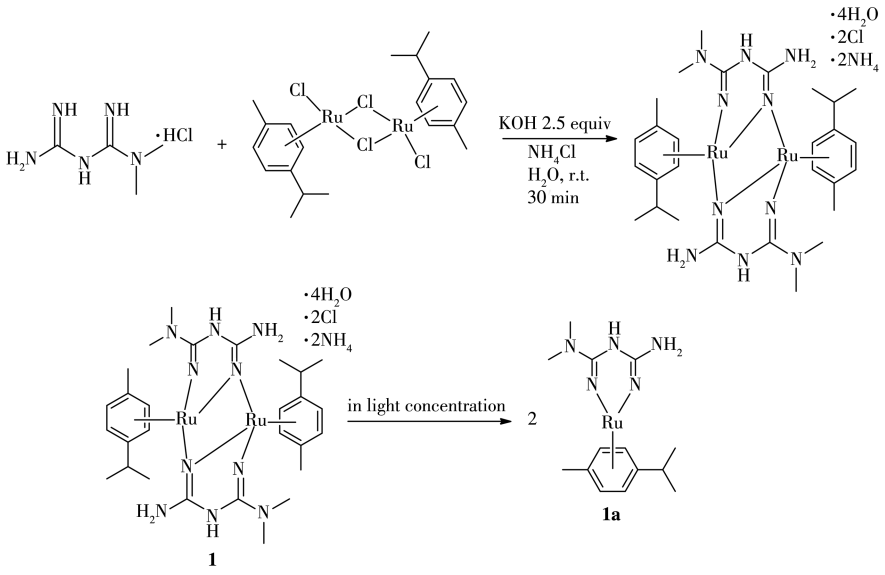
1.1 Materials and methods

NMR spectra were recorded on Bruker DRX-400 instrument with TMS as internal standard. Chemical shifts were reported as δ values, relative to internal DMSO (δ 2.50 for ^{1}H NMR and 39.50 for ^{13}C NMR). ESI-MS spectrum was determined on API QSTAR Pulsari spectrometer. X-ray diffraction was obtained by APEX DUO. Infrared spectra

were recorded on a FT-IR spectrometer with KBr pellets. Yield referred to spectroscopically (^{1}H NMR) homogeneous material. Unless otherwise noted, materials obtained from commercial suppliers were used without further purification.

1.2 Synthesis of the complex

Metformin hydrochloride (330 mg, 2 mmol), dichloro(*p*-cymene)ruthenium(Ⅱ) dimer (613 mg, 1 mmol), massive KOH (448 mg, 8 mmol), NH_4Cl (107 mg, 2 mmol) and distilled H_2O (40 mL) were placed into the reaction vessel, sealed and stirred for 30 min at room temperature, during which time the reation turned dark red. Then the magnetic stir bar was removed, and the mixture was crystallized in refrigerator (4 ℃) for ten days furnishing the desired complex **1**, $(NH_4)_2[Ru(Cym)(L)]_2Cl_2\cdot 4H_2O$ (Cym=*p*-cymene=1-isopropyl-4-methylbenzene, H_2L=1,1-dimethylbiguanide), as a dark red triclinic crystal. Yield: 483 mg, 65%. Anal. Calcd. for $C_{28}H_{62}Cl_2N_{12}O_4Ru_2$(%): C, 37.21; H, 6.91; N, 18.59. Found(%):C, 37.42; H, 6.91; N, 18.36. ESI-MS: *m*/*z* 364.0, Calcd. for [**1a**+H]$^+$: 364.10 (indicating the formation of **1a** in light methanol solution: monomeric species of **1**, Scheme 1). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.80 (d, *J*=6.08 Hz, 4H), 5.68 (d, *J*=6.08 Hz, 4H), 5.67 (s, 1H), 5.45 (s, 4H), 5.06 (s, 2H), 2.94 (s, 12H), 2.78~2.67 (m, 2H), 2.15 (s, 6H), 1.15 (d, *J*=6.88 Hz, 12H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 159.56, 158.27, 109.15, 104.58, 87.57, 86.49, 37.85, 29.76, 22.00, 17.53. IR (KBr, cm^{-1}): 3 340, 3 278, 3 200, 3 155, 2 961, 2 925, 2 869, 1 617, 1 584, 1 493, 1 431, 1 402, 1 303, 1 215, 1 110, 1 025, 865, 803, 775, 706, 671, 520, 470.



Scheme 1　Synthesis route of complex **1** and formation of **1a**

1.3 Crystal structural determination

The crystal data for complex **1** was collected on APEX DUO using Cu *Kα* radiation. Absorption corrections were applied using multi-scan methods. This structure was solved by direct methods and refined by full-matrix least-squares using the SHELXL-2018/1[9]. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located geometrically and treated as a riding atom. The diffraction data and selected bond lengths and bond angles are listed in Table 1 and 2, respectively.

CCDC: 2058702.

1.4 Cancer cell growth inhibition assay

1.4.1 Cell culture

Cancer cell lines (HepG-2, Hela, A549, MCF-7) were routinely grown in Dulbecco′s modified Eagle′s medium (DMEM/H) containing 10% heat-inactivated fetal bovine serum (FBS) at 37 ℃ in 5% (*V*/*V*) CO_2. Cell suspensions were seeded in 96-well plates at a density of 8 000 cells per well for

Table 1 Crystallographic data of complex 1

Empirical formula	$C_{28}H_{62}Cl_2N_{12}O_4Ru_2$	F(000)	468
Formula weight	903.93	Crystal size / mm	0.670×0.550×0.350
Temperature	100(2) K	θ range for data collection / (°)	4.21~53.93
Wavelength	0.154 178 nm	Index ranges	$-12 \leqslant h \leqslant 12$, $-13 \leqslant k \leqslant 12$, $-11 \leqslant l \leqslant 14$
Crystal system	Triclinic	Reflection collected	15 524
Space group	$P\bar{1}$	Independent reflection	4 046 (R_{int}=0.064 4)
a / nm	0.972 87(3)	Completeness / %	98.0
b / nm	1.018 26(3)	Refinement method	Full-matrix least-squares on F^2
c / nm	1.175 98(3)	Data, restraint, parameter	4 046, 31, 234
V / nm^3	0.945 99(5)	Goodness-of-fit on F^2	1.151
Z	1	Final R indices [$I>2\sigma(I)$] R_1, wR_2	0.082 3, 0.207 5
D_c / (Mg·m^{-3})	1.587	R indices (all data)	R_1=0.082 4, wR_2=0.207 6
Absorption coefficient / mm^{-1}	8.174	Largest diffraction peak and hole / (e·nm^{-3})	3 933 and −2 519

Table 2 Selected bond distances (nm) and angles (°) for complex 1

C15—Ru1	0.222 3(5)	C16—Ru1	0.221 5(5)	C21—Ru1	0.219 8(5)
C25—Ru1	0.219 2(5)	C47—Ru1	0.214 6(6)	C49—Ru1	0.218 2(5)
N7—Ru1	0.207 3(4)	N11—Ru1	0.212 2(5)	C31—N11	0.140 5(7)
N23—C31	0.130 2(7)	C32—N7	0.131 7(7)	C2—C15	0.150 9(8)
C45—C16	0.150 9(8)	N17—C31	0.134 1(7)	N20—C32	0.135 6(7)
C16—Ru1—C15	80.921(2)	C21—Ru1—C15	68.135(2)	C25—Ru1—C15	37.504(2)
C47—Ru1—C15	37.663(2)	C49—Ru1—C15	67.961(2)	N7—Ru1—C15	111.547(2)
N11—Ru1—N7	81.954(18)	C31—N11—Ru1	114.033(3)	N23—C31—N11	126.375(5)
C32—N7—Ru1	127.668(4)	C2—C15—Ru1	132.183(4)	C45—C16—Ru1	131.227(4)
N17—C31—N11	115.856(5)	N20—C32—N7	121.285(5)		

12 h. Then, a fresh complete drug-containing medium with 5% FCS was added, and incubated for another 48 h.

1.4.2 Determination of IC_{50} values

An MTT assay was used to evaluate cell viability. Following drug exposure (the required concentration varied from 0~100 μg·mL^{-1}), MTT solution (final concentration 0.5 mg·mL^{-1}) was added to each well, and staining for 4 h. The optical density, which was directly proportional to the number of surviving cells, was measured at 490 nm using microplate reader (Molecular Devices, Inc.). The percentages of surviving cells were calculated by using absorbance ratios of drug-treated cells versus untreated cells. The IC_{50} values for the inhibition of cell growth were calculated by fitting the plot of the logarithmic percentage of surviving cells against the logarithm of drug concentration with a linear regression function.

2 Results and discussion

2.1 Synthesis

The dinuclear complex **1** has been prepared by reaction between the precursor complex $[Ru(Cym)Cl_2]_2$ with the proligand metformin hydrochloride in aqueous base condition at room temperature. In alkaline solution, deprotonation of the proligand (1, 1-dimethylbiguanide) occured and the corresponding neutral ruthenium complex **1** was obtained by exchange reaction. Good dark red triclinic crystal suitable for X-ray diffraction studies was grown from stock solution. Orgnometallic ruthenium(Ⅱ) complex encompassing *N*,*N*-bidentate ligand of 1,1-dimethylbiguanide is stable, because the vacant *d* orbitals of the metal in oxidated state may overlap with the filled π orbitals of the ligand

which are considered as strong σ- and π-donating system. Interestingly, in light concentration of methanol solution, compound **1** was converted into compound **1a**, indicating the formation of monomeric species, that can be reflected in the ESI-MS spectrum.

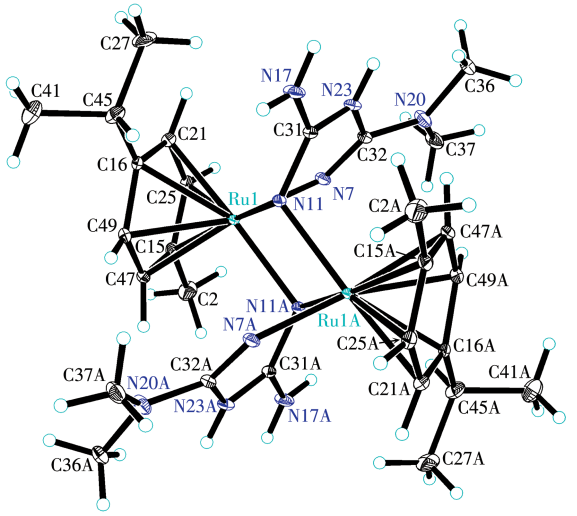
2.2 Spectroscopy

The infrared spectrum of the complex exhibited an intense absorption band in a range of 3 100~3 500 cm^{-1} assignable to the stretching vibration of the NH groups. It is probable that inter- or intramolecular hydrogen bonds overlap with NH vibrations and are responsible for this broad band. A set of strong bands observed in a range 1 400~1 700 cm^{-1} may be attributed to C═N stretch and NH deformation. A new band appearing at 1 320~1 220 cm^{-1} is assigned to ring vibration and supports the formation of a chelate ring.

The proton NMR spectra of the metal complex, recorded in DMSO-d_6 solution, showed a downfield shift of the aromatic protons resonances with respect to those of the dichloro(*p*-cymene) ruthenium(Ⅱ) dimer, while aliphatic protons did not undergo significant chemical shifts. The same pattern was also observed in the carbon NMR spectra of the complex. This fact may be attributed to π-electron delocalisation on the chelate ring. A total of four singlets were observed, among them δ=5.45 (s, 4H), 5.06 (s, 2H), 2.94 (s, 12H) are attributable to 2(—NH_2), 2(—NH—), 2$(CH_3)_2$N— protons, respectively, δ =2.15 (s, 6H) is assignable to 2 (CH_3—Ar). The carbon NMR spectra appearing at δ=159.56, 158.27 are assignable to C═N carbons.

2.3 Crystal structure

An ORTEP representation of the coordination environment of complex **1** including the atom labeling scheme is shown in Fig.2. Single-crystal X-ray structure analysis reveals that complex **1** crystallizes in the triclinic system space group $P\bar{1}$. The asymmetric unit (Fig. 3) of **1** contains a crystallographically unique Ru(Ⅱ) ion, one L^{2-} block, one *p*-cymene moiety, a lattice chloride anion, an ammonium cation and two water molecules. View of the pack drawing of **1** is shown in Fig.4. The Ru1—N7 and Ru1—N11 bond length are 0.207 3(4), 0.212 2(5) nm, respectively, significantly longer than the values reported in the dinuclear [(μ-abpy){Ru(acac)$_2$}$_2$], due to the effect of the π-accepting ancillary ligand, Cym. The N7—Ru1—N11 angle is 81.954(18)°, close to the values found in some reported Ru (Ⅱ) compounds[7]. The average



Water molecules, ammonium and chloride ions are omitted for clarity

Fig.2 Drawing of complex **1** with the atom-labelling scheme with 30% probability displacement ellipsoids

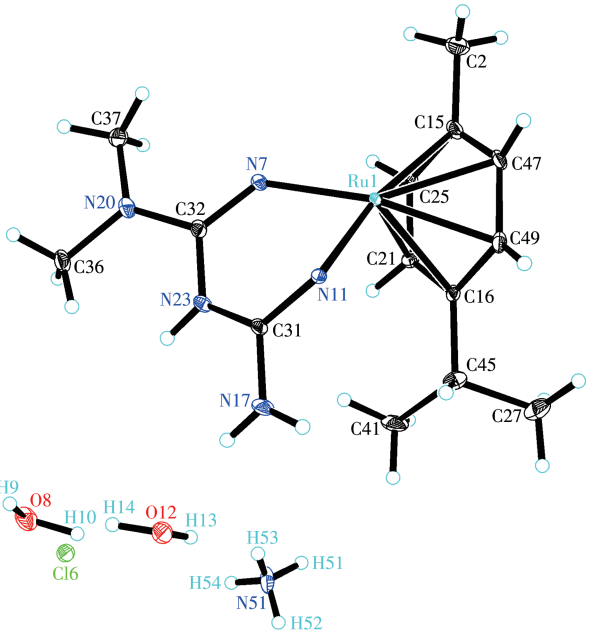


Fig.3 Drawing of asymmetric unit of complex **1** with 30% probability displacement ellipsoids

Ru—C(Cym) bond distance of 0.219 3 nm is comparable to the values reported in other {Ru-Cym} complexes[8].

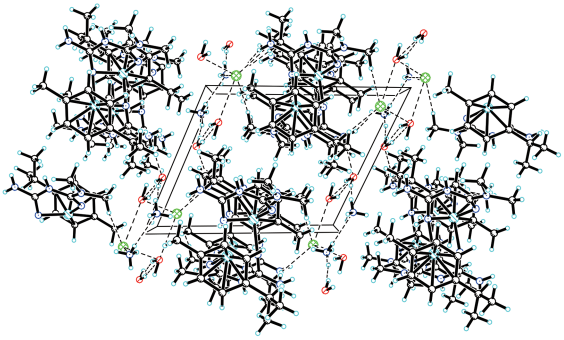


Fig.4 Pack drawing of complex **1** with hydrogen-bonds shown as dashed lines

2.4 *In vitro* proliferation assays

The anticancer efficacies of **1** *in vitro* were assessed by an MTT assay in four human cancer cell lines, including HepG-2 (hepatocellular carcinoma, HCC), A549 (lung cancer), Hela (cervical), MCF-7 (breast). The clinically prescribed platinum based therapeutic cisplatin was tested as well for comparison. These cells were treated with various concentrations of complex **1** and cisplatin for 48 h, and the IC_{50} values are given in Table 3. From the data, it is obvious that complex **1** displayed comparable cytotoxic potency toward HepG-2 (hepatocellular carcinoma, HCC) compared to cisplatin.

Table 3 IC_{50} values of metal complexes cisplatin and 1 against HepG-2, A549, Hela and MCF-7 cell lines for 48 h

Complex	IC_{50} / (μmol·L^{-1})			
	HepG-2 (HCC)	A549 (lung)	Hela (cervical)	MCF-7 (breast)
Cisplatin	118.98±100.449 9	133.31±8.565	115.81±7.998	129.58±23.596
1	105.79±25.37	509.35±117.67	474.69±147.87	792.59±171.87

3 Conclusions

In summary, a novel symmetrical dinuclear bridging complex encompassing the framework of metformin has been synthesized, characterized and tested against four cancer cell lines (HepG-2, A549, Hela, MCF-7). It is worth noting that the complex shows similar cytotoxicity toward hepatocellular carcinoma cell line to cisplatin. In most countries, hepatocellular carcinoma (HCC) occupies 70%~85% of all cases of liver cancer. Drug companies are pushing hard for ideal drugs to cure advanced HCC, though blows kept coming. We report herein a novel ruthenium complex which would provide good handle for further development.

Supporting information is available at http://www.wjhxxb.cn

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