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研究简报

新型钯配合物[Pd(Phen)(TsserNO)]·H_2O 的合成、晶体结构和体外抗肿瘤活性

张金超* 李路伟 王立伟 张芳芳 秦新英 李小六
(河北大学化学与环境科学学院,河北省化学生物学重点实验室,保定 071002)

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Synthesis, Crystal Structure and Cytotoxicity of a Novel Palladium(Ⅱ) Complex [Pd(Phen)(TsserNO)]·H_2O

ZHANG Jin-Chao* LI Lu-Wei WANG Li-Wei ZHANG Fang-Fang QIN Xin-Ying LI Xiao-Liu
(*College of Chemistry & Environmental Science, Chemical Biology Key Laboratory of Hebei Province, Hebei University, Baoding, Hebei* 071002)

Abstract: A novel palladium(Ⅱ) complex [Pd(Phen)(TsserNO)]·H_2O (Phen=1,10-phenanthroline; TsserNO=4-toluenesulfonyl-*L*-serinate dianion) has been prepared and structurally characterized, the cytotoxicity *in vitro* has also been investigated by MTT and SRB assays. The complex crystallizes in the monoclinic system, space group $P2_1$ with cell parameters a=0.618 64(14) nm, b=1.768 9(4) nm, c=0.990 2(2) nm, β=102.392(4)°, V=1.058 3(4) nm^3 and Z=2. The complex had selectivity against HL-60, BGC-823, Bel-7402 and KB cells lines, its cytotoxicity is equal to that of cisplatin against BGC-823 and Bel-7402 cells lines, however it is less potent than cisplatin against HL-60 and KB cell lines. CCDC: 756220.

Key words: antitumor; palladium(Ⅱ) complex; crystal structure

Metals, in particularly, transition metals offer potential advantages over the more common organic-based drugs, including a wide range of coordination numbers and geometries, accessible redox states, ′tuneability′ of the thermodynamics and kinetics of ligand substitution. Medicinal inorganic chemistry is a thriving area of research, which was initially fueled by the discovery of cisplatin[1-2]. By now, cisplatin has become one of the most commonly used compounds for the treatment of a wide spectrum of human malignancies. Unfortunately, cisplatin has several major drawbacks. Common problems include cumulative toxicities of nephrotoxicity, ototoxicity and peripheral neuropathy. In addition to the serious side effects, the therapeutic efficacy of cisplatin is also limited by inherent or treatment-induced resistant tumor cell sub-populations.

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*通讯联系人。E-mail:jczhang6970@yahoo.com.cn
第一作者:张金超,男,41 岁,博士,教授;研究方向:无机药物化学和细胞生物无机化学。

Driven by the impressive impact of cisplatin on cancer chemotherapy, great efforts have been made to develop metal-based anticancer drugs with improved pharmacological properties[3].

On the basis of the structural and thermodynamic analogy between platinum(Ⅱ) and palladium(Ⅱ) complexes, there is also much interest in the study of palladium(Ⅱ) derivatives as potential anticancer drugs[4-6]. In the 1980′s, Puthraya et al. synthesized several Pd(Ⅱ) complexes with amine ligands which have shown promising anticancer activity. These complexes have good water solubility, reduced nephrotoxicity, and potential future drugs against gastrointestinal tumors[7-8]. Numerous palladium complexes with aromatic *N*-containing ligands were shown to be effective against tumors in human and experimental tumors in animals[9]. Ligands like pyridine, quinoline, 1,10-phenanthroline and their derivatives have been widely used. Because of their planar nature, they have the ability to participate as a DNA intercalator. Some palladium compounds with *L*-amino acid ligands, $[Pd(bipy)(AA)]^{n+}$, (where bipy is 2,2′-bipyridine; AA is an anion of *L*-cysteine, *L*-aspartic acid, *L*-glutamic acid, *L*-methionine, *L*-histidine, *L*-arginine, *L*-phenylalanine, *L*-tyrosine, or *L*-tryptophan, and n=0 or 1) have been synthesized, some complexes have shown promising anticancer activity[4]. In this paper, the synthesis, crystal structure and cytot-oxicity of a novel palladium(Ⅱ) complex [Pd(Phen)(TsserNO)]·H_2O (Phen=1,10-phenanthroline; TsserNO=4-toluenesulfonyl-*L*-serinate dianion) was reported for the first time.

1 Experimental

1.1 Materials and instruments

4-toluenesulfonyl chloride and $K_2[PdCl_4]$ were of chemical grade, 1,10-phenanthroline and L-serine were of analytical grade. RPMI-1640 medium, trypsin and fetal bovine serum were purchased from Gibco. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), SRB (sulforhodamine B), benzylpenicillin and streptomycin were from Sigma. Four different human carcinoma cell lines: HL-60 (immature granulocyte leukemia), Bel-7402 (liver carcinoma), BGC-823 (gastrocarcinoma) and KB (nasopharyngeal carcinoma) were obtained from American Type Culture Collection.

Elemental analysis were determined on a Elementar Vario EL Ⅲ elemental analyzer. The ^{1}H NMR spectra were measured on a Bruker AV Ⅲ 600 NMR spectrometer in dimethyl sulfoxide-d_6 with solvent peaks as references. X-ray single crystal structure was performed on a Bruker SMART APEX Ⅱ CCD diffractometer. The optical density (OD) at 570 nm was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA).

1.2 Preparation of [Pd(Phen)(TsserNO)]·H_2O

Tsser (4-toluenesulfonyl-*L*-serine) and [Pd(Phen)Cl_2] were prepared by the reported procedure[10-11]. The title complex was prepared as follows: [Pd(Phen)Cl_2] (15 mg) was added to a 3 mL CH_3OH/H_2O (volume 1∶1) solution of Tsser (20 mg) until the solution temperature was heated to 50 ℃, the mixture was adjusted to pH=8~9 by NaOH solution, then stirred for 2 h. By evaporating the filtered solutions at room temperature, the yellow crystal suitable for X-ray diffraction was obtained after a few days (Fig.1). Elemental analysis for [Pd(Phen)(TsserNO)]·H_2O ($C_{22}H_{21}N_3O_6PdS$), calc.(%): C 47.03, H 3.77, N 7.48, found(%): C 47.48, H 3.73, N 7.51. ^{1}H NMR (600 MHz, DMSO-d_6) [Pd(Phen)TsserNO]·H_2O, δ=2.36 (s, 3H), 3.61 (m, 1H), 3.67 (m, 1H), 3.86 (m, 1H), 4.94 (m, 1H), 7.30 (d, J=8.0 Hz, 2H), 8.01 (d, J=8.2 Hz, 2H), 8.10 (dd, J=5.1 and 8.2 Hz, 1H), 8.18 (dd, J=5.3 and 8.2 Hz, 1H), 8.32~8.27 (m, 2H), 8.66 (dd, J=1.2 and 5.1 Hz, 1H), 8.98 (m, 2H), 9.40 (dd, J=1.2 and 5.3 Hz, 1H).

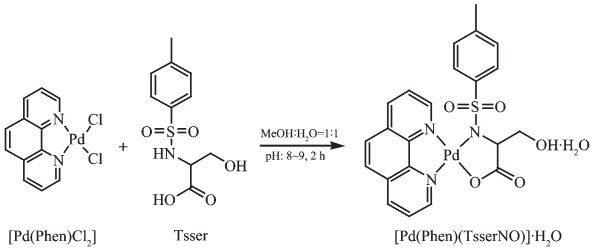

Fig.1 Synthetic routine of [Pd(Phen)(TsserNO)]·H_2O

1.3 Crystal structure determination

The single crystal of the title complex with approximate dimensions of 0.33 mm×0.16 mm×0.16 mm was selected for X-ray diffraction analysis. Data

collection was performed on a Bruker SMART APEX Ⅱ CCD diffractometer equipped with a graphite monochromatized Mo $K\alpha$ radiation (λ=0.071 073 nm) at 296(2) K. The maximum and minimum transmission factors are 0.857 6 and 0.727 0, respectively. Multi-scan absorption corrections were applied using the SADABS program. The structure was solved by the direct method using the SHELXS-97 program. Refinements on F^2 were performed using SHELXL-97 by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms. The hydrogen atoms of the ligand were generated geometrically, while the H atoms of the coordination water molecules were located from difference Fourier synthesis and refined with restraint parameters. A summary of crystallographic data and refinement parameters is given in Table 1.

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Table 1　Crystallographic data of title complex [Pd(Phen)(TsserNO)]·H_2O

Empirical formula	$PdC_{22}H_{21}N_3SO_6$	V / nm^3	1.058 3(4)
Formula weight	561.88	Z	2
Temperature / K	296(2)	Crystal size /mm	0.33×0.16×0.16
Color / shape	Yellow / block	F(000)	568
Crystal system	Monoclinic	D_c / (g·cm^{-3})	1.763
Space group	$P2_1$	Absorption coefficient / mm^{-1}	1.023
θ range for data collection / (°)	2.11~25.99	Reflections collected	5 819
a / nm	0.618 64(14)	Independent reflections (R_{int})	4 033 (0.011 8)
b / nm	1.768 9(4)	Goodness-of-fit on F^2	1.409
c / nm	0.990 2(2)	Final R indices [$I>2\sigma(I)$]	R_1=0.017 5, wR_2=0.041 7
β / (°)	102.392(4)	R indices (all data)	R_1=0.018 2, wR_2=0.041 7

1.4　Cell culture

Four human carcinoma cell lines were used for cytotoxicity determination: HL-60, Bel-7402, BGC-823 and KB. They were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units·mL^{-1} of penicillin and 100 μg·mL of streptomycin. Cells were maintained at 37 ℃ in a humidified atmosphere of 5% CO_2 in air.

1.5　Cytotoxicity assay

The cells harvested from exponential phase were plated equivalently into a 96-well plate, complex was added, control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. The plates were incubated at 37 ℃ in a 5% CO_2 incubator for 44 h. The MTT assay was performed as described by Mosmmann[12]. Upon completion of the incubation, stock MTT dye solution (20 μL, 5 mg·L^{-1}) was added to each well. After 4 h incubation, 2-propanol (100 μL) was added to solubilize the MTT formazan. The OD was measured on a microplate spectrophotometer at a wavelength of 570 nm. The SRB assay was performed as previously described[13]. Upon completion of the incubation, the cells were fixed in 10% trichloroacetic acid (100 μL) for 30 min at 4 ℃, washed five times in tapwater and stained with 0.1% SRB in 1% acetic acid (100 μL) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mmol·L^{-1} unbuffered Tris base (100 μL) and OD was measured at 540 nm as above. The IC_{50} value was determined from plots of % viability against dose of complex added.

2　Results and discussion

2.1　Crystal structure of the complex

The molecular structure of the title complex is shown in Fig.2. The selected bond lengths and angels of the complex is given in Table 2. In the complex, the Pd^{2+} ion is coordinated to the two nitrogen atoms (N2 and N3) of the 1,10-phenanthroline molecule and the chelating modified amino acid dianion (N1 and O1). The latter ligand forms the normal five-membered chelate ring. The angle between planar N(2)-Pd(1)-N(3) and planar O(1)-Pd(1)-N(1) is 2.586(73)° which indicates that the Pd(1)-O(1) -N(1)- N(2)- N(3) plane is

Table 2 Selected bond lengths (nm) and angles (°) for [Pd(Phen)(TsserNO)]·H_2O

Pd(1)-O(1)	0.198 91(17)	Pd(1)-N(2)	0.200 4(2)	Pd(1)-N(1)	0.201 4(2)
Pd(1)-N(3)	0.201 7(2)				
O(1)-Pd(1)-N(1)	83.73(7)	O(1)-Pd(1)-N(3)	95.09(8)	O(1)-Pd(1)-N(2)	176.16(8)
N(2)-Pd(1)-N(3)	81.46(8)	N(2)-Pd(1)-N(1)	99.76(8)	N(1)-Pd(1)-N(3)	177.88(9)

slightly distorted. The bond distances of Pd(1)-O(1) and Pd(1)-N(1) are 0.198 91(17) and 0.201 4(2) nm.

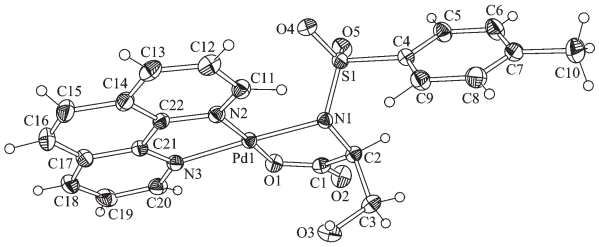

Fig.2 Crystal structure for the title complex (H_2O being omitted)

2.2 Cytotoxicity

As listed in Table 3, the complex [Pd(Phen)(TsserNO)]·H_2O had selectivity against HL-60, BGC-823, Bel-7402 and KB cells lines. The cytotoxicity of the complex [Pd(Phen)(TsserNO)]·H_2O is equal to that of cisplatin against BGC-823 and Bel-7402 cells lines (P >0.05). However it is less potent than cisplatin against HL-60 and KB cell lines. This suggests that it may be a new class metal-based anticancer drug.

3 Conclusions

Table 3 Cytotoxicity of the complex against various human carcinomas

Complex	IC_{50} / (μmol·L^{-1})			
	HL-60	BGC-823	Bel-7402	KB
Cisplatin	2.89	6.48	8.12	2.65
[Pd(Phen)(TsserNO)]·H_2O	9.83	6.85	8.39	36.82

In summary, a novel palladium(Ⅱ) complex [Pd(Phen)(TsserNO)]·H_2O has been prepared by reacting Tsser with [Pd(Phen)Cl_2]. The title complex was characterized by X-ray diffraction, elemental analysis, spectroscopic (IR, ^{1}H NMR) studies. The complex had selectivity against HL-60, BGC-823, Bel-7402 and KB cells lines, its cytotoxicity is equal to that of cisplatin against BGC-823 and Bel-7402 cells lines, however it is less potent than cisplatin against HL-60 and KB cell lines. This suggests that it may be a new class metal-based anticancer drug.

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