کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Cytotoxicity of Diimine Palladium (II) Complexes of Alkyldithiocarbamate Derivatives on Human Lung, Ovary and Liver Cells

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Abstract

Three new Complexes of formula \([\text{pd(bpy)(R-NH-CSS)}] \text{Cl}\) (where bpy is 2/2′- bipyridine, and R-NH-CSS is butylamine, hexylamine- and octyamine-dithiocarbamate anion) have been synthesized by University of Sistan and Blachostan. These complexes have been characterized by spectroscopic methods such as ultraviolet-visible, infrared and \(^1\text{H}-\text{NMR} as well as conductivity measurements and chemical analysis. In these complexes, each of the dithiocarbamate ligands coordinates to Pd (II) center as bidentate with two sulfur atoms. We have found a 1:1 electrolyte in water conductivity test for the above mentioned compounds. To measure the biologic activity and potential anticancer efficacy of these compounds, they have been compared with cisplatin and its palladium analogue of \([\text{Pd (NH}_3]^2\text{Cl}_2]\) on three different cell lines of human hepatocarcinoma HepG2, human ovarian carcinoma OV2008, and human lung adenocarcinoma A549. Clonogenic assay has shown \(LD_{50}\) s in the range of 0.131±0.025 to 0.934 ± 0.194 for these compounds on above cell lines. In comparison, cisplatin has shown \(LD_{50}\) s of 0.838 ± 0.074, 2.196 ± 0.220, and 2.799 ± 0.733 on OV2008, HepG2 and A549 cell lines, respectively. As a conclusion, above three new complexes have shown higher cytotoxicities compared to cisplatin on three different human cell lines. Based on biological tests, these compounds may potentially be considered as good anticancer candidates for further pharmacological studies.

Keywords: Palladium complex; Dithiocarbamates; Cytotoxicity; Liver cells.

Introduction

Currently, cis-diamminedichloroplatinum (II) (cisplatin) is being used as an anticancer drug against several human cancers, such as: testicular cancer, Ovarian and bladder cancer, osteogenic Sarcoma, head and neck cancer, endometrial and cervical cancer and non- small cell lung cancer (1). Due to the frequent resistant to this drug during cancer chemotherapy (2), as well as dose limiting toxic side effects of platinum anticancer drugs, there have been many attempts to find complexes with greater potency and less toxicity than the existing clinical drugs. As a consequence of this, attention has naturally turned to the other platinum group metals, ruthenium, rhodium, palladium, osmium and iridium (3). Among them, preparation and study of cis-dichloropalladium
alkyldithiocarbamate derivatives and the effect of hydrocarbon chain length present in the structure of these complexes on cytotoxicity. Also, the cytotoxic data obtained from these complexes have been compared with cisplatin and its palladium analogue.

**Experimental**

**Materials**

Butylamine, hexylamine and octylamine were bought from Merck and distilled before use. Other reagents like PdCl$_2$, NaOH, NaCl, and 2/2'-bipyridine were also purchased from Merck and used without further purification. Solvents used were reagent grade and purified before use by standard procedures. Dichloro-2,2'-bipyridinepalladium (II), [Pd (bpy) Cl$_2$], cisplatin, cis-[Pt (NH$_3$)$_2$Cl$_2$] and palladium analog of cisplatin, cis-[Pd (NH$_3$)$_2$Cl$_2$] were prepared as reported earlier (10).

Human ovarian adenocarcinoma OV2008, human hepatocarcinoma HepG2, and human lung adenocarcinoma A549 cell lines were obtained from Pharmacology lab, Ottawa Regional Cancer Center (Ottawa, Canada). DMEM/F12 medium, fetal bovine serum and trypsin and tripan blue dye were purchased from Gibco BRL, USA.

**Methods**

Infrared spectra of ligands and metal complexes were recorded on a Nicolet 5-DXB FT-IR spectrophotometer in the range of 4000-400 cm$^{-1}$ in KBr pellets. Electronic absorption spectra of palladium complexes were measured on a Shimadzu UV-265 recording spectrophotometer. Conductivity measurements were carried out on a Systronics conductivity bridge, model 305, with a cell (cell constant 0.59) using water conductivity as a solvent. Microchemical analysis (CHN) was done in the CHN-rapid Herause. $^1$H- NMR spectra were recorded on a Brucker DRX-500 Avance spectrometer at 500 MHz in DMSO-d$_6$ using sodium 2,2-dimethyl -2- silapentane-5- sulphonate (DSS) as internal references. Melting points were measured on a Unimelt capillary melting point apparatus and reported uncorrected.
Synthesis of ligands and complexes

**Butylamine dithiocarbamate sodium salt (Bu-dtcNa)**

A modified literature method (10) was followed for the synthesis of this compound: A mixture of butylamine (10.00 mL, 100 mmol) and NaOH (4.00 g, 100 mmol) in 80 mL acetone was stirred for 1 h. Stirring continued at 0°C in an ice bath and carbon disulfide (10.00 mL excess) was added dropwise over 15 min, after which the solution become cruddy and yellow. The reaction mixture was stirred for another 5 h at 0°C and overnight at room temperature. It was then filtered, and 100 mL ether was added to the filtrate and the solution was placed in the refrigerator overnight. The resulting white precipitate of crude product was filtered off and vacuum dried. For recrystallization, the crude product was stirred with 60 mL acetone and undissolved particles were filtered out. Dichloromethane (50 mL) was added to the filtrate and then left in a refrigerator overnight. The desired product was collected by filtration as white needle-like crystals and washed with small amount of dichloromethane and vacuum dried. Yield was 13.68 g (80%) with a melting point of 73°C.

Analytical Calculation for C$_{5}$H$_{10}$NS$_{2}$Na is as (171.12): C, 35.09; H, 5.85; N, 8.19. Found: C, 35.02; H, 5.84; N, 8.18%.

**Hexylamine dithiocarbamate sodium salt (Hex-dtcNa)**

The synthesis procedure adopted for Bu-dtcNa was used here except that hexylamine (13.25 mL, 100 mmol) was used in place of butylamine. Yield: 15.52g (78%) and melting point is 138°C. Anal. Calc. for C$_{7}$H$_{14}$NS$_{2}$Na (199.36): C, 42.21; H, 7.04; N, 7.04. Found: C, 42.20; H, 7.02; N, 7.05%.

**Octylamine dithiocarbamate sodium salt (Oct-dtcNa)**

The synthesis method adopted here is the same as for Bu-dtcNa, only octylamine (16.53 mL, 100 mmol) was used instead of butylamine. Yield: 17.03 g (75%) and melting point is 173°C. Anal. Calc. for C$_{9}$H$_{18}$NS$_{2}$Na (227.29): C, 47.58; H, 7.93; N, 6.17. Found: C, 47.57; H, 7.92; N, 6.16%.

**2,2′-Bipyridinebutylaminedithiocarbamatopalladium (II) Chloride. [Pd(bpy) (Bu-dtc)]Cl**

[Pd (bpy) Cl$_{2}$] (0.333g, 1 mmol) was well suspended in 150 mL acetone by vigorous stirring for 2h and then temperature was adjusted at 40°C. To this was added dropwise Bu-dtcNa (0.205 g, 1.2 mmol) dissolved in acetone (50 mL), after which the colour of suspension was yellow. The reaction mixture was refluxed for 1 h and stirred for another 10-12 h at room temperature. It was then filtered and the volume of yellow filtrate reduced to 20 mL on a rotary evaporator. It was allowed to cool to room temperature and the yellow solid formed was filtered and washed with small amount of cold water, acetone and vacuum dried. Yield: 0.268 g (60%) and decomposes at 170°C. Anal. Calc. for C$_{15}$H$_{18}$N$_{3}$S$_{2}$Cl Pd (445.50): C, 40.40; H, 4.04; N, 9.43. Found: C, 40.30; H, 4.05; N, 9.45%.

**2,2′-bipyridinehexylaminedithiocarbamatopalladium (II) Chloride, [Pd(bpy)(Hex-dtc)] Cl**

This compound was synthesized and purified by following the procedure as given for [Pd(bpy) (Bu-dtc)]Cl complex except that Hex-dtcNa (0.239g, 1.2 mmol) was used in place of Bu-dtcNa. Yield: 0.289 g (61%) and decomposes at 180°C. Anal. Calc. for C$_{17}$H$_{22}$N$_{3}$S$_{2}$ClPd (473.50): C, 43.08; H, 4.65; N, 8.87. Found: C, 43.05; H, 4.62; N, 8.84%.

**2,2′-bipyridineoctylaminedithiocarbamatopalladium (II) chloride, [Pd(bpy) (Oct-dtc)] Cl**

This complex was synthesized and isolated by following the procedure as given for [Pd(bpy) (Bu-dtc)]Cl complex, except that Oct-dtcNa (0.272 g, 1.2 mmol) was used instead of Bu-dtcNa. Yield: 0.316 g (63%) and decomposes at 178°C. Anal. Calc. for C$_{19}$H$_{26}$N$_{3}$S$_{2}$ClPd (501.50): C, 45.46; H, 5.18; N, 8.37. Found: C, 45.42; H, 5.20; N, 8.30%.

**Cell culture experiments and clonogenic assay**

Cells were cultured in DMEM/F12 medium supplied with 10% fetal calf serum of 37°C in humidified incubator with 5% CO$_{2}$. All cellular experiments were carried out in triplicates. To examine cells’ growth in this condition, 30,000 cells per well in 6 well petridishes were...
seeded and growth curves were drawn for each cell line. All experiments were performed on the exponentially growing cells, which were prepared by minimum three passages of the initial seed of frozen stock.

For the clonogenic assay, cells from above three different cell lines were harvested with trypsin, washed with medium, and plated in quadruplicate onto 60 mm plastic tissue culture dishes at a density of 500 cells/dish in 4 mL culture medium. The cells were incubated overnight and allowed to attach on the surface of the dishes. Cells were then exposed to the various concentrations of each of above synthesized compounds, and/or cisplatin for one hour at 37°C. Three controls were used to normalize resulting data. To do so, a quadruplicate set of dishes was treated with saline, another set with DMSO solvent of these compounds as controls for experiments. The medium was then aspirated and cells were twice rinsed with saline and then the fresh medium was added to each dish. Each experiment was performed in triplicates. After 7–14 days incubation (based on the cell line growth parameters) the medium was aspirated, and cells were fixed and stained with tripan blue dye. Colonies of cells containing at least 50 cells were counted under a microscope. Percentages of colonies for each concentration compared to the appropriate control were assigned as the measurement of cytotoxicity for different concentrations.

### Results and Discussion

Three complexes of formula [Pd(bpy)(R-NH-CSS)]Cl (Where bpy is 2,2'-bipyridine and R-NH-CSS is an anion of butylamine-, hexylamine-, and octylamine–dithiocarbamate) were prepared by interaction of [Pd (bpy) Cl] with sodium salt of dithiocarbamate in molar ratio of 1:1. The molar conductance values of these complexes in water conductivity are in the range of 9197- cm² ohm⁻¹ mol⁻¹ (Table 1). These values suggest that they are 1:1 electrolytes (11). The chemical analysis and molar conductance data support the above formulation of the palladium complexes.

In the electronic absorption spectra of above three complexes, four bands are observed. The band maxima with their extinction coefficients are given in Table 1. The band I and II show blue shifts of 67- nm from less polar chloroform to more polar water. Therefore, These bands may tentatively be assigned to charge transfer from metal to 2,2′-bipyridine ligand. Band III and IV are assigned to first and higher internal π→π* transition of 2,2′- bipyridine (11).

Two most significant bands in the IR spectra of the ligands and the complexes are of interest; the ligands Bu-dtcNa, Hex-dtcNa and Oct–dtcNa showed a strong absorption at 1480, 1491 and 1492 cm⁻¹ respectively, which is assigned to N-CSS stretching mode, while the complexes [Pd (bpy) (Bu-dtc)] Cl, [Pd (bpy)(Hex-dtc)] Cl and [Pd (bpy)(Oct-dtc)] Cl showed absorption at 1534, 1551 and 1539 cm⁻¹ respectively. These absorption data suggested that the N-CSS bond order is in between a single bond (1350-1250=υ cm⁻¹) and a double bond (1690-1640=υ cm⁻¹). Also, on passing from free dithiocarbamate ligands to corresponding complexes, the ν (N-CSS) mode shifted to higher energies on coordination, showing an increase in the nitrogen-carbon double bond character, caused by electron delocalization towards the palladium center. Thus the above alkylthi dithiocarbamate ligands coordinate to Pd (II) centers through sulfur atoms. Second, the presence of a single strong band at 923, 983 and 930 for Bu-dtcNa, Hex-dtcNa and Oct-dtcNa, respectively, and at 1026, 1027 and 1030 cm⁻¹ for [Pd(bpy)(Bu-

| Compound | Molar conductance of 5×10⁻⁴ solution cm² ohm⁻¹ mol⁻¹ | Band maxima in nm |
|----------|-------------------------------------------------|------------------|
| [Pd(bpy)(Bu-dtc)]Cl | 96 | 317(2.19) | 307(1.96) | 248(4.71) | 303(2.76) |
| [Pd(bpy)(Hex-dtc)]Cl | 92 | 319(2.13) | 308(2.06) | 249(5.71) | 201(3.57) |
| [Pd(bpy)(Oct)]Cl | 97 | 318(1.88) | 307.4(1.82) | 247(6.50) | 203(3.78) |

*aExtinction coefficients in liter mole⁻¹ cm⁴×10⁻⁴ are given in parenthesis.*
Cytotoxicity of Diimine Palladium (II) Complexes

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1H NMR spectra of sodium salts of butylamine dithiocarbamate (Bu-dtcNa), hexylamine dithiocarbamate (Hex-dtcNa) and octyamine dithiocarbamate (Oct-dtcNa) in DMSO-d6 were recorded using DSS as internal reference. The 1H NMR spectrum of Bu-dtcNa shows five peaks at 0.82, 1.21, 1.39, 3.31 and 8.08 ppm which are assigned to three H-a, Two H-b, two H-c two H-d and one H-e protons respectively (Table 2 and figure 1-I). Similar assignments have been done for analogous ligands Hex-dtcNa and Oct-dtcNa which data are collected in Table 2. The integrated areas under the peaks correspond to the ratio 3 : 2 : 2 : 1 for Bu-dtcNa, 3 : 6 : 2 : 2 : 1 for Hex-dtcNa and 3 : 10 : 2 : 2 : 1 for Oct-dtcNa and thus support the proposed structures.

The 1H NMR spectra of [Pd(bpy)(Bu-dtc)]Cl complexes were recorded in deuterated dimethyl sulphoxide (DMSO-d6) using DSS as internal reference. Their chemical shifts along with the coupling constants are summarized in Table 2 and proposed structure and NMR numbering scheme are given in Figure 1. The protons of 2,2'-bipyridine moieties in [Pd(bpy)(Bu-dtc)]Cl appear as a doublet at 8.50 ppm, a triplet at 8.25 ppm, quartet at 8.06 ppm and triplet at 7.64 ppm which are assigned to H-6,6', H-4,4', H-3,3' and H-5,5' protons, respectively (10). Similar assignments for protons of 2,2'-bipyridine moieties in [Pd(bpy)(Hex-dtc)]Cl and [Pd(bpy)(Oct-dtc)]Cl are given in Table 2. In the [Pd(bpy)(Bu-dtc)]Cl Complex two multiplets observed at 0.86 and 1.99 ppm are assigned to three H-a and two H-b protons (Figure 1). The two other multiplets appeared at 1.55 and 3.37 ppm is assigned to H-c and H-d protons of diethiocarbamate moieties. Another broad singlet (possibly multiplet) resonates at 11.51 ppm is assigned to H-e proton of coordinated diethiocarbamate. This peak disappear on D2O exchange and also shows an upfield shift of 3.35 ppm in the complex as compared to its value in sodium diethiocarbamate. This suggests the bonding of diethiocarbamate ligand to palladium (II) through both sulphur atoms. Similar assignments have been done for protons of diethiocarbamate moieties in the analogous complexes [Pd(bpy)(Hex-dtc)]Cl and [Pd(bpy)(Oct-dtc)]Cl (Table 2). The integrated areas under the peaks of 2,2'-bipyridine and diethiocarbamate protons in the ratio 8 : 10 for[Pd(bpy)(Bu-dtc)]Cl and 8:14 for [Pd(bpy)(Hex-dtc)]Cl and 8 : 18 for [Pd(bpy)(Oct-dtc)]Cl are further supporting the proposed structures.

Finally, 1H NMR spectra of the complexes dissolved in DMSO-d6, and recorded after 24 h observed no changes in these spectra, suggesting no dissociation of diethiocarbamate anions.

DNA binding and antitumor activities of these compounds on human K562 cell line have been published before (10). Here, using the same derivatives, we have examined the cytotoxicity effects of above mentioned compounds on three different human cell lines.

Table 1. 1H NMR spectral data of R-NH-CSSNa ligands and [Pd(bpy)(R-NH-CSS)]Cl complexes in deuterated DMSO.

| compounds          | 2,2'-bipyridine protons | Dithiocarbamate proton |
|--------------------|-------------------------|------------------------|
|                    | H-6,6'                  | H-4,4'                 |
|                    | H-3,3'                  | H-5,5'                 |
|                    | H-a                     | H-b                    |
|                    | H-c                     | H-d                    |
| Bu-dtcNa           | 0.82 (t)                | 1.21(m)                |
| [Pd(bpy)(Bu-dtc)]Cl| 8.50(d)                 | 8.25(t)                |
| Hex-dtcNa          | 0.84 (m)                | 1.23 (m)               |
| [Pd(bpy)(Hex-dtc)]Cl| 8.54(d)                | 8.32(t)                |
| Oct-dtcNa          | 0.84 (t)                | 1.23 (m)               |
| [Pd(bpy)(Oct-dtc)]Cl| 8.51(d)               | 8.26(t)                |

α chemical shift in ppm. β,δ,ε,ζ,η and ρ are singlet, doublet, triplet, quartet, multiplet and singlet broad, respectively.
as potential candidates for human cancer therapy with the results shown in Table 3. In this table, clonogenic results (mean ± SEM) are listed as the percentages of colonies survived after exposure to different concentrations of compounds in comparison with the control. As is shown in this table from the clonogenic assay, a typical dose-response curve with LD$_{50}$s of 0.838 ± 0.074 µg/mL, 2.196 ± 0.220 µg/mL and 2.799 ± 0.733 µg/mL, have been shown on OV2008, HepG2 and A549, respectively for cisplatin. The rank order of cisplatin cytotoxicity on these cell lines is in well agreement with the previous publication and the cell lines sensitivities to this anticancer drug. This may be counted as a proof for the accuracy of cytotoxicity assay. As is shown in Table 3, the Pd analogue of cisplatin is not following the same ranking order of cytotoxicity on these cell lines, being most toxic for OV2008 and A549 and less for the HepG2. This compound may be a good candidate for further studies as a potential anticancer drug for the lung adenocarcinoma which is considered as a very resistant cancer to chemotherapy. Other synthetic Pd compounds

Figure 1. Proposed structures and numbering scheme of (1) Bu-dtcNa (2) Hex-dtcNa (3) Oct-dtcNa (4) [Pd(bpy)(Bu-dtc)]Cl (5) [Pd(bpy)(Hex-dtc)]Cl and (6) [Pd(bpy)(Oct-dtc)]Cl compounds.
Cytotoxicity of Diimine Palladium (II) Complexes

Table 3. Cytotoxicity in LD₅₀ of synthesized Pd compounds (abbreviations as per Figure 1), as well as cisplatin and its Pd analogue on three different human cancer cell lines in µg/mL.

| CELL LINE | A549      | HepG2     | OV2008    |
|-----------|-----------|-----------|-----------|
| IV        | 0.379 ± 0.107 | 0.934 ± 0.194 | 0.234 ± 0.023 |
| V         | 0.605 ± 0.054 | 0.131 ± 0.052 | 0.914 ± 0.097 |
| VI        | 0.482 ± 0.152 | 0.172 ± 0.060 | 0.258 ± 0.043 |
| Pd Analogue | 1.721 ± 0.793 | 8.833 ± 0.729 | 1.53 ± 0.557 |
| cisplatin | 2.799 ± 0.733 | 2.196 ± 0.220 | 0.838 ± 0.074 |

are in the same cytotoxic range as cisplatin for different cell lines, but with a distinguished effect on lung adenocarcinoma. Above biological study may suggest these Pd analogues of cisplatin as good candidates to be considered for the treatment of human lung cancer. Further cellular and animal studies are suggested aiming toward the potential clinical application of these compounds.

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References

(1) Prestayko AW, Crooke ST and Carter SK(eds) In: Cisplatin: Current Status and New Developments. Academic Press, New York, 1980.
(2) Zendehdel R, Masoudi-Nejad A, Mohammadzadeh J and Shirazi FH. Cisplatin resistant patterns in ovarian cell line using ftir and principle component analysis. Iranian J. Pharm. Res. (2012) 11: 235-240.
(3) Bruijninx PCA and Sadler PJ. Controlling platinum, ruthenium and osmium reactivity for anticancer drug design. Adv. Inorg. Chem. (2009) 61: 1-62.
(4) Ho Y, Au-Yeung SCF, To KW. Platinum-based anticancer agents: in novative design strategies and biological perspectives. Medicinal Research Reviews (2003) 23: 633-655.
(5) MartinRB. In:platinum, GoldandotherChemotherapeutic Agents. Lippard SJ (ed) ACS Symposium Series No. 209 Washington DC. (1993) 231-244.
(6) González ML, Tercero JM and Matilla M. Cis-dichloro (r,r-diamino carboxylate ethyl ester) palladium (II) as palladium (II) versus platinum (II) model anticancer drugs: synthesis, solution equilibria of their aqua, hydroxo, and/or chloro species, and in-vitro/in-vivo DNA-binding properties. Inorg. Chem. (1997) 36: 1806-1812.
(7) Sobrero A, Guglielmi A, Aschele C and Rosso R. Current strategies to reduce cisplatin toxicity. J Chemother. (1990) 2:3-7.
(8) Das B, Antoon R and Tsuchida R. Squalene selectively protects mouse bone marrow progenitors against cisplatin and carboplatin-induced cytotoxicity in-vivo without protecting tumor growth. neoplasia. (2008) 10: 1105-1119.
(9) Konstantinov S, Topushka-Ancheva M, Kariaenova M, Zoneva G and Galova I. Antitumor, nephrotoxic and clastogenic effect of cis-DDP with DDTC or NAC. Neoplasma. (1994) 41: 253-8.
(10) Mansouri-Torshizi H, Saeidifar M, Khosravi F, Divsalar A, Saboury AA and Hassani F. DNA binding and antitumor activity of α-diimineplatinum(ii) and palladium (ii) dithiocarbamate complexes. Bioinorg Chem Appl. (2011):11.
(11) Gidincy PM, Gillard RD and Heaton BT. Complexes of the platinum metals containing weak donor ligands J. chem. Soc. Dalton Trans.(1973) 132.
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