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Antitumor effects of a tetradentate amido-carboxylate ligands and corresponding square-planar palladium(II) complexes toward some cancer cells. Crystal structure, DFT modeling and ligand to DNA probe Docking simulation

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ABSTRACT

Novel square-planar palladium(II) complexes with O–N–N–O-type ligands H_4mda (H_4mda = malamido-*N*,*N*′-diacetic acid) and H_4obp (H_4obp = oxamido-*N*,*N*′-di-3-propionic acid) were prepared and characterized. The ligands coordinate to the palladium(II) ion via two pairs of deprotonated ligating atoms with square chelation. A four coordinate, square-planar geometry was verified crystallographicaly for the K_2[Pd(mda)]·H_2O complex. The binary and ternary systems of Pd(II) ion with H_4mda or H_4obp (L) as primary ligands and guanosine (A) as secondary ligand were studied in aqueous solutions in 0.1 M NaCl ionic medium at 25 °C by potentiometric titrations. In addition, calculations based on density functional methods (DFT) were carried out. A natural bonding orbital analysis indicated that the Pd–N bonds are three-centric in nature and mainly governed by charge transfer via a strong delocalization of the oxygen lone pair with "p" character into the bonding Pd–N orbital. Mononuclear palladium(II) complexes together with amido acid *N*,*O*-containing ligands were tested against several tumor cells and reveal significant antitumor activity and lower resistance of tumor cells *in vitro* than *cis*platin. In this paper, interactions of palladium complexes with DNA are discussed in order to provide guidance and determine structure and antitumor activity relationships for continuing studies of these systems. Docking simulation on DNA dodecamer or 29-mer (Lippard solved crystal structures), suggests several favorable interactions with the hydrogen pocket/binding site for the incoming ligands. These results support amidoacids/Pd complexes as novel antitumor drugs and suggest that their potent cell life inhibition may contribute to its anti-cancer efficacy.

1. Introduction

Oxamide type compounds comprise a very interesting family in co-ordination chemistry. Furthermore, in the presence of transition-metal ions and provided that the substituted oxamide can form five- or six-membered chelate rings, the amide deprotonates and co-ordinates simultaneously at low pH. *N*,*N*′-*bis*(substituted)oxamidates are well known as versatile bridging ligands, which can afford symmetric and asymmetric oxamidate bridges by their *cis*–*trans* conformational change [1,2].

The complexes of copper(II) with oxamido-derived tetradentatate ligands, composed of oxalic and amino acid (β-alaninate, glycine or anthranilic acid) [3–11] have been studied by many research groups. The d-electron configuration and size of the central metal ion [12–14] could have significant influence on the geometry in O–N–N–O-type and related complexes. The complexes, especially of copper(II) and nickel(II), with malonamide-derived ligands (Scheme 1), readily accessible *via* aminolysis reactions of malonic esters with polyamines, have been studied extensively [11–17]. At the same time, a rather limited number of complexes of the late transition metals, e.g. palladium(II) [18,19], platinum(II) [20,21], rhenium(V) [22] and technetium(V) [23], with this kind of ligand have been thoroughly characterized up to date [24].

Extensible research efforts have been made to develop novel metal-based antitumor complexes with the aim of improving effectiveness and reducing the severe side effects of the current clinical platinum chemotherapeutic agents such as *cis*platin and its analogues [25,26]. Special attention has been paid to platinum complexes with different structural features from those of already used drugs [27–31] and complexes of the other platinum-group metals like ruthenium, rhodium, palladium and iridium [32–35].

The coordination chemistry of palladium(II) is very similar to that of platinum(II), but the higher liability in ligand exchange at Pd centre (10^5 times greater than Pt) may cause rapid hydrolytic processes leading to the dissociation of the complex and formation of very reactive

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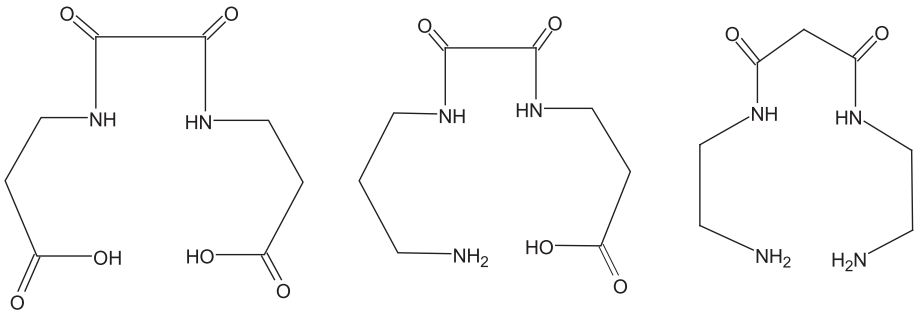

Scheme 1. Amide derived ligands of oxalic and malonic acids.

species unable to reach their biological targets. These problems could be overcome by using the bulky heterocyclic and chelating ligands. A number of palladium complexes with aromatic *N*- and *N*,*N*-containing ligands [33,36–38] and N,O-containing ligands [39] as well as those with N,S-chelating ligands have shown very promising antitumor characteristics [40–42].

Considerable efforts have also been devoted to characterize and predict structure of novel complexes with potential antitumor activity by applying theoretical methods. Computational chemistry encompasses not only quantum mechanics but also molecular mechanics which results in minimization, simulations, conformational analysis and other computer-based methods for understanding and predicting the behavior of molecular systems. For analysis of quantum-mechanical output files, a third generation program, such as NBO or NEDA use is to be considered [43,44].

With this in mind, we have used amide-containing ligands, especially derivates of malonic and oxalic acid, to synthetize palladium(II) complexes. We expected to obtain palladium(II) complexes of the square-planar geometry, used for further investigation of their biological activity. Therefore, the aim of the present paper is to quantitatively examine the equilibria in ligand solution (mda = 2,2′-[(1,3-dioxopropane-1,3-diyl) diimino]diacetic acid or obp = 3,3′-[(1,2-dioxoethane-1,2-diyl)diimino] dipropanoic acid) in the presence of palladium ion in order to gain a better understanding of the identity, stability and speciation in aqueous solutions. Accordingly, the complex formation between palladium(II) ion and H_4mda or H_4obp as primary ligands and guanosine (Guo) as secondary ligand has been studied by using potentiometric measurements. In addition, we report the results of experimental (synthesis of ligands and related metal complexes and their cytotoxicity *in vitro* examinations toward tumor cell lines) and theoretical (modeling-density functional theory (DFT) and simulation-docking) studies of ligands and their new palladium(II) complexes. The ligands and complexes were characterized by IR, UV and ^{1}H and ^{13}C NMR spectrums. The molecular structure of K_2[Pd(mda)] · H_2O complex was confirmed by single-crystal X-ray structure analysis.

2. Experimental

2.1. Materials

Reagent grade, commercially available, chemicals were used without further purification. H_4mda (**1**) and H_4obp (**2**) acids were prepared by applying the method of Matović et al. [11]. Potassium hydroxide, potassium-tetrachloro-palladate(II), palladium(II) chloride and guanosine were purchased from Sigma-Aldrich and used as supplied.

2.2. Physical measurements

C, H, and N analyses were performed with Carlo Erba EA1108 elemental analyser. IR spectra in the 400–4000 cm^{-1} region were measured in KBr pellets on a Perkin–Elmer FTIR spectrophotometer Spectrumone; electronic spectra were measured on a Perkin–Elmer Lambda 35 spectrophotometer. For these measurements, 1×10^{-4} M of aqueous solutions of the complexes to be explored were used. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300 MHz spectrometer using D_2O solutions.

Suitable yellow-colored parallelepiped-shaped crystals of K_2[Pd(mda)] · H_2O were obtained by recrystallisation from water. A crystal with the dimensions of $0.21\times0.20\times0.08$ mm was mounted on top of a glass fiber and aligned on a *Bruker SMART APEX CCD* diffractometer [45]. A total of 1800 frames were collected with an exposure time of 10.0 s per frame. The overall data collection time was 8.0 h. The final unit cell was obtained from the *xyz* centroids of 6222 reflections after integration. Intensity data were corrected for Lorentz and polarization effects, scale variation, for decay and absorption: a multi-scan absorption correction was applied, based on the intensities of symmetry-related reflections measured at different angular settings and reduced to F_o^2. The program suite *SHELXTL* was used for space group determination (*XPREP*) [45]. The unit cell was identified as monoclinic; the space group $P2_1/a$, was derived from the systematic extinctions. The structure was solved by direct methods with *SIR-97* [46]. The positional and anisotropic displacement parameters for the non-hydrogen atoms were refined. A subsequent difference Fourier synthesis resulted in the location of all the hydrogen atoms, whose coordinates and isotropic displacement parameters were refined. Final refinement on F^2 carried out by full-matrix least-squares techniques converged at $wR(F^2) = 0.1331$ for 2900 reflections and $R(F) = 0.0469$ for 2680 reflections with F_o 4.0 (F_o) and 204 parameters and 2 restraints. The final difference Fourier map was essentially featureless: no significant peaks (0.9(3) e/Å^3) having chemical meaning above the general background were observed, except one free peak of 2.4(3) e/Å^3 and one hole of 3.0(3) e/Å^3 within 0.5 Å from K2, but were neglected/rejected, being artefacts. The positional and anisotropic displacement parameters for the non-hydrogen atoms and isotropic displacement parameters for hydrogen atoms were refined on F^2 with full-matrix least-squares procedures minimizing the function $Q = \sum_h [w((F_o^2) - k(F_c^2))^2]$, where $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$, $P = [\max(F_o^2, 0) + 2F_c^2]/3$, F_o and F_c are the observed and calculated structure factor amplitudes, respectively; ultimately the suggested a (=0.0638) and b (=15.9131) were used in the final refinement (Table 1). Neutral atom scattering factors and anomalous dispersion corrections were taken from *International Tables for Crystallography* [47]. All refinement calculations were performed on a HP XW6200 (Intel XEON 3.2 GHz)/Debian-Linux computer at the University of Groningen with the program packages *SHELXL* [48] (least-square refinements).

2.3. Synthesis of the 2,2′-[(1,3-dioxopropane-1,3-diyl)diimino]diacetic acid, H_4mda (1)

Glycine (9.75 g, 0.1298 mol) was suspended in H_2O (10 ml) and then dissolved by adding a solution of NaOH (5.0 g, 0.1298 mol) in H_2O (10 cm^3). To this solution an EtOH solution of diethylmalonate [10.4 g, 0.0649 mol in 96% EtOH (20 ml)] was added. The reaction mixture was then heated under reflux (65 °C) for 3 h with stirring.