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Palladium(II) oxalato complexes involving N6-(benzyl)-9-isopropyladenine-based N-donor carrier ligands: Synthesis, general properties, ^{1}H, ^{13}C and $^{15}N\{^{1}H\}$ NMR characterization and *in vitro* cytotoxicity

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ABSTRACT

Reactions of potassium bis(oxalato)palladate dihydrate, $K_2[Pd(ox)_2]\cdot 2H_2O$, with two molar equivalents of N6-(benzyl)-9-isopropyladenine-based organic molecules (L_{1-7}), i.e. 2-chloro-N6-(2-methoxybenzyl)-9-isopropyladenine (L_1), 2-chloro-N6-(3-methoxybenzyl)-9-isopropyladenine (L_2), 2-chloro-N6-(3,5-dimethoxybenzyl)-9-isopropyladenine (L_3), 2-(1-ethyl-2-hydroxyethylamino)-N6-(benzyl)-9-isopropyladenine (L_4), 2-(1-ethyl-2-hydroxyethylamino)-N6-(2-methoxybenzyl)-9-isopropyladenine (L_5), 2-(1-ethyl-2-hydroxyethylamino)-N6-(3-methoxybenzyl)-9-isopropyladenine (L_6) and 2-(1-ethyl-2-hydroxyethylamino)-N6-(4-methoxybenzyl)-9-isopropyladenine (L_7), provided a series of seven palladium(II) oxalato (ox) complexes of the general formula $[Pd(ox)(L_{1-7})_2]\cdot nH_2O$ (**1**–**7**; $n = 0$ for **4**, **5** and **7**, ¾ for **1** and **2**, 1 for **6**, and 3 for **3**). The compounds were characterized by elemental analysis, IR, Raman, ^{1}H, ^{13}C and $^{15}N\{^{1}H\}$ NMR spectroscopy, ESI+ mass spectrometry, molar conductivity and TG/DTA thermal analysis. The geometry of $[Pd(ox)(L_2)_2]$ (**2**) was optimized on the B3LYP/6-311G*/LANL2DZ level of theory. The complexes **4**–**7** represent the first palladium(II) oxalato complexes with a PdN_2O_2 donor set, which involve highly potent purine-based cyclin-dependent kinase (CDK) inhibitors (L_{4-7}) as carrier N-donor ligands. The selected complexes **1**, **3**–**5** and **7** were tested by an MTT assay for their *in vitro* cytotoxic activity against human osteosarcoma (HOS) cancer cell line. The highest activity was found for the complexes **5** ($IC_{50} = 34.9$ μM) and **7** ($IC_{50} = 39.2$ μM).

1. Introduction

The plant hormone N6-(benzyl)adenine [6-(benzylamino)-purine] [1] and its derivatives were found to be suitable N-donor ligands of transition metal complexes. In the field of palladium(II) complexes, the *cis*-$[PdCl_2(L)_2]$, *trans*-$[PdCl_2(L)_2]$, $[PdCl_3(L^+)]$, $[PdCl_2(H_2O)(L)]$, $[PdCl(H_2O)_2(L^-)]$ and $[Pd(ox)(L)_2]$ types of compounds were prepared in our laboratory (see lit. [2–5] and the reference cited therein), where L, L^+ and L^- stand for an electroneutral, protonated, and deprotonated N6-(benzyl)adenine derivative, respectively, and ox symbolizes an oxalate dianion.

Talking about the $[Pd(ox)(L)_2]$ compounds in more detail, five complexes with 2-chloro-N6-(benzyl)-9-isopropyladenine, or its analogues with the substituted benzyl group, have recently been published [3]. The molecular and crystal structures of two complexes involving 2-chloro-N6-(4-methoxybenzyl)-9-isopropyladenine (L_I; complex **I**) and 2-chloro-N6-(4-methylbenzyl)-9-isopropyladenine (L_{II}; complex **II**), were determined by a single-crystal X-ray analysis. The mentioned complexes **I** and **II** have the tetra-coordinated central Pd(II) ion which is surrounded by one bidentate-coordinated oxalate dianion and by two monodentate bonded adenine-based molecules (L_I or L_{II}) in a PdN_2O_2 donor set. Moreover, these complexes were tested by a calcein acetoxymethyl (AM) assay for their *in vitro* cytotoxic activity against breast adenocarcinoma (MCF-7) and chronic myelogenous leukaemia (K562) human cancer cell lines. Two of the tested substances showed promising *in vitro* cytotoxicity (IC_{50} values of 6.2 and 6.8 μM), which is higher than those of the commercially used platinum-based anticancer drugs *Cisplatin* ($IC_{50} = 10.9$ μM) and *Oxaliplatin* ($IC_{50} = 18.2$ μM). To our best knowledge, only the $[Pd(ox)(Hoen)_2]\cdot 0.5H_2O$ and $[Pd(ox)(Clen)_2]$ complexes (Hoen = *N,N′*-bis(hydroxyethyl)ethylenediamine, Clen = *N,N′*-bis(chloroethyl)ethylenediamine) [6], besides the above-mentioned $[Pd(ox)(L)_2]$ compounds prepared in our laboratory, were tested for their *in vitro* cytotoxicity within a group of monomeric palladium(II) oxalato complexes, however, these substances were inactive against mice leukaemia (P388) cells. On the other hand, the results obtained in the case of $[Pd(ox)(L)_2]$ showed that this type of complexes represents a promising group of compounds in

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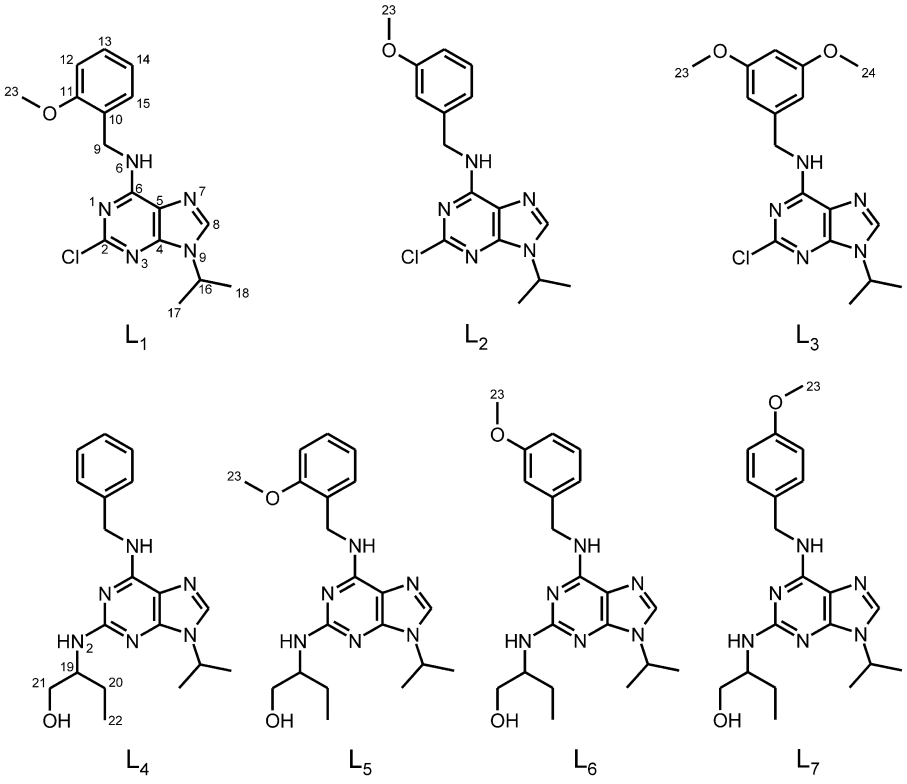

Scheme 1. The derivatives of N6-(benzyl)-9-isoropyladenine (L_{1-7}) used for the preparation of the $[Pd(ox)(L_{1-7})]\cdot nH_2O$ palladium(II) oxalato complexes.

connection with their *in vitro* cytotoxicity. For comparison, other types of biologically active palladium complexes have been reviewed in the literature [2,4].

In this paper, we present results following from our ongoing research of palladium(II) oxalate complexes involving N6-(benzyl)-9-isopropyladenine-based N-donor carrier ligands. We prepared and characterized seven $[Pd(ox)(L)_2]\cdot nH_2O$ complexes of which the compounds **1**–**3** represent analogues of recently reported palladium(II) oxalato complexes [3] varying in the substitution on a benzene ring of the 2-chloro-N6-(benzyl)-9-isopropyladenine moiety (L_{1-3}; see Scheme 1). On the other hand, the complexes **4**–**7** involve differently substituted type of N6-(benzyl)adenine derivatives with 2-amino-1-butanol at the C2 position of a purine ring instead of the chlorine atom, namely 2-(1-ethyl-2-hydroxyethylamino)-N6-(benzyl)-9-isopropyladenine (*Roscovitine*, L_4) [7] and its benzyl-substituted analogues (L_{5-7}; Scheme 1). It is known that *Roscovitine* and its derivatives belong to the group of highly potent cyclin-dependent kinase (CDK) inhibitors, and thus the presented complexes **4**–**7** represent the first palladium(II) oxalato complexes with purine-based CDK inhibitors acting as N-donor carrier ligands.

Based on the above-mentioned statements, we decided to carry out an *in vitro* cytotoxicity screening of the prepared complexes against human osteosarcoma cancer cell line (HOS). The obtained results showed that the complexes **5** and **7**, involving the potent CDK inhibitors, have *in vitro* cytotoxicity comparable with *Cisplatin*, as discussed below. These findings motivated us to evaluate deeply the *in vitro* cytotoxicity of these complexes, and thus, the named palladium(II) oxalato complexes are currently tested against a variety of human cancer cell lines, e.g. MCF-7, lung carcinoma (A549), cervix epithelioid carcinoma (HeLa), ovarian carcinoma (A2780), *Cisplatin*-resistant ovarian carcinoma (A2780*cis*) or malignant melanoma (G-361).

2. Experimental

2.1. Starting materials

Chemicals and solvents were purchased from the commercial sources (Sigma-Aldrich Co., Acros Organics Co., Lachema Co. or Fluka Co.) and they were used as received. Dimethyl sulfoxide (DMSO) was dried using $MgSO_4$.

Potassium bis(oxalato)palladate(II) dihydrate, $K_2[Pd(ox)_2]\cdot 2H_2O$, was prepared from potassium tetrachloropalladate(II), $K_2[PdCl_4]$, as formerly described [3,8]. Syntheses of 2-chloro-N6-(2-methoxybenzyl)-9-isopropyladenine (L_1), 2-chloro-N6-(3-methoxybenzyl)-9-isopropyladenine (L_2), 2-chloro-N6-(3,5-dimethoxybenzyl)-9-isopropyladenine (L_3), 2-(1-ethyl-2-hydroxyethylamino)-N6-(benzyl)-9-isopropyladenine (L_4), 2-(1-ethyl-2-hydroxyethylamino)-N6-(2-methoxybenzyl)-9-isopropyladenine (L_5), 2-(1-ethyl-2-hydroxyethylamino)-N6-(3-methoxybenzyl)-9-isopropyladenine (L_6) and 2-(1-ethyl-2-hydroxyethylamino)-N6-(4-methoxybenzyl)-9-isopropyladenine (L_7) were inspired by several literature sources, and their structures are depicted in Scheme 1 [9–12]. A scheme of the synthetic pathway of the L_{1-7} compounds, as well as the results of IR, Raman and NMR spectroscopies, are given in Appendix A in Supplementary material.

2.2. Preparation of $[Pd(ox)(L_1)_2]\cdot{}^3\!/_4H_2O$ (**1**), $[Pd(ox)(L_2)_2]\cdot{}^3\!/_4H_2O$ (**2**), $[Pd(ox)(L_3)_2]\cdot 3H_2O$ (**3**), $[Pd(ox)(L_4)_2]$ (**4**), $[Pd(ox)(L_5)_2]$ (**5**), $[Pd(ox)(L_6)_2]\cdot H_2O$ (**6**) and $[Pd(ox)(L_7)_2]$ (**7**)

The palladium(II) oxalato complexes **1**–**7** were prepared according to a general synthetic procedure recently published and depicted in Scheme 2 [3]. Briefly, a $K_2[Pd(ox)_2]\cdot 2H_2O$ distilled water solution (15 mL, 40 °C) was mixed together with an acetone solu-