Available online at www.sciencedirect.com
ScienceDirect
Journal of Inorganic Biochemistry 101 (2007) 477–492
Journal of Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

# Synthesis, characterization and assessment of the cytotoxic properties of *cis* and *trans*-[Pd(L)$_2$Cl$_2$] complexes involving 6-benzylamino-9-isopropylpurine derivatives

Zdeněk Trávníček [a,b,*], Lucie Szűčová [a,b], Igor Popa [b]

[a] *Department of Inorganic Chemistry, Palacký University, Křížkovského 10, CZ-771 47 Olomouc, Czech Republic*
[b] *Laboratory of Growth Regulators, Palacký University & Institute of Experimental Botany AS CR, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic*

Received 9 June 2006; received in revised form 15 November 2006; accepted 16 November 2006
Available online 23 November 2006

**Abstract**

A series of square-planar Pd(II) complexes of the composition *cis*-[Pd(L$_n$)$_2$Cl$_2$] {L$_1$ = 2-chloro-6-benzylamino-9-isopropylpurine (**1**), L$_2$ = 2-chloro-6-[(4-methoxybenzyl)amino]-9-isopropylpurine (**2**), L$_3$ = 2-chloro-6-[(2-methoxybenzyl)amino]-9-isopropylpurine (**3**) and 2-[(chloropropyl)amino]-6-benzylamino-9-isopropylpurine (**6**)} has been synthesized by the reaction of PdCl$_2$ with L$_n$ in a 1:2 molar ratio. In contrast, the same reaction followed by recrystallization of the product from *N*,*N*′-dimethylformamide (DMF) leads to *trans*-[Pd(L$_n$)$_2$Cl$_2$] · *n*DMF {L$_3$, $n = 0$ (**4**), $n = 1$(**4*DMF**); L$_4$ = 2-chloro-6-[(2,3-dimethoxybenzyl)-amino]-9-isopropylpurine, $n = 0$ (**5**), $n = 1.5$ (**5*DMF**). The compounds have been characterized by elemental analyses, conductivity measurements, electrospray mass spectra in the positive ion mode (ES + MS), FTIR, $^1$H and $^{13}$C NMR spectra, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Moreover, the complexes **2** and **6** have been also investigated by $^{15}$N NMR spectroscopy. The molecular structures of L$_5$, {(H$^{2+}$L$_5$)(Cl$^-$)$_2$} · H$_2$O, i.e. the protonated form of L$_5$, *trans*-[Pd(L$_3$)$_2$Cl$_2$] (**4**) and *trans*-[Pd(L$_4$)$_2$Cl$_2$] (**5**) have been determined by single crystal X-ray analysis. NMR data and X-ray structures revealed that the organic molecules are coordinated to Pd via N7 atom of a purine moiety. All the complexes and the corresponding ligands have been tested in vitro for their cytotoxicity against four human cancer cell lines: breast adenocarcinoma (MCF7), malignant melanoma (G361), chronic myelogenous leukaemia (K562) and osteogenic sarcoma (HOS). Promising in vitro cytotoxic effect has been found for *cis*-[Pd(L$_2$)$_2$Cl$_2$] (**2**), having the IC$_{50}$ values of 12, 10, 25, and 14 μM against MCF7, G361, K562, and HOS, respectively, and for *trans*-[Pd(L$_3$)$_2$Cl$_2$] · DMF (**4**) with the IC$_{50}$ value of 15 μM against G361.


*Keywords:* Palladium(II) complexes; 6-Benzylamino-9-isopropylpurines; Cytotoxicity; X-ray structure analysis

## 1. Introduction

After the expansion of platinum-based anticancer drugs, it has been shown that not only Pt(II), but also another VIIIB group metal ions like Ru(II) [1–4], Ru(III) [5–9], Rh(I) [10,11], Rh(III) [7,11], Ir(I) [10], Ir(III) [12], Os(II) [13] or Pd(II) [14–18] may form the complexes with substantive antitumour activity, which are not supposed to cause such undesirable side effects as *cis*-diamminedichloroplatinum(II) (*Cisplatin*) and its congeners [19,20]. For that reason, the main interest is paid to Pd(II), mainly due to the evident structural analogy between Pt(II) and Pd(II) complexes. Other derivatives of *cis*-[Pd(L)$_2$Cl$_2$] have been described, where L = pyrazole [21], triazolopyrimidine [22] or inosine derivatives [23], with significant antitumour activity. Many *trans*-[Pd(L)$_2$Cl$_2$] complexes have also

* Corresponding author. Address: Laboratory of Growth Regulators, Palacký University & Institute of Experimental Botany AS CR, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic. Tel.: +420 58 563 4944; fax: +420 58 563 4954.
*E-mail addresses:* zdenek.travnicek@upol.cz, trav@aix.upol.cz (Z. Trávníček).

0162-0134/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2006.11.010

been published, where L = 2,6-dimethyl-4-nitropyridine [24], pyrazole [21], aminobenzonitrile [25], inosine, guanosine, adenosine, xanthosine [23,26] or 6-benzylaminopurine derivatives [18,27,28], with moderate anticancer properties. It is known that the mechanism of action of *trans*-diamminedichloroplatinum(II) (*Transplatin*) derivatives has been studied and described in greater details [20,29,30] than in case of *trans*-Pd(II) analogues [24]. However, the promising antitumor activity found for some square-planar *trans*-palladium complexes [24,31] might be taken as a challenge to continue in their research.

C2- and N9-substituted 6-benzylaminopurine derivatives, used as ligands in the present work, have been intensively investigated for last fifteen years due to their ability to behave as cyclin-dependent kinase (*CDKs*) inhibitors [32–34]. *CDKs* belong to the family of protein kinase enzymes responsible for the regulation of the cell cycle [33–36]. Some of the first artificially prepared derivatives of the above type, especially 2-{[1-(hydroxymethyl)propyl]amino}-6-benzylamino-9-isopropylpurine (*Roscovitine, Seliciclib®*, *CYC202*) and 2-[3-(hydroxypropyl)-amino]-6-benzylamino-9-isopropylpurine (*Bohemine*), have shown very promising anticancer properties, e.g. *Roscovitine* has terminated IIb phase of clinical trials recently (www.cyclacel.com). As for their in vitro cytotoxicity against selected human cancer cell lines (MCF7, K562, HOS, and G361), the $IC_{50}$ values range from 11 to 40 μM, and from 28 to 113 μM, respectively [37]. It was found and described previously, that the cytotoxicity of the above-mentioned organic molecules can be improved by the coordination of the molecule to appropriate transition metal ions [18,27,28,38–41]. In our preceding work, we have prepared and characterized Pd(II) complexes of the type *trans*-$[Pd(L_n)_2Cl_2]$ ($L_n$ = 2-{[1-(hydroxymethyl)propyl]amino}-6-[(3-hydroxybenzyl)amino]-9-isopropylpurine, 2-{[1-(hydroxymethyl)-2-(methyl)propyl]amino}-6-[(3-hydroxybenzyl)amino]-9-isopropylpurine, 2-chloro-6-[(3-hydroxybenzyl)amino]-9-isopropylpurine and 2-chloro-6-[(2-hydroxy-3-methoxybenzyl)amino]-9-isopropylpurine). We have found that the cytotoxicity of the complexes was significantly improved as compared to that for the free organic molecules [18].

In this study, we want to pick up the threads of our preceding work and wish to intensify the knowledge on Pd(II) complexes bearing the molecules structurally very similar to CDK-inhibitors, i.e. 2-chloro-6-benzylamino-9-isopropylpurine ($L_1$), 2-chloro-6-[(4-methoxybenzyl)amino]-9-isopropylpurine ($L_2$), 2-chloro-6-[(2-methoxybenzyl)amino]-9-isopropylpurine ($L_3$), 2-chloro-6-[(2,3-dimethoxybenzyl)-amino]-9-isopropylpurine ($L_4$) and 2-[(chloropropyl)-amino]-6-benzylamino-9-isopropylpurine ($L_5$), of which a schematic representation is shown in Fig. 1. To provide insight into the preparation and the properties of these complexes, we have synthesized and characterized six novel Pd(II) complexes of the type $[Pd(L)_2Cl_2]$ (**1**–**6**) where the mononuclear nature was confirmed by X-ray structures of **4** and **5**.

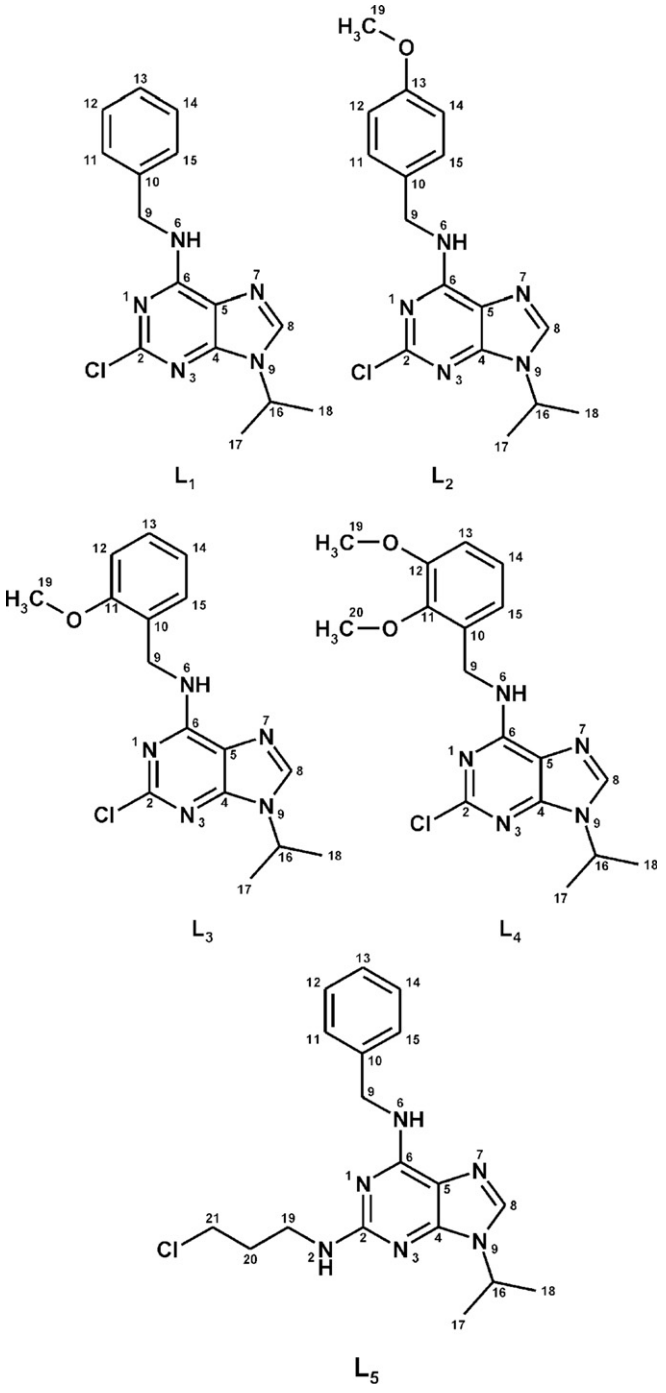


Fig. 1. The organic molecules $L_1$–$L_5$ used as ligands in this study.

## 2. Experimental

### 2.1. Materials

$PdCl_2$, $SOCl_2$, triethylamine (TEA), *N*,*N*′-dimethylformamide-$d_7$ (DMF-$d_7$) (99.5%) and other common organic solvents used for the syntheses were purchased from Aldrich Co. and Lachema Co., and used as received. 2-[3-(Hydroxypropyl)amino]-6-benzylamino-9-isopropylpurine (*Bohemine*) was prepared according to the literature