SYNTHESIS, CHARACTERIZATION AND ANTIVIRAL PROPERTIES OF Pd(II) COMPLEXES WITH PENCICLOVIR

A. Garoufis [1], K. Karidi [1], N. Hadjiliadis*[1], S. Kasselouri [2], J. Kobe [3], J. Balzarini [4] and E. De Clercq[4]

[a] Laboratory of Inorganic and General Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece
[b] Department of Chemistry, Physics and Material Technology, Technological Institution of Athens, 12210 Egaleo , Greece
[c] National Institute of Chemistry, Hajdrihova 19, P.O.B. 3430, Ljubljana, Slovenia
[d] Katholieke Universiteit Leuven, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium

ABSTRACT.

With the aim to improve and extend the antiviral activity of the antiherpic drug penciclovir, to a wider spectrum of viruses, we have synthesized and characterized new binary and ternary complexes of Pd(II) of formulae *cis*-$(pen)_2PdCl_2$ and *cis*-$[(nucl)_2Pd(pen)_2]Cl_2$, where nucl = guanosine, inosine, cytidine or penciclovir. The characterization was mainly based on IR and ^1H NMR spectroscopy, and the results showed that in all prepared complexes, penciclovir coordinates to the metal through N7. The *far*-i.r. spectrum of the complex *cis*-$(pen)_2PdCl_2$ confirmed the *cis*- geometry around Pd(II). All the prepared complexes were markedly active against HSV-1 and HSV-2 strains, but not against thymidine kinase-deficient HSV-1 strains.

1. Introduction.

Acyclic nucleoside analogues are well known for their antiviral activity[1]. The antiherpic drug, acyclovir (ACV), was the first acyclic nucleoside analogue shown to be antivirally effective [2]. Various other guanosine analogues have been synthesized, among which penciclovir or 9(4-hydroxy-3-(hydroxymethyl)but-1-yl)guanine (Fig. 1). Like acyclovir, penciclovir acts through a selective inhibition of viral DNA synthesis and replication [3]. For the acyclic nucleoside analogues to be antivirally active they must be enzymatically metabolized within the herpes virus-infected cells [4]. Thus the interactions of metal ions with acyclic nucleosides and their derivatives present a great interest, because the majority of enzymes, in virus-infected and uninfected cells, require metal ions for their activity [5]. Although several metal complexes of acyclovir have been synthesized, characterized and tested against a variety of viruses [6-10], to our knowledge, until today, there are not reports on the interaction of penciclovir with metal ions.

Herein we report on the synthesis, characterization and antiviral properties of some Pd(II) complexes with penciclovir.

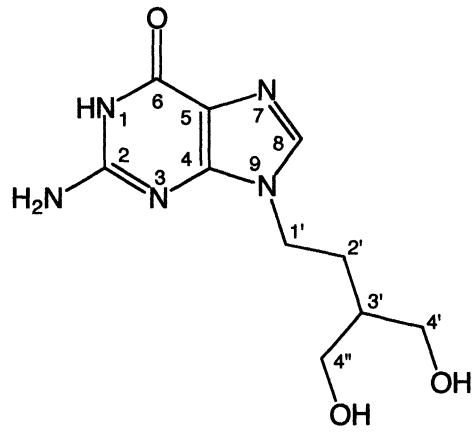

Figure 1 Molecular structure of Penciclovir

2. Materials and Methods

2.1. Materials and physical measurements.

The nucleosides guanosine, inosine, cytidine were purchased from Sigma and used without further purification. Palladium(II) chloride was obtained from Fluka A.G. Penciclovir [11], and the complexes $(guo)_2PdCl_2$, $(ino)_2PdCl_2$ and $(cyd)_2PdCl_2$ were prepared according to the literature [12].

Mid- and far-i.r. spectra were recorded on a Perkin Elmer GX spectrophotometer in KBr and polyethylene pellets, respectively. ^1H NMR spectra were obtained on a Bruker AMX 400 MHz and on Bruker AC 250 MHz instrument. The sample temperature was set at 298^0 K.

2.2 Preparation of the complexes.

cis-$[Pd(L)_2(pen)_2]Cl_2$, cis-[bis(L)bis(penciclovir)palladium(II)]Dichloride (L = guanosine , inosine, cytidine). (I), (II), (IV)

General procedure: cis-[bis(L)dichloropalladium(II)] (L:guo, ino, cyd) (0.5 mmol) was mixed with (1 mmol) of penciclovir in the solid state, and 2 ml of D_2O was added. After stirring for about 20 min at 50 ^{0}C complete dissolution was achieved. The ^{1}H-NMR spectrum of the mixture showed the end of the reaction. The complex was purified by chromatographic methods with Sephadex G-10. It was then precipitated with acetone, filtered off, washed with acetone (2 x 5 ml) and ether (2 x 5 ml) and finally dried at 110 ^{0}C under vacuum over silica.

(I) $[Pd(ino)_2(pen)_2]Cl_2$. *Anal.* Calc. C, 40.3; H, 4.5; N, 21.1.Found: C, 39.9; H, 4.9: N, 20.1.
(II) $[Pd(guo)_2(pen)_2]Cl_2$. *Anal.* Calc. C, 39.3; H, 4.6; N, 22.9. Found: C, 38.6; H, 4.9; N, 22.5.
(IV) $Pd(cyd)_2(pen)_2]Cl_2$. *Anal.* Calc. C, 39.9; H, 4.9; N, 19.6. Found: C, 39.2; H, 5.2: N, 19.3.

$[Pd(pen)_4]Cl_2$,] tetrakis(penciclovir)palladium(II) Dichloride. (III)

cis-[bis(penciclovir)dichloropalladium(II)] (0.5 mmol) was mixed with (1 mmol) of penciclovir in the solid state and 2 ml of D_2O was added. After stirring for about 20 min at 50 ^{0}C, complete dissolution was achieved. The evolution of ^{1}H-NMR spectra showed the end of the reaction. The complex was then precipitated with acetone, filtered, washed with acetone (2 x 5 ml) and ether (2 x 5 ml), and dried at 110 ^{0}C under vacuum over silica. The complex was purified by chromatography on Sephadex G-10

(III) $[Pd(pen)_4]Cl_2$. *Anal.* Calc. C, 42.3; H, 5.3; N, 24.7. Found: C, 41.2; H, 4.9; N, 23.8.

cis-$[Pd(pen)_2Cl_2]$. cis-[bis(penciclovir) dichloropalladium(II)]. (V)

Palladium chloride (0.5 mmol) was dissolved in 10 ml of 0.5N HCl by heating to about 50 ^{0}C. Penciclovir (1 mmol) was dissolved in 20 ml of 0.5N HCl. The two solutions were mixed at room temperature and stirred for 2 h. The yellow precipitate that formed was filtered, washed with cold acetone and ether and dried at 110 ^{0}C under vacuum.

(V) $Pd(pen)_2Cl_2$. *Anal.* Calc. C, 36.4; H, 4.6; N, 14.6. Found: C, 36.2; H, 4.4; N, 14.8.

3. Results and discussion.

3.1 Synthesis.

The reaction of H_2PdCl_4 with penciclovir at molar ratio 1:2, in strong acidic solutions (0.5N HCl), produces the cis-$(pen)_2PdCl_2$, because the trans- influence of pen is comparable to that of pyridine [13] (eq 1). The cis- configuration around the metal was confirmed by a Kurnakoff test [14]. A mixture of the above complex and excess of thiourea, were mixed in the solid state and dissolved in D_2O. The ^{1}H NMR spectrum of the mixture showed the presence of free penciclovir.

$$H_2PdCl_4 + 2\ pen \longrightarrow cis\text{-}(pen)_2PdCl_2 + 2\ HCl \qquad (1)$$

The reaction of cis-$(pen)_2PdCl_2$ with penciclovir in a molar ratio of 1:2 in aqueous media, formed the soluble complex [tetrakis(penciclovir)palladium(II)] Dichloride, $[Pd(pen)_4]Cl_2$, according to eq. 2

$$cis\text{-}(pen)_2PdCl_2 + 2\ pen \longrightarrow [(pen)_4Pd]Cl_2 \qquad (2)$$

Mixed nucleoside palladium(II) complexes were prepared by a similar procedure upon the reaction of cis-$(guo)_2PdCl_2$, cis-$(ino)_2PdCl_2$ and cis-$(cyt)_2PdCl_2$ with free penciclovir.

3.2 Spectroscopic characterization of the complexes.

3.2.1. Infrared spectroscopy.

The i.r. spectra of the complexes showed a strong band invariably at 1697 to 1726 cm^{-1} assigned to free _(C=O) of the 6th position of the purine ring or the 3th position of the pyrimidine ring, in the case of cyd containing complexes, excluding the formation of a Pd-O bond through the carbonyl group or the coordination through the neighboring N1. A similar behavior was observed in the infrared spectra of trans- and cis-$(nucl)_2PdCl_2$ or mixed trans- or cis-$[(nucl)_2Pd(nucl')_2]Cl_2$, were nucl or nucl_ = guanosine, inosine or cytidine [12, 13]. In addition all spectra of the prepared complexes, exhibited a broad band at about 425 to 430 cm^{-1}, attributed to Pd-N stretching vibration, suggesting coordination through N7. The far-i.r. spectrum of the binary complex cis-$(pen)_2PdCl_2$ exhibited two strong to medium intensity bands at 325 and 329 cm^{-1} assigned to Pd-Cl stretching vibration, confirming the cis- configuration of the chlorine atoms [16].

The characteristic i.r. bands for the complexes and the

TABLE I. Characteristic i.r.[a] and far-i.r.[a] bands (cm^{-1}) for the complexes and the free ligand.

Compounds	ν_a(C=O)	$\delta(NH_2)$	ν(C=C, C=N)[b]	ν (Pd-N)	ν (Pd-Cl)
Penciclovir	1692 s	1645 m	1604 s 1546 m		
cis-$(pen)_2PdCl_2$	1726 s	1642 s	1625 m 1550 b	430 m 419 w	325 s 339 m
$[(pen)_4Pd]Cl_2$	1712 s	1640 s	1620-1530 b	425 w	
cis-$[(guo)_2Pd(pen)_2]Cl_2$	1697 s	1637 s	1598 s	430 w	
cis-$[(ino)_2Pd(pen)_2]Cl_2$	1701 s	1635 s	1592 b	428 w	
cis-$[(cyd)_2Pd(pen)_2]Cl_2$	1720 s	1678 s 1664 s	1620 w 1592 b	430 m	

[a] Fourier-transform spectra. [b] skeletal vibrations. Abbreviations: b = broad, m = medium, s = strong, w = weak.

3.2.2 ^1H NMR spectroscopy.

The H8 resonance of penciclovir, in the ^1H NMR spectra of all five prepared complexes showed a downfield shift by 0.38 to 0.46 ppm, compared to the free ligand in D_2O, indicating a covalent interaction of the Pd(II) ion with the neighboring to H8, nitrogen atom of the purine ring (N7). Similar strong downfield shifts of the guanosine's H8 proton, were observed in the binary or ternary complexes of the ligand with Pd(II) [12,13]. It is noticeable that in the spectrum of cis-$(pen)_2PdCl_2$ (in DCl 1N) the H8 proton resonance shifts upfield, compared with the protonated at N7 form of penciclovir, pen (in DCl 1N), by about 0.47 ppm, indicating that the H^+ causes higher electron deshielding in the magnetic environment of the H8 nucleus than the palladium ion.

TABLE II gives the ^1H NMR chemical shifts of the prepared complexes.

TABLE II. 400 MHz ^1H NMR chemical shifts (ppm) [a,b] of the prepared complexes and the free ligand.

		PENCICLOVIR PROTONS					NUCLEOSIDE PROTONS		
Compounds	solvent	H8	H1'	H2'	H3'	H4' & H4"	H8	H2	H5
Penciclovir	D_2O	7.90	3.99	1.70	1.45	3.40			
	DCl 1N	8.69	4.06	1.67	1.46	3.49			
cis-$(pen)_2PdCl_2$	DCl 1N	8.22	4.02	1.67	1.45	3.52			
[c] $[(pen)_4Pd]Cl_2$	D_2O	8.36	4.01	1.69	1.45	3.42			
[c] cis-$[(guo)_2Pd(pen)_2]Cl_2$	D_2O	8.39	4.09	1.70	1.39	3.45	8.94		
[c] cis-$[(ino)_2Pd(pen)_2]Cl_2$	D_2O	8.28	4.06	1.72	1.45	n.a.	8.98	8.20	
[c] cis-$[(cyd)_2Pd(pen)_2]Cl_2$	D_2O	8.23	4.05	1.71	1.36	n.a.			8.18

[a] Spectra recorded at ambient temperature. [b] The values are referenced to the HDO peak which has been set at 4.82 ppm.
[c] 250 MHz spectra. n.a = not assigned.

3.3 Antiviral properties.

All prepared penciclovir complexes were markedly active against HSV-1 and HSV-2 strains but not against thymidine kinase-deficient (TK^-) HSV in E_6SM cell cultures (TABLE III). The compounds were also inactive against a variety of other viruses including vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus in HeLa cell cultures (TABLE IV) and against parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures (TABLE V). They were also not active against human immunodeficiency virus type 1 (III_B) and type 2 (ROD) in CEM and MT-4 cell cultures (data not shown). None of the compounds proved markedly cytostatic against murine leukemia L1210, murine mammary carcinoma FM3A and human lymphocyte Molt4 and CEM cells (50% inhibitory concentration > 100 μM) (except for compound II that inhibited Molt4 and CEM cell proliferation at 30-34 μM) (TABLE VI). Clearly, the compounds I-V displayed a similar antiviral spectrum as the parent compound penciclovir. However, they were not superior to penciclovir in inhibiting herpes virus-induced cytopathicity in cell culture. Also, the test compounds lost marked activity against a TK-deficient herpes simplex virus as also penciclovir did. In general, the most active compound was III that contained four penciclovir molecules for each Pd atom in the entire molecule.

In conclusion, the Pd containing penciclovir derivatives had a comparable antiviral spectrum as penciclovir (i.e. herpes simplex virus type 1 and 2), but were not superior to the parent compound.

3.4 Broad-spectrum antiviral activity assays

For herpes simplex viruses (HSV), vaccinia virus (VV), Coxsackie virus type B4, vesicular stomatitis virus (VSV), parainfluenza virus type 3, respiratory syncytial virus (RSV), Sindbis virus, Punta Toro virus and reovirus type 1, the origin of the virus stocks [17] and the assay procedures [18, 19] have been described previously. HSV assays were carried out against HSV-1 TK^+ (KOS, F and McIntyre) and HSV-2 (G and Lyons) and against HSV-1 TK^- (B2006) in embryonic skin muscle (E_6SM) and human embryonic lung (HEL) cell cultures. Ribavirin, ganciclovir, penciclovir, (S)-DHPA, BVDU and ACV were used as reference compounds.

The cytostatic activity measurements were basically described [20]. Briefly, tumor cells were seeded at ~ 250,000-300,000 cells/ml in 200 μl-wells of 96-wells microtiter plates and incubated for 2 days (L1210, FM3A) or 3 days (Molt4/C8, CEM) at 37°C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, cells were counted with a Coulter counter and the IC_{50} (50% inhibitory concentration) determined as the compound concentration required to inhibit tumor cell proliferation by 50%.

TABLE III. Cytotoxicity and antiviral activity of test compounds in E_6SM cell cultures

COMPOUND	Minimum cytotoxic concentration (_g/ml)	Minimum inhibitor concantration[b] (_g/ml)								
		Herpes simplex virus-1 TK- KOS ACV	Herpes simplex virus-1 (KOS)	Herpes simplex virus-1 (F)	Herpes simplex virus-1 (McIntyre)	Herpes simplex virus-2 (G)	Herpes simplex virus-2 (196)	Herpes simplex virus-2 (Lyons)	Vaccinia virus	Vesicular stomatitis virus
I	240	32	0.076	0.384	0.076	1.15	0.640	0.384	> 80	> 80
II	≥ 400	240	0.384	0.076	0.076	1.92	0.384	0.64	> 400	> 400
III	≥ 400	32	0.076	0.076	0.076	0.384	0.640	0.384	> 400	> 400
IV	> 400	64	0.384	0.640	0.384	1.15	1.92	1.92	> 400	> 400
V	400	48	0.256	0.076	0.076	1.28	1.92	1.92	> 400	> 400
BVDU	≥ 400	64	0.015	0.015	0.025	160	> 80	> 80	3.2	> 80
Ribavarin	≥ 400	144	144	48	240	48	240	240	48	9.6
ACG	≥ 400	48	0.076	0.076	0.076	0.051	0.076	0.076	> 400	> 400
Ganciclovir	≥ 100	1.44	0.002	0.003	0.001	0.003	0.003	0.003	> 100	> 100
Penciclovir [c]	> 100	> 100	0.14	-	-	0.40	-	-	-	

[a]Required to cause a microscopically detectable alteration of normal cell morphology.

[b]Required to reduce virus-induced cytopathogenicity by 50 %.

[c]Data obtained in human embryonic lung (HEL) cells.

3.4 Broad-spectrum antiviral activity assays

For herpes simplex viruses (HSV), vaccinia virus (VV), Coxsackie virus type B4, vesicular stomatitis virus (VSV), parainfluenza virus type 3, respiratory syncytial virus (RSV), Sindbis virus, Punta Toro virus and reovirus type 1, the origin of the virus stocks [17] and the assay procedures [18, 19] have been described previously. HSV assays were carried out against HSV-1 TK^+ (KOS, F and McIntyre) and HSV-2 (G and Lyons) and against HSV-1 TK^- (B2006) in embryonic skin muscle (E_6SM) and human embryonic lung (HEL) cell cultures. Ribavirin, ganciclovir, penciclovir, (S)-DHPA, BVDU and ACV were used as reference compounds.

The cytostatic activity measurements were basically described [20]. Briefly, tumor cells were seeded at ~ 250,000-300,000 cells/ml in 200 μl-wells of 96-wells microtiter plates and incubated for 2 days (L1210, FM3A) or 3 days (Molt4/C8, CEM) at 37°C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, cells were counted with a Coulter counter and the IC_{50} (50% inhibitory concentration) determined as the compound concentration required to inhibit tumor cell proliferation by 50%.

TABLE IV. Cytotoxicity and antiviral activity of test compounds in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4 virus	Respiratory syncytial
I	> 400	> 400	240	> 400
II	> 400	> 400	> 400	> 400
III	> 400	> 400	> 400	> 400
IV	> 400	> 400	> 400	> 400
V	> 400	240	240	> 400
BVDU	> 400	> 400	> 400	> 400
(S)-DHPA	> 400	240	> 400	> 400
Ribavirin	> 400	48	48	0.64

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50 %.

TABLE V. Cytotoxicity and antiviral activity of test compounds in Vero cell cultures.

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitor concantration[b] (μg/ml)				
		Punta Toro virus	Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4
I	≥ 400	240	> 400	> 400	240	240
II	≥ 400	> 400	> 400	> 400	> 400	240
III	400	> 80	> 80	> 80	> 80	240
IV	≥ 400	> 400	> 400	> 400	> 400	400
V	≥ 400	400	> 400	> 400	400	240
BVDU	≥ 400	> 80	> 80	> 80	> 80	> 80
(S)-DHPA	> 400	> 400	16	240	> 400	400
Ribavarine	> 400	9.6	48	80	80	240

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce a virus-induced cytopathogenicity by 50 %.

TABLE VI. Inhibitory effects of test compounds on the proliferation of murine leukemia cells (L1210/0), murine mammary carcinoma cells (FM3A) and human T-lymphocyte cells (Molt4/C8, CEM/0)

Compound	IC_{50} (μM)			
	L1210	FM3A	Molt4/C8	CEM
I	196 ± 76	> 250	≥ 250	199 ± 72
II	115 ± 50	170 ± 3	34 ± 2	30 ± 5
III	175 ± 105	> 250	198 ± 5	207 ± 37
IV	> 250	> 250	> 250	> 250
V	110 ± 16	112 ± 3	125 ± 12	132 ± 12

[a]50% inhibitory concentration.

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