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

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ABSTRACT

With the aim to improve and extend the antiviral activity of the antiherpetic 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)2PdCl2 and cis-[nucleo]2Pd(pen)2Cl2, where nucleo = 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)2PdCl2 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 antiherpetic 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.

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)2PdCl2, (ino)2PdCl2 and (cyd)2PdCl2 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 250 MHz instrument. The sample temperature was set at 298°K.

2.2 Preparation of the complexes.
cis-[Pd(L)₂(pen)₂]Cl₂, 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, cd) (0.5 mmol) was mixed with (1 mmol) of penciclovir in the solid state, and 2 ml of D₂O was added. After stirring for about 20 min at 50 °C complete dissolution was achieved. The ¹H-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 °C under vacuum over silica.

(I) [Pd(ino)₂(pen)₂]Cl₂, 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]Cl₂, 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]Cl₂, Anal. Calc. C, 39.9; H, 4.9; N, 19.6. Found: C, 39.2; H, 5.2; N, 19.3.

 cis-[bis(penciclovir)dichloropalladium(II)] (0.5 mmol) was mixed with (1 mmol) of penciclovir in the solid state and 2 ml of D₂O was added. After stirring for about 20 min at 50 °C complete dissolution was achieved. The evolution of ¹H-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 °C under vacuum over silica. The complex was purified by chromatography on Sephadex G-10.

(III) [Pd(pen)₄]Cl₂, Anal. Calc. C, 42.3; H, 5.3; N, 24.7. Found: C, 41.2; H, 4.9; N, 23.8.

 cis-[Pd(pen)Cl₂], 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 °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 °C under vacuum.

(V) Pd(pen)₂Cl₂. 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 H₂PdCl₄ with penciclovir at molar ratio 1:2, in strong acidic solutions (0.5N HCl), produces the cis-(pen)₂PdCl₂, 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 D₂O. The ¹H NMR spectrum of the mixture showed the presence of free penciclovir.

H₂PdCl₄ + 2 pen → cis-(pen)₂PdCl₂ +2 HCl (1)

The reaction of cis-(pen)₂PdCl₂ with penciclovir in a molar ratio of 1:2 in aqueous media, formed the soluble complex [tetrakis(penciclovir)palladium(II)]Dichloride, [Pd(pen)₄]Cl₂, according to eq. 2

cis-(pen)₂PdCl₂ + 2 pen → [(pen)₄Pd]Cl₂ (2)

Mixed nucleoside palladium(II) complexes were prepared by a similar procedure upon the reaction of cis-(guo)₂PdCl₂, cis-(ino)₂PdCl₂ and cis-(cyt)₂PdCl₂ 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)₂PdCl₂ or mixed trans- or cis-{(nucl)₂Pd(nucl')₂}Cl₂, 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)₂PdCl₂ 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

| Compounds                  | ν(C=O) (cm⁻¹) | δ(NH₂) (cm⁻¹) | ν(C=C, C=N)² | ν(Pd-N) (cm⁻¹) | ν(Pd-Cl) (cm⁻¹) |
|----------------------------|--------------|--------------|-------------|---------------|---------------|
| Penciclovir                | 1692 s       | 1645 m       | 1604 s 1546 m |                |               |
| cis-(pen)₂PdCl₂            | 1726 s       | 1642 s       | 1625 m 1550 b | 430 m 419 w    | 325 s 339 m   |
| [(pen)₂PdCl₂               | 1712 s       | 1640 s       | 1620-1530 b  | 425 w          |               |
| cis-{(guo)₂Pd(pen)₂}Cl₂    | 1697 s       | 1637 s       | 1598 s      | 430 w          |               |
| cis-{(ino)₂Pd(pen)₂}Cl₂    | 1701 s       | 1635 s       | 1592 b      | 428 w          |               |
| cis-{(cyd)₂Pd(pen)₂}Cl₂    | 1720 s       | 1678 s 1664 s| 1620 w 1592 b| 430 m          |               |

¹ Fourier-transform spectra. ² Skeletal vibrations. Abbreviations: b = broad, m = medium, s = strong, w = weak.
3.2.2 $^1$H NMR spectroscopy.

The H8 resonance of penciclovir, in the $^1$H 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$_2$O, 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)$_2$PdCl$_2$ (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 $^1$H NMR chemical shifts of the prepared complexes.

| TABLE II. 400 MHz $^1$H NMR chemical shifts (ppm)$^{ab}$ of the prepared complexes and the free ligand. |
|---|---|---|---|---|---|---|---|---|---|
| Compounds solvent | H8 | H1' | H2' | H3' | H4' & H4'' | H8 | H2 | H5 |
| Penciclovir D$_2$O | 7.90 | 3.99 | 1.70 | 1.45 | 3.40 |
| DCI 1N | 8.69 | 4.06 | 1.67 | 1.46 | 3.49 |
| cis-(pen)$_2$PdCl$_2$ DCI 1N | 8.22 | 4.02 | 1.67 | 1.45 | 3.52 |
| [(pen)$_2$PdCl] | D$_2$O | 8.36 | 4.01 | 1.69 | 1.45 | 3.42 |
| cis-[(guo)$_2$Pd(pen)$_2$]Cl$_2$ D$_2$O | 8.39 | 4.09 | 1.70 | 1.39 | 3.45 | 8.94 |
| cis-[(ino)$_2$Pd(pen)$_2$]Cl$_2$ D$_2$O | 8.28 | 4.06 | 1.72 | 1.45 | n.a. | 8.98 | 8.20 |
| cis-[(cyd)$_2$Pd(pen)$_2$]Cl$_2$ D$_2$O | 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.

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$_0$SM 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 (IIIb) 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 $\mu$M) (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$_0$SM) 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 $\mu$-wells of 96-wells microtiter plates and incubated for 2 days (L1210, FM3A) or 3 days (Molt4/C8, CEM) at 37$^\circ$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 E6SM cell cultures

| COMPOUND | Minimum cytotoxic concentration (µg/ml) | Minimum inhibitor concentration (µg/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 | 480 | 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 | > 100 | > 100 |
| Penciclovir<sup>a</sup> | > 100 | > 100 | 0.14 | - | - | 0.40 | - | - | - |

<sup>a</sup>Required to cause a microscopically detectable alteration of normal cell morphology.

<sup>b</sup>Required to reduce virus-induced cytopathogenicity by 50%.

<sup>c</sup>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_SM) and human embryonic lung (HEC) 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 (Mol4/C8, CEM) at 37°C in a humidified CO2-controlled atmosphere. At the end of the incubation period, cells were counted with a Coulter counter and the IC₅₀ (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 concentrationa (µg/ml) | Minimum inhibitory concentrationb (µg/ml) |
|----------|----------------------------------------|----------------------------------------|
|          | Vesicular stomatitis virus              | Coxsackie virus B4 virus                | Respiratory syncytial virus |
| 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\(^a\) (\(\mu\)g/ml) | Minimum inhibitor concentration\(^b\) (\(\mu\)g/ml) |
|----------|---------------------------------------------|---------------------------------------------|
|          | Punta Toro virus | Parainfluenza-3 virus | Reovirus-1 | Sindbis virus | Coxsackie virus B4 |
| I        | \(\geq 400\)     | 240                          | \(> 400\)  | \(> 400\)    | 240                  |
| II       | \(\geq 400\)     | \(> 400\)                    | \(> 400\)  | \(> 400\)    | 240                  |
| III      | 400               | \(> 80\)                     | \(> 80\)   | \(> 80\)     | \(> 80\)             |
| IV       | \(\geq 400\)     | \(> 400\)                    | \(> 400\)  | \(> 400\)    | 400                  |
| V        | \(\geq 400\)     | 400                           | \(> 400\)  | \(> 400\)    | 240                  |
| BVDU     | \(\geq 400\)     | \(> 80\)                     | \(> 80\)   | \(> 80\)     | \(> 80\)             |
| (S)-DHPA | \(> 400\)        | \(> 400\)                    | 16         | 240          | 400                  |
| Ribavirine | \(> 400\)     | 9.6                           | 48         | 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 |

*50% inhibitory concentration.

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