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Palladium(II) and platinum(II) complexes of (1*R*,2*R*)-(−)-1,2-diaminocyclohexane (DACH) with various carboxylato ligands and their cytotoxicity evaluation

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Several palladium(II) and platinum(II) complexes analogous to oxaliplatin, bearing the enantiomerically pure (1*R*,2*R*)-(−)-1,2-diaminocyclohexane (DACH) ligand, of the general formula {MX_2[(1*R*,2*R*)-DACH]}, where M = Pd or Pt, X = $\frac{1}{2}$ $(COO)_2$, $\frac{1}{2}$ $CH_2(COO)_2$, ½ $CH_2CH_2C(COO)_2$, ½ $CH_2(CH_2)_2C(COO)_2$, $\frac{1}{2}${1,1′-$C_5H_8(CH_2COO)_2$}, $\frac{1}{2}$[1,1′-$C_6H_{10}(CH_2COO)_2$], $\frac{1}{2}$[1,1′-$(COO)_2$ferrocene], CH_2CH_2CHCOO, $CH_2(CH_2)_2CHCOO$, $CH_2(CH_2)_3CHCOO$, MeCOO and Me_3CCOO, were synthesized. All the complexes prepared were characterized physicochemically and spectroscopically. Some selected complexes were screened *in vitro* against several tumor cell lines and the results were compared with reference standard drug, oxaliplatin.

Keywords: platinum and palladium complexes; DACH; carboxylates

Introduction

The potent anti-neoplastic agent oxaliplatin, {$Pt(C_2O_4)$[(1*R*, 2*R*)-DACH]}, after discovery of cisplatin, *cis*-[$PtCl_2(NH_3)_2$], and the discovery of carboplatin, {$Pt[(OOC)_2CCH_2CH_2CH_2](NH_3)_2$}, attracts the interest of the researchers all over the world in synthesizing a large number of platinum complexes and to a lesser extent palladium complexes. Some of these complexes are already undergoing preclinical and clinical trials aiming to find a more active and less toxic target complex and these have mostly been covered by a book[1] and recent reviews[2–4] and articles.[5–8] In continuation of our previous findings in this field of research; summarized in a review[9] and recently in a reported work,[10–13] we attempted to synthesize a new set of palladium(II) and platinum(II) complex analogs to oxaliplatin, bearing the enantiomerically pure (1*R*,2*R*)-DACH ligand with different carboxylato groups, i.e. complexes **1**–**26** (Fig. 1). Some of the prepared complexes were investigated for *in vitro* antitumor activity in a panel of 12 human tumor cell lines.

Materials and Methods

General

Elemental analyses were performed on EA 1110 CHNS-O CE instrument and ^{1}H- and ^{13}C-NMR spectra were recorded on 200 MHz Varian Unity 500 and Gemini 200 spectrometers at room temperature, respectively, using $CDCl_3$ or DMSO-d_6 or D_2O as solvents with Me_4Si as an internal reference. The NMR spectra and the elemental analyses were determined at the Institut für Anorganische Chemie, Martin-Luther-Universität Halle-Wittenberg, Germany. IR spectra were recorded for KBr disks on a Pye-Unicam FTIR spectrophotometer.

Starting Materials

The K_2PtCl_4 and K_2PdCl_4 and the ligands (1*R*, 2*R*)-DACH were purchased from Fluka. The acids $(COOH)_2 \cdot 2H_2O$, $CH_2(COOH)_2$, $CH_2CH_2C(COOH)_2$, $CH_2CH_2CH_2C(COOH)_2$, 1,1′-ferrocene dicarboxylic acid [1,1′-$C_5H_8(CH_2COOH)_2$], 1,1′-$C_6H_{10}(CH_2COOH)_2$, CH_3COOH, Me_3CCOOH, $CH_2CH_2CHCOOH$, $CH_2(CH_2)_2CHCOOH$, $CH_2(CH_2)_3CHCOOH$ and $CH_2(CH_2)_4CHCOOH$ were commercial products and were used as supplied. The silver acetate was a commercial product and the K-carboxylates were prepared by adding one equivalent of KOH for each COOH group in ethanol until complete precipitation of the salt occurred. The solid formed

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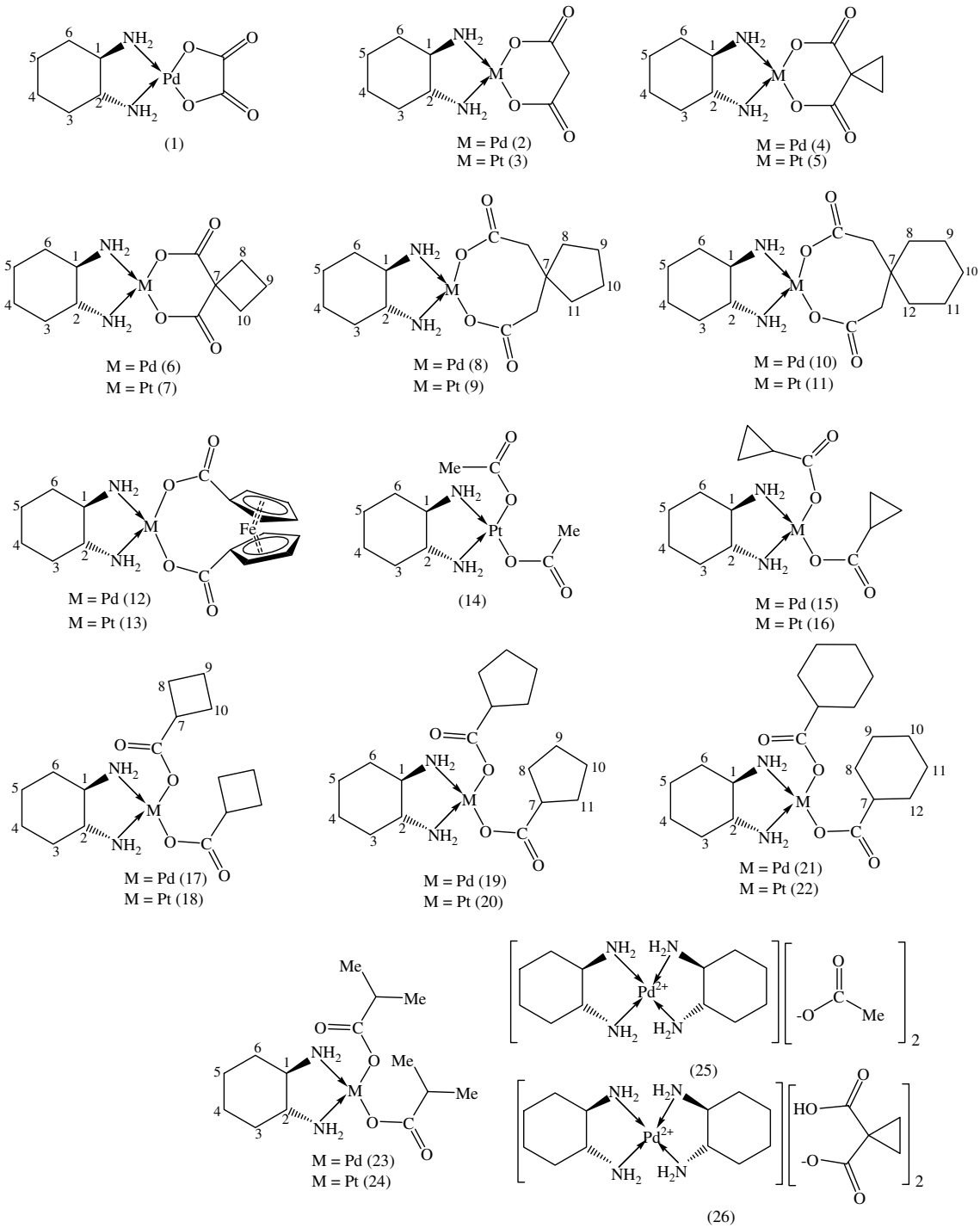

Figure 1. The palladium(II) and platinum(II) complexes (**1–26**) of (1*R*,2*R*)-DACH used in this study.

was filtered off, washed with ethanol and ether, and dried under vacuum. The yield in all cases was >80%.

Preparation of Complexes

{MCl$_2$[(1R,2R)-DACH]}, M = Pd or Pt

These starting complexes were prepared according to a standard method from K_2MCl_4 (M = Pd or Pt) and (1*R*,2*R*)-DACH (1 : 1 molar ratio) in H_2O in *ca* >90% yield.

{Pd[(OOC)$_2$][(1R,2R)-DACH]} (1 · 3/4H$_2$O)

The {PdCl$_2$[(1*R*, 2*R*)-DACH]} complex (0.32 g, 1.1 mmol) was suspended in water (10 ml) and a solution of $AgNO_3$ (0.34 g, 0.2 mmol) in water (5 ml) was added. The mixture was heated gently with stirring for *ca* 2 h away from light and filtered while hot. The pale yellow filtrate was heated with charcoal and filtered. To the colorless filtrate was added a pre-prepared solution of $K_2C_2O_4$ (0.30 g, 0.18 mmol) in water (5 ml). The mixture was heated gently until

yellow solid started to deposit. In a similar manner, complexes **2–13** and **15–24** were prepared.

*Pt[(OOCCH$_3$)$_2$][(1*R*,2*R*)-DACH]} (**14**)*

The {PtCl$_2$[(1*R*, 2*R*)-DACH]} complex (0.30 g, 0.8 mmol) was suspended in water (10 ml) and a solution of AgOOCCH$_3$ (0.25 g, 1.5 mmol) in water (10 ml) was added. The mixture was heated gently with charcoal away from light for *ca* 2 h and the mixture was then filtered while hot. The yellowish solution was evaporated until the solid started to deposit. This was left aside for few hours until complete precipitation occurred. The solid was filtered off, washed with small portions of cold water and dried in vacuum to give complex **14**. A similar procedure was repeated by using palladium instead of platinum, the reaction gave complex **25** as the ionic bis(DACH) complex, i.e. {Pd[(1*R*, 2*R*)-DACH]$_2$}(OOCCH$_3$)$_2$.

{Pd[(OOC)$_2$CCH$_2$CH$_2$][(1*R*,2*R*)-DACH]} (**4**)
and {Pd[(1*R*,2*R*)-DACH]$_2$}[O(OH)(CO)CCH$_2$CH$_2$]$_2$ (**26**)

The {PdCl$_2$[(1*R*, 2*R*)-DACH]} (0.30 g, 1.0 mmol) was suspended in water (10 ml) and a solution of AgNO$_3$ (0.32 g, 1.9 mmol) in water (5 ml) was added. The mixture was heated gently with stirring for *ca* 2 h away from light and filtered while hot. The pale yellow filtrate was heated with charcoal and filtered. To the colorless filtrate was added a pre-prepared solution of K$_2$(OOC)$_2$CCH$_2$CH$_2$ (0.25 g, 1.2 mmol) in water (5 ml). The mixture was heated gently until the solution became turbid. It was left in the refrigerator for several hours to give a pale yellow solid which was filtered off, washed with small portions of cold water and dried in vacuum. Analysis of this product showed it to be complex **4**. The yield was 40% based on the palladium starting material. On leaving the mother liquor aside for slow evaporation, colorless crystals started to deposit. After complete crystallization, the crystals were collected by decantation and dried in air. Analyses of theses crystals including preliminary X-ray diffraction showed it to be the ionic bis(DACH), i.e. complex **26** with a yield of 24% (further X-ray details about complex **26** will be reported elsewhere in the literature).[14]

IR (cm^{-1}) of complexes **1–26**: 1600–1650s (C═O), 3100–3300 s, sharp (NH) and the frequency of water of solvation of complexes **1, 4, 5, 7–18, 20, 22–26** appeared as a broad band centered at *ca* 3450 cm^{-1}.

^{1}H-NMR of complex **1** (DMSO-d$_6$): δ (ppm); 2.1 (m, 2H, α-H), 1.1 (m, 2H, β-H), 1.6(m, 2H, γ-H), 4.7–5.3 (m, 4H, NH$_2$). ^{13}C-NMR of complex **1**: δ (ppm); 59.9 (α-C), 23.7 (β-C), 32.4 (γ-C), 166.0 (C═O). (Hydrogens α, β, γ and the corresponding carbons are for 1,2, 3,6 and 4,5 positions of 1,2-cyclohexyl group, respectively.)

^{1}H-NMR of complex **7** (DMSO-d$_6$): δ (ppm); 2.1 (m, 2H, α-H), 1.0–1.2 (m, 2H, β-H), 1.8 (m, 2H, γ-H), 5.1–5.8 (m, 4H, NH$_2$), 1.65 (q, 2H, cyclobutyl-C$_9$-H), 2.5 (m, 4H, cyclobutyl-C$_{8,10}$-H). ^{13}C-NMR of complex **7**: δ 62.0 (α-C), 24.0 (β-C), 31.4 (γ-C), 177.1 (C═O), 55.4 (cyclobutyl-C$_7$), 30.1(cyclobutyl-C$_{8,10}$), 14.8 (cyclobutyl-C$_9$).

^{1}H-NMR of complex **15** (DMSO-d$_6$): δ (ppm); 2.2 (b, 2H, α-H), 0.9–1.1 (m, 2H, β-H), 1.8 (m, 2H, γ-H), 5.0–5.1 (m, 4H NH$_2$), 0.51 (m, 8H, cyclopropyl-C$_{8,9}$-H), 4.4 (m, 2H, cyclopropyl-C$_7$-H). ^{13}C-NMR of complex **15**: δ 60.3 (α-C), 23.8 (β-C), 32.3 (γ-C), 180.0 (C═O), 14.5 (cyclopropyl-C$_7$), 6.9 (cyclopropyl-C$_{8,9}$).

^{1}H-NMR of complex **18** (DMSO-d$_6$): δ (ppm); 2.0 (m, 2H, α-H), 1.3–1.5 (m, 2H, β-H), 1.7 (m, 2H, γ-H), 5.6–6.1 (m, 4H, NH$_2$), 1.0 (m, 4H, cyclobutyl-C$_9$-H), 2.1 (m, 8H, cyclobutyl-C$_{8,10}$-H), 3.0 (q, 2H, cyclobutyl-C$_7$-H). ^{13}C-NMR of complex **18**: δ 60.4 (α-C), 23.8 (β-C), 31.5 (γ-C), 180.4 (C═O), 61.5 (cyclobutyl-C$_7$), 25.6 (cyclobutyl-C$_{8,10}$), 17.7 (cyclobutyl-C$_9$).

^{1}H-NMR of complex **19** (CDCl$_3$): δ (ppm); 1.9 (m, 2H, α-H), 1.3–1.5 (m, 2H, β-H), 1.7 (m, 2H, γ-H), 4.7–5.2 (m, 4H, NH$_2$), 2.5 (q, 8H, cyclopentyl-C$_{9,10}$-H), 2.82 (m, 8H, cyclopentyl-C$_{8,11}$-H), 2.75 (q, 2H, cyclopentyl-C$_7$-H). ^{13}C-NMR of complex **19**: δ 60.7 (α-C), 24.1 (β-C), 33.2 (γ-C), 184.2 (C═O), 46.3 (cyclopentyl-C$_7$), 30.6 (cyclopentyl-C$_{8,11}$), 25.9 (cyclopentyl-C$_{9,10}$).

^{1}H-NMR of complex **21** (CDCl$_3$): δ (ppm); 2.0 (m, 2H, α-H), 1.14 (m, 2H, β-H), 1.73 (m, 2H, γ-H), 4.6–5.3 (m, 4H, NH$_2$), 2.85 (m, 8H, cyclohexyl-C$_{8,12}$-H), 2.4 (m, 2H, cyclohexyl-C$_7$-H) and cyclohexyl-C$_{9,10,11}$-H obscured by other signals. ^{13}C-NMR of complex **21**: δ 60.7 (α-C), 24.0 (β-C), 33.5 (γ-C), 183.7 (C═O), 45.7 (cyclohexyl-C$_7$), 30.5 (cyclohexyl-C$_{8,12}$), 25.9 (cyclohexyl-C$_{9,11}$), 26.1 (cyclohexyl-C$_{10}$).

^{1}H-NMR of complex **23** (DMSO-d$_6$): δ (ppm); 2.2 (m, 2H, α-H), 1.18–1.47 (m, 2H, β-H), 1.84 (m, 2H, γ-H), 4.34–5.20 (m, 4H, NH$_2$), 1.0 (s, 18H, CH$_3$). ^{13}C-NMR of complex **23**: δ 60.0 (α-C), 23.6 (β-C), 32.2 (δ-C), 183.6 (C═O), 38.6 (*C*-Me$_3$), 28.3 (CH$_3$).

^{1}H-NMR of complex **24** (DMSO-d$_6$): δ (ppm); 2.3 (m, 2H, α-H), 1.3–1.47 (m, 2H, β-H), 1.9 (m, 2H, γ-H), 5.7–6.1 (m, 4H, NH$_2$), 1.0 (s, 18H, CH$_3$). ^{13}C-NMR of complex **24**: δ 60.4 (α-C), 23.9 (β-C), 31.5 (γ-C), 183.4 (C═O), 38.25 (*C*-Me$_3$), 28.1 (CH$_3$).

^{1}H-NMR of complex **25** (D$_2$O): δ (ppm); 2.3 (m, 4H, α-H), 1.0–1.1 (m, 8H, β-H), 1.5–1.85 (m, 8H, γ-H), signals due to NH$_2$ exchanged with D$_2$O, 1.8 (s, 6H, CH$_3$). ^{13}C-NMR of complex **25**: δ 60.0 (α-C), 23.4 (β-C), 32.9 (γ-C), 181.5 (C═O), 23.3 (CH$_3$).

^{1}H-NMR of complex **26** (D$_2$O): δ (ppm); 2.4 (m, 4H, α-H), 1.1–1.2 (m, 8H, β-H), 1.6–2.0 (m, 8H, γ-H), signals due to NH$_2$ and OH exchanged with D$_2$O, signals due to cyclopropyl hydrogens obscured by other signals. ^{13}C-NMR of complex **26**: δ 60.2 (α-C), 23.6 (β-C), 33.1 (γ-C), 179.4 (C═O), 60.3 (cyclopropyl-C$_7$), 20.4 (cyclopropyl-C$_{8,9}$).

Cytotoxicity of the Complexes

Cell lines

Nine cell lines were derived from the Oncotest collection comprising gastric (GXF 251L), lung (LXFA 629L, LXFL 529L), mammary (MAXF 401NL), renal (RXF 486L, RXF 944L) and uterine cancer (UXF 1138L) as well as melanoma (MEXF 462NL, MEXF 514L). They were established from human tumor xenografts as described by Roth *et al*.[15] The origin of the donor xenografts was described by Fiebig *et al*.[16] The other three cell lines (H460, MCF-7, and PC3M) were kindly provided by the National Cancer Institute (Bethesda, MD, USA). Human tumor cells were grown at 37 °C in a humidified atmosphere (95% air, 5% CO$_2$) in RPMI 1640 medium (PAA, Cölbe, Germany) supplemented with 10% fetal calf serum (PAA, Cölbe, Germany) and 0.1% gentamicin (PAA, Cölbe, Germany). Cells were routinely passaged once or twice weekly. They were maintained no longer than 20 passages in culture.

Assay

A modified propidium iodide assay was used to assess the effects of the complexes on the growth of the human tumor cell lines.[17,18] Briefly, cells were harvested from exponential phase cultures by trypsinization, counted and plated in 96-well flat-bottomed microtiter plates at a cell density dependent on the cell line (4–10,000 viable cells per well). After 24 h recovery to allow the cells to resume exponential growth, 10 µl of culture medium

(six control wells per plate) or culture medium containing the test complexes were added to the wells. Each concentration was plated in triplicate. Following 4 days of continuous drug exposure, cell culture medium with or without drug was replaced by 200 µl of an aqueous propidium iodide (PI) solution (7 µg ml^{-1}). Since PI only passes leaky or lysed cell membranes, DNA of dead cells will be stained and measured, while living cells will not be stained. To measure the proportion of living cells, cells were permeabilized by freezing the plates, resulting in death of all cells. After thawing of the plates fluorescence was measured using the Cytofluor 4000 microplate reader (excitation 530 nm, emission 620 nm), giving a direct relationship to the total cell number. Growth inhibition/stimulation was expressed as treated/control × 100 (%T/C). Antitumor activity was defined as inhibition of tumor growth to less than 30% to the medium-treated control cells. IC_{50}, IC_{70} and IC_{90} values were determined by plotting complex concentration vs cell viability. IC_{50} and IC_{70} values are shown as median of three independent experiments.

Results and Discussion

Synthesis of Complexes

The reaction of 1 : 2 molar ratio of $\{MCl_2\{[1R,2R)$-DACH]}, M = Pd or Pt, and $AgNO_3$ in water afforded the diaqua complex $\{M[(1R,2R)$-DACH$](H_2O)_2\}^{2+}$ which upon treatment with the potassium salts of carboxylic and dicarboxylic acids afforded complexes **1**–**24** (Fig. 1). Some of the platinum complexes were previously reported elsewhere in the literature.[19,20] Complex **14** was an exceptional case; it could not be prepared from the reaction above but it could be prepared directly from $\{PtCl_2[(1R,2R)$-DACH]} and $AgOOCCH_3$ in water in 1 : 2 molar ratio. On the contrary, the reactions of $\{PdCl_2[(1R,2R)$-DACH]} with (a) $AgOOCCH_3$ (1 : 2 molar ratio) or (b) the potassium salt of $\overline{CH_2CH_2C(COOH)_2}$ (1 : 1 molar ratio) in water proceeded in different ways. The first reaction gave the ionic bis(DACH) complex **25** only while the second reaction gave both the mono(DACH), **4**, and the ionic bis(DACH), **26** (Figs 1 and 2). The course of the second reaction is outlined in Fig. 2, in which complex **4** was immediately precipitated on the addition of $(KOOC)_2\overline{CCH_2CH_2}$ to the solution of the cation $\{M[(1R,2R)$-DACH$](H_2O)_2\}^{2+}$ in a low yield. Upon slow evaporation of the mother liquor, complex **26** crystallized as a colorless product.

Almost all the complexes formed are solvated with a number of water molecules, as is clear from the elemental analysis, IR and ^{1}H-NMR spectral data. The physical properties of the complexes are compiled in Table 1 and the ^{1}H- and ^{13}C-NMR data of some of the complexes are discussed in the Experimental.

With few exceptions, all the complexes showed decomposition temperatures around 200 °C. The constitution of all complexes **1**–**26** follows from the results of elemental analyses (Table 1), IR and NMR spectral data (data given in the Experimental). The solvated H_2O was confirmed by the strong and broad IR band centred at *ca* 3450 cm^{-1}. Furthermore, IR spectra showed characteristic bands assigned to υ(N–H), υ(C–H) and υ(C═O) stretching frequencies. The latter appeared as strong bands with shoulders above 1600 cm^{-1}, being characteristic for CO of the carboxylato groups bonded to metals.[11,21]

The ^{1}H- and ^{13}C-NMR spectral data of some selected complexes (**1, 7, 15, 18, 19, 21, 23**–**26**) were recorded (see Experimental). The ^{1}H-NMR spectra revealed the presence of signals due to the protons of the cyclohexyl and NH_2 groups of the (1*R*,2*R*)-1,2-diaminocyclohexane ligands and the carboxylato ligands (except for complex **1,** having an oxalato ligand). As expected, the ^{13}C-NMR spectra of the complexes showed resonances of the cyclohexyl carbons and of the carboxylato carbons.[21,22]

Cytotoxic Activity

The selected complexes. **3, 5, 7, 9, 11, 16, 18, 20** and **22** together with the starting material $\{PtCl_2[(1R,2R)$-DACH]} and oxaliplatin (the third-generation anticancer drug)[23] were investigated for their antitumor activity *in vitro* in a cellular proliferation assay at five concentrations (0.003, 0.03, 0.3, 3.0 and 30 µg ml^{-1}) in a panel of 12 permanent human tumor cell lines, comprising gastric,

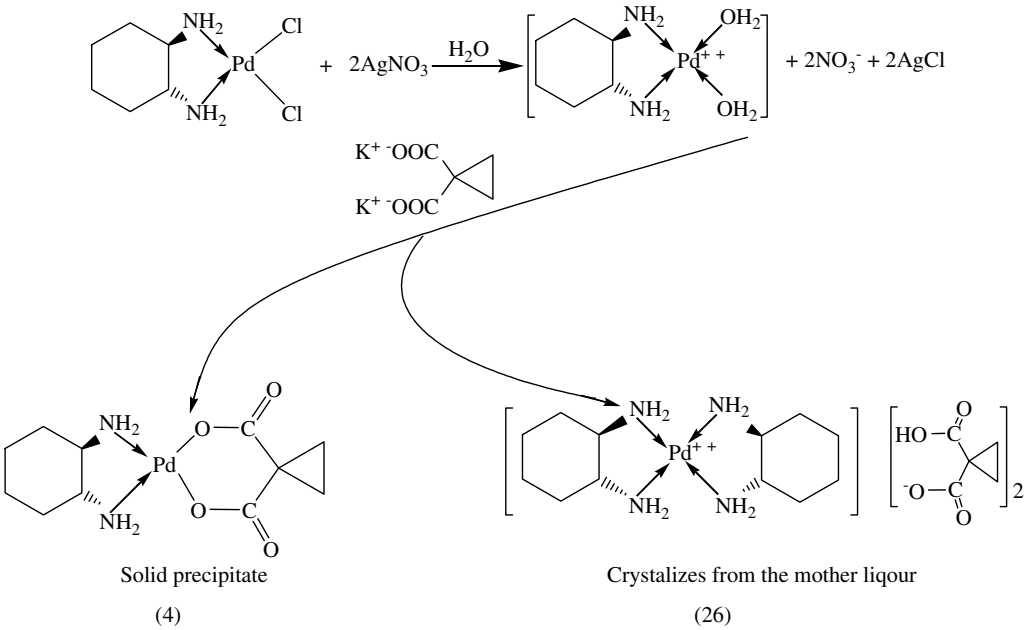

Figure 2. Schematic diagram of the reaction leading to the mono (DACH), **4** and the ionic bis(DACH), **26** products.

Table 1. Physical and analytical data of complexes **1**–**26**

Complex number	Color	$T_{dec.}$ (°C)	Yield (%)	Molecular formula (Molecular Weight)	Analysis: Found (Calcd) (%) C	H	N
1	Bright yellow	200–202	70	$C_8H_{14}N_2O_4Pd \cdot 3/4H_2O$ (322.13)	29.50(29.82)	5.03(4.85)	8.40(8.70)
2	Yellow	204–206	60	$C_9H_{16}N_2O_4Pd$ (322.65)	33.62(33.50)	5.26 (5.00)	8.68(8.68)
3	White	230–232	65	$C_9H_{16}N_2O_4Pt$ (411.34)	26.14(26.28)	3.99(3.92)	6.81(6.81)
4	Pale yellow	200–202	40	$C_{11}H_{18}N_2O_4Pd \cdot 0.5H_2O$ (355.68)	36.88 (37.15)	5.46(5.38)	7.80(7.88)
5	Off-white	228–230	72	$C_{11}H_{18}N_2O_4Pt \cdot H_2O$ (453.38)	29.29(29.14)	4.46(4.45)	6.20(6.18)
6	Pale yellow	198–200	60	$C_{12}H_{20}N_2O_4Pd$ (362.71)	39.90 (39.74)	5.50(5.56)	7.74(7.73)
7	Milky	229–232	70	$C_{12}H_{20}N_2O_4Pt \cdot H_2O$ (469.42)	30.69(30.70)	4.75(4.72)	5.94(5.97)
8	Pale yellow	198–201	81	$C_{15}H_{26}N_2O_4Pd \cdot H_2O$ (422.81)	42.77(42.61)	6.70 (6.67)	6.56(6.63)
9	Milky	194–196	77	$C_{15}H_{26}N_2O_4Pt \cdot 2H_2O$ (529.51)	33.73(34.02)	5.64(5.71)	5.56 (5.29)
10	Pale yellow	200–202	72	$C_{16}H_{28}N_2O_4Pd \cdot H_2O$ (436.84)	44.24(44.00)	6.61(6.92)	6.27(6.41)
11	Off-white	218–220	68	$C_{16}H_{28}N_2O_4Pt \cdot 2H_2O$ (543.54)	34.93(35.36)	5.59(5.56)	5.62(5.16)
12	Yellow-orange	188–190	63	$C_{18}H_{24}N_2O_4FePd \cdot H_2O$ (512.68)	42.70(42.17)	5.03(5.11)	5.20(5.47)
13	Yellow-brown	190–192	66	$C_{18}H_{24}N_2O_4FePt \cdot 2H_2O$ (619.38)	34.32(34.91)	4.40(4.56)	4.47(4.52)
14	Off-white	210–212	57	$C_{10}H_{20}N_2O_4Pt \cdot 5H_2O$ (545.40)	26.15(26.43)	5.02(5.10)	6.20(6.17)
15	Pale yellow	194–196	71	$C_{14}H_{24}N_2O_4Pd \cdot H_2O$ (408.77)	41.36(41.37)	6.28(6.41)	6.88(6.85)
16	White	200–202	70	$C_{14}H_{24}N_2O_4Pt \cdot H_2O$ (497.46)	33.61(33.80)	5.18(5.27)	5.67(5.63)
17	Pale yellow	190–192	69	$C_{16}H_{28}N_2O_4Pd \cdot H_2O$ (436.84)	44.17 (44.00)	6.67(6.92)	6.27(6.41)
18	Milky	192–194	73	$C_{16}H_{28}N_2O_4Pt \cdot 2H_2O$ (543.54)	35.47(35.36)	5.63(5.93)	5.40(5.16)
19	Yellow-green	170–182	64	$C_{18}H_{32}N_2O_4Pd$ (446.87)	48.61(48.38)	7.20 (7.22)	5.95(6.27)
20	Creamy	212–214	67	$C_{18}H_{32}N_2O_4Pt \cdot 1.5H_2O$ (562.59)	38.48(38.43)	5.86 (6.23)	5.10(5.00)
21	Yellow-green	175–177	71	$C_{20}H_{36}N_2O_4Pd$ (474.93)	50.46(50.58)	7.71(7.64)	5.80 (5.90)
22	Milky	200–203	80	$C_{20}H_{36}N_2O_4Pt \cdot H_2O$ (581.63)	41.05(41.30)	6.45(6.58)	4.81 (4.82)
23	Pale yellow	210–212	77	$C_{16}H_{32}N_2O_4Pd \cdot H_2O$ (440.87)	43.90(43.59)	7.77 (7.77)	6.31(6.36)
24	White	218–220	83	$C_{16}H_{32}N_2O_4Pt \cdot H_2O$ (529.56)	35.74(36.29)	6.41(6.47)	5.18(5.29)
25	Bright yellow	194–196	42	$C_{16}H_{34}N_4O_4Pd \cdot 4H_2O$ (524.95)	36.44 (36.61)	7.68(8.06)	10.43 (10.67)
26	Colorless	198–200	24	$C_{22}H_{38}N_4O_8Pd \cdot 0.5H_2O$ (601.99)	43.90(43.89)	6.46(6.53)	9.32 (9.31)

Table 2. *In vitro* antitumor activity of some selected oxaliplatin derivatives[a]

Complex no.	IC_{50} ($\mu g\ ml^{-1}$)	IC_{70} ($\mu g\ ml^{-1}$)	Active/total[b] at 3 $\mu g\ ml^{-1}$		at 30 $\mu g\ ml^{-1}$	
3	15.7	23.5	0/12	0%	3/12	25%
5	8.1	17	2/12	17%	5/12	42%
7	4.6	18.4	3/12	25%	3/12	25%
9	28.4	>30	0/12	0%	1/12	8%
11	>30	>30	0/12	0%	0/12	0%
16	26.3	>30	0/12	0%	0/12	0%
18	26.6	&gt30	0/12	0%	0/12	0%
20	>30	&gt30	0/12	0%	0/12	0%
22	21.5	&gt30	0/12	0%	2/12	17%
$[PtCl_2(DACH)]$[c]	>30	>30	0/10	0%	0/10	0%
Oxaliplatin	1.7	3.9	4/12[d]	33%	10/12[d]	83%

[a] Compounds were tested at 0.003, 0.03, 0.3, 3 and 30 $\mu g\ ml^{-1}$ in a panel of 12 cell lines comprising gastric (GXF 251L), lung (LXFA 629L, LXFL 529L, H460), mammary (MAXF 401NL, MCF-7), prostate (PC3M), renal (RXF 486L, RXF 944L) and uterine cancer (UXF 1138L) as well as melanoma (MEXF 462NL, MEXF 514L).
[b] Responsive (T/C $\leq$ 30%)/total cell lines.
[c] The starting material.
[d] Oxaliplatin was tested at 0.0015, 0.015, 0.15. 1.5 and 15 $\mu g\ ml^{-1}$.

lung, mammary, prostate, renal and uterine cancer as well as melanoma. Oxaliplatin was chosen as reference standard because all of the complexes used in this study are analogs to it from the view point of the ligand (1*R*,2*R*)-DACH. Only the three new complexes **3, 5** and **7** showed slight antitumor activity (mean IC_{70} in the range from 17 up to 23 $\mu g\ ml^{-1}$), but in comparison to the standard agent oxaliplatin (mean $IC_{70} = 3.9\ \mu g\ ml^{-1}$), these complexes were significantly less antitumor active in this cell line panel (Table 2).

Conclusions

In this paper, we have explored the synthesis and characterizations of 26 palladium(II) and platinum(II) complexes analogous to oxaliplatin, bearing the anantiomerically pure (1*R*,2*R*)-(−)-1,2-diaminocyclohexane ligand. These complexes were screened *in vitro* against nine tumor cell lines and the results obtained were compared with those of the reference standard, oxaliplatin, a known antitumor drug. However, these complexes were significantly less antitumor-active in this cell line panel.

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