Journal of Inorganic Biochemistry 105 (2011) 1630–1637

Contents lists available at SciVerse ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Lipophilic Pt(II) complexes with selective efficacy against cisplatin-resistant testicular cancer cells

Bernhard Biersack [a], Andrea Dietrich [b], Miroslava Zoldakova [a], Bernd Kalinowski [c], Reinhard Paschke [c], Rainer Schobert [a,*], Thomas Mueller [b,**]

[a] Organic Chemistry Laboratory, University Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany
[b] Department of Internal Medicine IV, Oncology/Hematology, Martin-Luther-University Halle-Wittenberg, 06120 Halle/Saale, Germany
[c] Biocenter of the Martin-Luther-University Halle-Wittenberg, 06120 Halle/Saale (Germany) , 06120 Halle/Saale, Germany

ARTICLE INFO

Article history:
Received 14 May 2011
Received in revised form 1 August 2011
Accepted 22 August 2011
Available online 14 September 2011

Keywords:
Platinum complexes
Terpenes
Cisplatin resistance
Testicular cancer

ABSTRACT

A series of dichloridoplatinum(II) complexes with selective and high cytotoxicity [IC_{90}(96 h) ≤ 3 μM] against cisplatin-resistant 1411HP testicular cancer cells were identified. They bear stationary 6-aminomethylnicotinate or 2,4-diaminobutyrate ligands esterified with lipophilic terpenyl residues, i.e., (−)/(+)-menthyl, (+)-cedrenyl, (−)-menthoxypropyl, or with a decyl-tethered 1,1,2-triphenylethene. They accumulated to a larger extent in 1411HP cells than in cells of the cisplatin-sensitive H12.1 germ cell tumour. Their mechanism of apoptosis induction differed from that of cisplatin by being independent of p53 and of caspase-3 activation and by an early loss of the mitochondrial membrane potential. The new complexes are promising candidates for the treatment of cisplatin-resistant testicular tumours.

1. Introduction

The three FDA-approved platinum compounds, cisplatin, carboplatin, and oxaliplatin epitomise the progress that metallodrug-based chemotherapy has made over the past four decades. Cisplatin, an accidental discovery, was rashly approved for clinical use despite its severe side effects, mainly for want of alternative treatments. In contrast, carboplatin, a second generation modification of cisplatin with fewer side effects and oxaliplatin, a derivative with high efficacy against metastasised colorectal cancer were the products of rational structure–activity deliberations and pharmacological studies [1–5]. The search continues for platinum complexes with patterns of cytotoxicity significantly different from those of the three FDA-approved platinum drugs and in particular for such with activity against cisplatin-resistant tumours. New promising derivatives of this type currently in clinical trials are picoplatin (ZD0473) [6] which features a sterically shielding α-picoline ligand and the Pt(IV) complex satraplatin (JM216) [7]. Attempts at circumventing the cisplatin resistance were also made by attaching cancer-specific carrier or shuttle groups to the cytotoxic platinum complex fragment [4], [5], [8–11]. Recently, Schobert et al. published an estradiol-Pt(II) complex conjugate that accumulates in breast cancer cells by binding to the sex hormone binding globulin (SHBG) and to the oestrogen receptor [12]. They also disclosed a (−)-menthyl-Pt(II) conjugate **1a** preferentially concentrating in cisplatin-resistant 1411HP germ cell cancer cells [13]. A series of similar terpene–Pt(II) complex conjugates were finally developed with enhanced and selective efficacy against melanoma and colon carcinoma cell lines [14]. Paschke et al. reported undecyl-linked tetrahydropyran and cholic acid platinum complex conjugates (THPPt-11, ChAPt-11) with anticancer activities surpassing that of cisplatin against 1411HP cells due to enhanced uptake and to induction of alternative apoptosis pathways [15], [16]. Bérubé and co-workers reported several lipophilic conjugates of Pt(II) complexes with tamoxifen-like 1,1,2-triphenylethene moieties displaying increased activity against breast cancer cells [17–20]. The uptake-dependent breach of cisplatin-resistance can ideally be studied using the two germ cell tumour cell lines 1411HP which is cisplatin-resistant and H12.1 which is cisplatin-sensitive. The cisplatin resistance of 1411HP is due to an unusually high threshold for the activation of the apoptosis-relevant caspase-9 [21]. Herein we report on a collection of known and new lipophilic platinum complexes with terpenoid, steroidal and 1,1,2-trisarylethene appendages that exhibit a significantly higher cytotoxicity in the cisplatin-resistant 1411HP cells when compared to the sensitive H12.1 cells.

2. Experimental

2.1. General

Melting points were determined with a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer

* Corresponding author. Fax: +49 921 552671.
** Corresponding author. Fax: +49 345 5577279.
E-mail addresses: laganlad@yahoo.co.uk (R. Schobert), thomas.mueller@medizin.uni-halle.de (T. Mueller).

0162-0134/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2011.08.028

One FT ATR (attenuated total reflection) IR spectrophotometer. The metal content of cells was ascertained with a graphite furnace atomic absorption spectrometer model AAS5 EA solid (Jena GmbH, Germany). NMR spectra were recorded under conditions as indicated on a Bruker Avance 300 spectrometer. Chemical shifts (δ) are given in parts per million downfield from Me_4Si as internal standard for ^1H and ^{13}C and relative to $\Xi(^{195}Pt) = 21.4$ MHz for ^{195}Pt. Signal multiplicities are assigned as "multiplet (m)", "singlet (s)", "doublet" (d), "triplet" (t), or "quartet (q)". Mass spectra were recorded using a Thermo Finnigan MAT 8500 in electron impact (EI) mode. Elemental analyses were carried out with a Perkin-Elmer 2400 CHN elemental analyser. For column chromatography Merck silica gel 60 (230–400 mesh) was used. All starting compounds were purchased from the usual sources and used without further purification. The complexes **1a**–**k** and the 1,1,2-trisarylalkenols necessary for the preparation of esters **2l**–**n** were prepared following literature procedures [12–14], [17–20].

2.2. Chemistry

2.2.1. 11,12,12-Triphenyldodec-11-enyl 6′-(t-butoxycarbonylaminomethyl) nicotinate (**2l**)

6-*t*-Butoxycarbonylaminomethylnicotinic acid (140 mg, 0.56 mmol) was dissolved in dry DMF (2 mL) and treated with Et_3N (80 μL, 0.58 mmol) and 2,4,6-trichlorobenzoyl chloride (95 μL, 0.59 mmol). The resulting suspension was stirred under argon at room temperature for 20 min. A solution of 9,10,10-triphenyldec-9-en-1-ol (230 mg, 0.56 mmol) and DMAP (143 mg, 1.18 mmol) in dry toluene (20 mL) was added and the resulting mixture was stirred under argon at room temperature for 16 h. After dilution with ethyl acetate and washing with water the organic phase was dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography (silica gel 60; ethyl acetate/hexane 1:3). Yield: 270 mg (75%); colourless oil; R_f 0.26 (ethyl acetate/hexane 1:3); ν_{max}/cm^{-1}: 2925, 2854, 1718, 1598, 1491, 1366, 1275, 1243, 1166, 1114, 758, 698; ^1H NMR (300 MHz, $CDCl_3$): δ 1.1–1.5 (m, 21H), 1.6–1.8 (m, 2H), 2.3–2.5 (m, 2H), 4.31 (t, $J = 6.7$ Hz, 2H), 4.48 (d, $J = 5.5$ Hz, 2H), 5.5–5.6 (m, 1H), 6.8–7.4 (m, 16H), 8.23 (d, $J = 8.2$ Hz, 1H), 9.12 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.9, 28.4, 28.6, 28.8, 29.2, 29.4, 29.6, 29.9, 35.8, 45.8, 65.5, 121.0, 125.0, 125.6, 126.1, 126.5, 126.8, 127.2, 127.3, 127.7, 128.1, 129.5, 129.6, 129.9, 130.2, 130.7, 137.7, 139.0, 141.1, 142.5, 143.0, 143.5, 150.4, 155.9, 161.8, 165.2; *m/z* 646 (25) [M^+], 590 (100), 546 (55), 269 (22), 191 (85), 91 (43), 57 (72); accurate mass (EIMS) for $C_{42}H_{50}N_2O_4$: calcd 646.37706, obsd 646.37700.

2.2.2. 11,12,12-Tris-(p-methoxyphenyl)dodec-11-enyl 6′-(t-butoxycarbonylaminomethyl)nicotinate (**2m**)

Analogously to the synthesis of **2l**, compound **2m** was obtained from 6-*t*-butoxycarbonylaminomethylnicotinic acid (109 mg, 0.43 mmol), Et_3N (70 μL, 0.51 mmol), 2,4,6-trichlorobenzoyl chloride (80 μL, 0.49 mmol), 11,12,12-triphenyldodec-11-en-1-ol (196 mg, 0.43 mmol) and DMAP (104 mg, 0.86 mmol). Yield: 230 mg (73%); colourless oil; R_f 0.24 (ethyl acetate/hexane 1:2); ν_{max}/cm^{-1}: 2926, 2854, 1717, 1603, 1507, 1282, 1239, 1170, 1032, 829; ^1H NMR (300 MHz, $CDCl_3$): δ 1.1–1.5 (m, 23H), 1.7–1.8 (m, 2H), 2.3–2.5 (m, 2H), 3.67 (s, 3H), 3.73 (s, 3H), 3.80 (s, 3H), 4.31 (t, $J = 6.7$ Hz, 2H), 4.48 (d, $J = 5.5$ Hz, 2H), 5.5–5.6 (m, 1H), 6.5–7.2 (m, 12H), 7.33 (d, $J = 8.1$ Hz, 1H), 8.23 (d, $J = 8.1$ Hz, 1H), 9.11 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 25.9, 28.3, 28.7, 29.0, 29.2, 29.3, 29.4, 29.7, 35.9, 45.8, 55.0, 55.1, 55.2, 65.5, 79.7, 112.7, 113.2, 113.4, 121.5, 125.0, 130.6, 131.9, 135.1, 136.1, 136.5, 137.5 (C-12), 137.7 (C-4′), 139.4 (C-11), 150.4 (C-2′), 157.3, 157.6, 158.1, 161.9, 165.2; *m/z* 736 (85) [M^+], 680 (47), 636 (100), 359 (74), 251 (40), 121 (37); accurate mass (EIMS) for $C_{45}H_{56}N_2O_7$: calcd 736.40875, obsd 736.40870.

2.2.3. 13,14,14-Tris-(p-methoxyphenyl)tetradec-13-enyl 6′-(t-butoxycarbonylaminomethyl)nicotinate (**2n**)

Analogously to the synthesis of **2l**, compound **2n** was obtained from 6-*t*-butoxycarbonylaminomethylnicotinic acid (160 mg, 0.63 mmol), Et_3N (100 μL, 0.72 mmol), 2,4,6-trichlorobenzoyl chloride (111 μL, 0.72 mmol), 13,14,14-tris-(*p*-methoxyphenyl)-13-en-1-ol (196 mg, 0.43 mmol) and DMAP (155 mg, 1.26 mmol). Yield: 400 mg (83%); colourless oil; R_f 0.26 (ethyl acetate/hexane 1:2); ν_{max}/cm^{-1}: 2925, 2853, 1717, 1603, 1507, 1282, 1239, 1170, 1110, 1032, 829; ^1H NMR (300 MHz, $CDCl_3$): δ 1.1–1.5 (m, 27H), 1.7–1.8 (m, 2H), 2.3–2.5 (m, 2H), 3.67 (s, 3H), 3.73 (s, 3H), 3.80 (s, 3H), 4.31 (t, $J = 6.7$ Hz, 2H), 4.48 (d, $J = 5.5$ Hz, 2H), 5.5–5.6 (m, 1H), 6.5–7.2 (m, 12H), 7.33 (d, $J = 8.1$ Hz, 1H), 8.23 (d, $J = 8.1$ Hz, 1H), 9.11 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 26.0, 28.4, 28.6, 29.0, 29.2, 29.3, 29.5, 29.6, 29.7, 35.9, 45.8, 55.0, 55.1, 55.2, 65.5, 79.8, 112.7, 113.2, 113.4, 121.0, 125.0, 130.6, 131.9, 135.1, 136.1, 136.6, 137.5, 137.7, 139.4, 150.4, 157.3, 157.6, 158.1, 161.9, 165.2; *m/z* 764 (59) [M^+], 690 (58), 664 (92), 359 (100), 251 (58), 227 (28), 121 (37), 59 (46); accurate mass (EIMS) for $C_{47}H_{60}N_2O_7$: calcd 765.004, obsd 765.005.

2.2.4. 11,12,12-Triphenyldodec-11-enyl 6′-aminomethylnicotinate dihydrochloride (**3l**)

2l (230 mg, 0.36 mmol) was treated with 4 M HCl/dioxane (15 mL) at room temperature for 1 h. After evaporation of the solvent the oily residue was treated with hexane giving a yellowish gum. The hexane was evaporated and the residue was dried in vacuum. Yield: 210 mg (94%); ν_{max}/cm^{-1}: 2923, 2853, 1724, 1644, 1600, 1490, 1442, 1294, 1122, 758, 698; ^1H NMR (300 MHz, D_6-DMSO): δ 1.1–1.4 (m, 14H), 1.6–1.8 (m, 2H), 2.3–2.4 (m, 2H), 4.2–4.4 (m, 4H), 6.8–7.4 (m, 15H), 7.65 (d, $J = 8.2$ Hz, 1H), 8.35 (d, $J = 8.2$ Hz, 1H), 8.4–8.6 (m, 3H), 9.08 (s, 1H); ^{13}C NMR (75.5 MHz, D_6-DMSO): δ 25.3, 28.0, 28.5, 28.6, 28.8, 35.1, 42.6, 65.2, 122.6, 125.3, 125.8, 126.2, 126.7, 127.5, 127.8, 128.3, 128.9, 129.2, 130.0, 137.7, 140.4, 141.8, 142.6, 142.9, 149.3, 157.8, 164.4; *m/z* 546 (100) [$M^+ - 2$ HCl], 269 (11), 191 (38), 91 (17).

2.2.5. 11,12,12-Tris-(p-methoxyphenyl)dodec-11-enyl 6′-aminomethylnicotinate dihydrochloride (**3m**)

Obtained analogously to **3l** from **2m** (230 mg, 0.31 mmol) as a yellowish gum. Yield: 220 mg (100%); ν_{max}/cm^{-1}: 2925, 2852, 1762, 1606, 1510, 1463, 1291, 1243, 1173, 1121, 1034, 831; ^1H NMR (300 MHz, D_6-DMSO): δ 1.0–1.4 (m, 14H), 1.6–1.8 (m, 2H), 2.3–2.4 (m, 2H), 3.62 (s, 3H), 3.67 (s, 3H), 3.75 (s, 3H), 4.2–4.4 (m, 4H), 6.5–7.1 (m, 12H), 7.66 (d, $J = 8.2$ Hz, 1H), 8.35 (d, $J = 8.2$ Hz, 1H), 8.4–8.6 (m, 3H), 9.08 (s, 1H); ^{13}C NMR (75.5 MHz, D_6-DMSO): δ 25.4, 28.0, 28.2, 28.5, 28.6, 28.7, 28.8, 28.9, 35.2, 42.6, 54.8, 55.0, 65.2, 112.9, 113.3, 113.5, 122.6, 125.3, 130.2, 130.3, 131.3, 134.3, 135.6, 135.8, 137.2, 137.7, 138.8, 149.3, 157.0, 157.3, 157.8, 164.0; *m/z* 636 (100) [$M^+ - 2$HCl], 359 (40), 251 (23).

2.2.6. 13,14,14-Tris-(p-methoxyphenyl)tetradec-13-enyl 6′-aminomethylnicotinate dihydrochloride (**3n**)

Obtained analogously to **3l** from **2n** (380 mg, 0.50 mmol) as a yellowish gum. Yield: 360 mg (0.49 mmol, 98%); ν_{max}/cm^{-1}: 2922, 2851, 1722, 1604, 1507, 1285, 1240, 1172, 1119, 1032, 828; ^1H NMR (300 MHz, D_6-DMSO): δ 1.0–1.5 (m, 18H), 1.6–1.8 (m, 2H), 2.3–2.4 (m, 2H), 3.62 (s, 3H), 3.68 (s, 3H), 3.75 (s, 3H), 4.2–4.4 (m, 4H), 6.5–7.1 (m, 12H), 7.67 (d, $J = 8.2$ Hz, 1H), 8.35 (d, $J = 8.2$ Hz, 1H), 8.5–8.7 (m, 3H), 9.08 (s, 1H); ^{13}C NMR (75.5 MHz, D_6-DMSO): δ 25.4, 28.0, 28.2, 28.5, 28.6, 28.7, 28.9, 35.2, 42.6, 54.8, 55.0, 65.2, 112.9, 113.3, 113.5, 122.6, 125.3, 130.2, 130.3, 131.3, 134.3, 135.5, 135.8, 137.2, 137.7, 138.8, 149.2, 157.0, 157.3, 157.8, 157.9, 164.4; *m/z* 664 (100) [$M^+ - 2$HCl], 359 (32), 251 (18); accurate mass (EIMS) for $C_{42}H_{52}N_2O_5$ (free base): calcd 664.38762, obsd 664.38760.