Platinum pyridinecarboxaldimine complexes containing boronate esters

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Abstract: Condensation of 2-pyridinecarboxaldehydes with 2-, 3-, and 4-$H_2NC_6H_4Bpin$ (pin = 1,2-$O_2C_2Me_4$) gave the corresponding boron-containing pyridinecarboxaldimines (N–N_{Bpin}). Addition of these ligands to $[PtCl_2(coe)]_2$ (coe = *cis*-cyclooctene) gave complexes of the type *cis*-$PtCl_2(N–N_{Bpin})$ in moderate yields. The platinum complexes have been examined for their potential cytotoxicities against OV2008 (human ovarian carcinoma) and the analogous cisplatin-resistant cell line C13.

Key words: boronate esters, pyridinecarboxaldimines, cytotoxicity, platinum, boron.

Résumé : La condensation des pyridine-2-carboxaldéhydes avec les 2-, 3- et 4-$H_2NC_6H_4Bpin$ (pin = 1,2-$O_2C_2Me_4$) conduit à la formation des pyridinecarboxaldimines contenant du bore (N–N_{Bpin}). L'addition de ces ligands au $[PtCl_2(coe)]_2$ (coe = *cis*-cyclooctène) conduit à la formation de complexes du type *cis*-$PtCl_2(N–N_{Bpin})$, avec des rendements modérés. On a étudié les cytotoxicités potentielles des complexes du platine vis-à-vis du OV2008 (carcinome ovarien humain) et la série de cellules C13 apparentées, qui est résistante au cisplatine.

Mots clés : esters de l'acide boronique, pyridinecarboxaldimines, cytotoxicité, platine, bore.

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Introduction

Interest in compounds containing boronic acids $[RB(OH)_2]$ or boronate esters $[RB(OR')_2]$ arises from their remarkable versatility in catalysed carbon–carbon bond formation reactions, solid-phase synthesis, macrocyclic chemistry, organometallic and organic syntheses, and as glucose sensors for diabetes therapy (1). Interest in these compounds also arises from their potent biological activities (2–11). For instance, α-aminoboronic acids are effective and reversible inhibitors of serine proteases, a diverse group of proteolytic enzymes whose numerous physiological functions include digestion, growth, differentiation, and apoptosis. Proteases are also vital in the generation of most disease processes and, as a result, much effort has focused on the synthesis of α-aminoboronic acids for possible applications as enzyme inhibitors. Indeed, the boron compounds boromycin (12) and aplasmomycin (13) are powerful antibiotics and analogous amino acids containing boronic acids, such as L-4-boronophenylalanine, have also been investigated for their use in boron neutron capture therapy (BNCT) for the treatment of cancer (14–16). BNCT is a bimodal form of therapy, which depends on selectively depositing boron-10 atoms into the cancerous tumour prior to irradiation by slow (thermal) neutrons. These known biological activities, along with their ability to transport water insoluble reagents through membranes (17, 18), prompted us to investigate the use of boronate ester compounds as carrier ligands for biologically-active metal complexes.

Cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, and a few related platinum-based complexes are currently used as anticancer agents against testicular and ovarian malignancies (19–25). However, there are several limitations to platinum therapy such as neural and kidney toxicity as well as intrinsic and acquired resistance of tumor cells to the drugs (19). These complications have provided incentive for further research in the development of platinum-based complexes with increased solubility and enhanced specificity towards cancer cells. Recent studies have shown that *cis*-amminedichloro(2-methylpyridine)platinum(II) (AMD473 or ZD0473, **1** in Fig. 1) shows considerable cytotoxicity in cisplatin-resistant cell lines (26, 27). Steric crowding from the methyl group is

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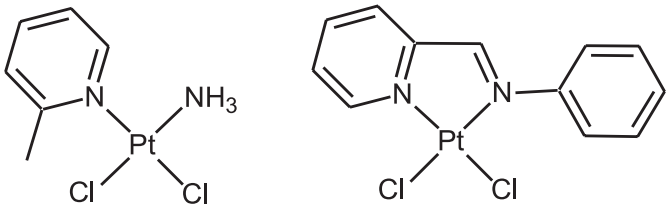
Fig. 1. Structure of AMD473 (**1**) and complex **2**.



1 **2**

believed to decrease the rates of hydrolysis, and substitution reactions of AMD473 thereby permit high selectivity in binding with DNA (28). The primary mechanism of action in these platinum drugs is believed to arise from the interaction of the metal with DNA.

We have begun to develop AMD473 analogues by replacing the NH_3 group with a pendant imine group. Previous studies have shown that the platinum complex derived from aniline, **2** (Fig. 1), has shown considerable activity against the hormone independent human mammary carcinoma cell line MDA-MB 231 (29). Varying the aniline functionality allows us to design compounds with a wide range of physical and chemical properties that may provide steric congestion around the platinum atom (29). As well, the use of bidentate ligands prevents trans labilization and undesired displacement of the ligands by sulfur and nitrogen donors in biomolecules, interactions believed responsible for some of the adverse side effects associated with cisplatin (19). We report herein our results on the synthesis and initial cytotoxic testing of *cis*-dichloro(pyridin-2-ylcarboxaldimine)platinum(II) compounds containing boronate esters.

Experimental

General

Reagents and solvents used were obtained from Aldrich Chemicals. K_2PtCl_4 was purchased from Precious Metals Online Ltd. (Melbourne, Australia) and $[PtCl_2(coe)]_2$ (coe = *cis*-cyclooctene) was made following an established procedure (30). 2-APBpin (pin = 1,2-$O_2C_2Me_4$) was synthesized by modification of a reported synthesis (31). Pyridinecarboxaldimine ligands were prepared as described elsewhere (32) and (6-methyl-pyridin-2-ylmethylene)-[4-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]amine was synthesized under a nitrogen atmosphere and used in situ. NMR spectra were recorded on a JEOL JNM-GSX270 FT or a Varian Mercury Plus 200 NMR spectrometer. ^{1}H NMR chemical shifts are reported in ppm and are referenced to residual protons in a deuterated solvent at 270 and 200 MHz. ^{11}B NMR chemical shifts are referenced to external $BF_3{\cdot}OEt_2$ at 87 MHz. ^{13}C NMR chemical shifts are referenced to solvent carbon resonances as internal standards at 68 and 50 MHz. Multiplicities are reported as singlet (s), doublet (d), triplet (t), multiplet (m), broad (br), and overlapping (ov). Infrared spectra were obtained using a Mattson Genesis II FT-IR spectrometer and are reported in cm^{-1}. Melting points were measured uncorrected with a Mel-Temp apparatus. Microanalyses for C, H, and N were carried out at Guelph Chemical Laboratories Ltd. (Guelph, Ont.).

Compound 3a

Under a nitrogen atmosphere, a THF (2 mL) solution of pyridin-2-ylmethylene-[2-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]amine (0.09 g, 0.29 mmol) was added to $[PtCl_2(coe)]_2$ (0.10 g, 0.13 mmol) in THF (3 mL). The reaction mixture was stirred for 18 h at which point a precipitate was collected by suction filtration and washed in turn with cold THF (3 × 1 mL) and Et_2O (3 × 5 mL) to afford **3a** (0.06 g, 39%) as a yellow-orange solid; mp 270–272 °C (decomposition). Spectroscopic NMR data (in DMSO-d_6): ^{1}H δ: 9.50 (d, $J_{H\text{-}H}$ = 7 Hz, $J_{H\text{-}Pt}$ = 62 Hz, 1H, Ar), 9.35 (s, $J_{H\text{-}Pt}$ = 92 Hz, 1H, C(*H*) = N), 8.47 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 8.22 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 8.01 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.74 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.60 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.42 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.34 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 1.21 (s, 6H, $O_2C_2(CH_3)_4$), and 1.15 (s, 6H, $O_2C_2(CH_3)_4$). ^{11}B{^{1}H} δ: 29.1 (br). ^{13}C{^{1}H} δ: 173.0, 157.7, 152.7, 149.7, 141.2, 135.4, 131.5, 130.1, 130 (br, *C*-B), 129.7, 128.3, 124.9, 84.5, 25.4, and 25.1. IR (cm^{-1}) (Nujol): 2926, 2856, 1601 (C=N), 1462, 1377, 1300, 1240, 1200, 1144, 1076, 964, 860, 779, and 654. Anal. calcd. (%) for $C_{18}H_{21}N_2BCl_2O_2Pt$, C 37.64, H 3.69, N 4.88; found: C 37.94, H 3.33, N 4.84.

Compound 3b

Pyridin-2-ylmethylene-[3-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]amine (0.09 g, 0.29 mmol) in CH_2Cl_2 (5 mL) was added to $[PtCl_2(coe)]_2$ (0.10 g, 0.13 mmol) in CH_2Cl_2 (5 mL) and the reaction mixture was stirred for 1 h at which point solvent was removed under vacuum to give an oil. Following trituration with hexane (3 × 10 mL) an orange solid was recovered and recrystallized from THF (5 mL) at room temperature to afford **3b** (0.08 g, 54%) as a red-orange solid; mp 316 °C (decomposition). Spectroscopic NMR data (in $CDCl_3$): ^{1}H δ: 9.76 (d, $J_{H\text{-}H}$ = 7 Hz, $J_{H\text{-}Pt}$ = 60 Hz, 1H, Ar), 8.88 (s, $J_{H\text{-}Pt}$ = 84 Hz, 1H, C(*H*) = N), 8.19 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.97 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.83 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.81–7.72 (ov m, 2H, Ar), 7.66 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.43 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), and 1.27 (s, 12H, $O_2C_2(CH_3)_4$). ^{11}B{^{1}H} δ: 32.5 (br). ^{13}C{^{1}H} δ: 168.3, 156.5, 150.0, 146.1, 139.5, 135.6, 129.0, 129 (br, *C*-B), 128.9, 128.4, 127.5, 127.4, 84.0, and 24.7. IR (cm^{-1}) (Nujol): 2895, 2856, 1610 (C=N), 1462, 1377, 1329, 1234, 1151, 933, 854, 816, 777, and 706. Anal. calcd. (%) for $C_{18}H_{21}N_2BCl_2O_2Pt$, C 37.64, H 3.69, N 4.88; found: C 37.25, H 3.62, N 4.73.

Compound 3c

Pyridin-2-ylmethylene-[4-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]amine (0.18 g, 0.58 mmol) in CH_2Cl_2 (5 mL) was added to $[PtCl_2(coe)]_2$ (0.20 g, 0.26 mmol) in CH_2Cl_2 (5 mL) and the reaction mixture was stirred for 7 h at which point solvent was removed under vacuum to give an oil. Following trituration with hexane (3 × 10 mL) an orange solid was recovered and recrystallized from THF (5 mL) at room temperature to afford **3c** (0.19 g, 64%) as an orange solid; mp 304 °C (decomposition). Spectroscopic NMR data (in DMSO-d_6): ^{1}H δ: 9.48 (d,

$J_{H\text{-}H}$ = 7 Hz, $J_{H\text{-}Pt}$ = 65 Hz, 1H, Ar), 9.33 (s, $J_{H\text{-}Pt}$ = 84 Hz, 1H, C(*H*) = N), 8.45 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 8.24 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 8.00 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.77 (d, $J_{H\text{-}H}$ = 7 Hz, 2H, Ar), 7.46 (d, $J_{H\text{-}H}$ = 7 Hz, 2H, Ar), and 1.32 (s, 12H, $O_2C_2(CH_3)_4$). $^{11}B\{^1H\}$ δ: 33.1 (br). $^{13}C\{^1H\}$ δ: 173.1, 157.6, 149.8, 149.6, 141.2, 134.8, 130.4, 130.2, 129.5 (br, *C*-B), 124.4, 84.5, and 25.2. IR (cm^{-1}) (Nujol): 2941, 2914, 2858, 1601 (C=N), 1460, 1377, 1356, 1315, 1263, 1144, 1084, 1018, 968, 858, 764, 723, and 656. Anal. calcd. (%) for $C_{18}H_{21}N_2BCl_2O_2Pt$, C 37.64, H 3.69, N 4.88; found: C 37.31, H 3.50, N 4.69.

Compound 4a

Under an atmosphere of nitrogen, a THF (1 mL) solution of (6-methyl-pyridin-2-ylmethylene)-[2-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]amine (0.09 g, 0.28 mmol) was added to $[PtCl_2(coe)]_2$ (0.09 g, 0.12 mmol) in THF (3 mL). The reaction mixture was stirred for 8 h at which point a precipitate was collected by suction filtration and washed with Et_2O (3 × 5 mL) to afford **4a** (0.06 g, 42%) as an orange solid; mp 285 °C (decomposition). Spectroscopic NMR data (in $CDCl_3$): ^1H δ: 8.77 (s, $J_{H\text{-}Pt}$ = 84 Hz, 1H, C(*H*) = N), 8.02 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.86 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.68 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.56 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.50 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.36 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.29 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 3.25 (s, 3H, CH_3), and 1.25 (br s, 12H, $O_2C_2(CH_3)_4$). $^{11}B\{^1H\}$ δ: 29.3 (br). $^{13}C\{^1H\}$ δ: 161.9, 159.5, 155.2, 138.4, 136.8, 133.1, 129.1, 128.5, 126 (br, *C*-B), 119.6, 115.6, 114.8, 83.7, 25.2, and 24.3. IR (cm^{-1}) (Nujol): 2924, 2854, 1603 (C=N), 1462, 1377, 1348, 1144, 1072, 1018, 962, 858, 725, 654, and 596. Anal. calcd. (%) for $C_{19}H_{23}N_2BCl_2O_2Pt$, C 38.79, H 3.95, N 4.76; found: C 38.95, H 4.05, N 4.78.

Compound 4b

(6-Methyl-pyridin-2-ylmethylene)-[3-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]amine (0.08 g, 0.25 mmol) in CH_2Cl_2 (5 mL) was added to $[PtCl_2(coe)]_2$ (0.08 g, 0.11 mmol) in CH_2Cl_2 (5 mL) and the reaction was heated at reflux for 24 h. Removal of solvent under vacuum gave an oil which was triturated with hexane (3 × 10 mL) to afford a red-orange solid which was dissolved in a CH_2Cl_2:Et_2O (5:5 mL) solution and stored at 0 °C for 18 h. The resulting precipitate was collected by suction filtration to afford **4b** (0.08 g, 62%) as an orange solid; mp 264 °C (decomposition). Spectroscopic NMR data (in $CDCl_3$): ^1H δ: 8.91 (s, $J_{H\text{-}Pt}$ = 80 Hz, 1H, C(*H*) = N), 7.97 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.81–7.77 (ov m, 2H, Ar), 7.70 (s, 1H, Ar), 7.59–7.51 (ov m, 2H, Ar), 7.40 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 3.21 (s, 3H, CH_3), and 1.33 (s, 12H, $O_2C_2(CH_3)_4$). $^{11}B\{^1H\}$ δ: 29.6 (br). $^{13}C\{^1H\}$ δ: 168.6, 167.9, 157.6, 146.5, 138.8, 136.0, 131.8, 129 (br, *C*-B), 128.7, 127.8, 127.7, 126.3, 84.3, 28.1, and 25.0. IR (cm^{-1}) (Nujol): 2927, 2856, 1599 (C=N), 1568, 1462, 1428, 1377, 1319, 1252, 1228, 1166, 1144, 1101, 970, 924, 854, 798, 731, 704, and 606. Anal. calcd. (%) for $C_{19}H_{23}N_2BCl_2O_2Pt$, C 38.79, H 3.95, N 4.76; found: C 38.97, H 3.90, N 4.73.

Compound 4c

(6-Methyl-pyridin-2-ylmethylene)-[4-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)phenyl]amine (0.08 g, 0.25 mmol) in CH_2Cl_2 (5 mL) was added to $[PtCl_2(coe)]_2$ (0.08 g, 0.11 mmol) in CH_2Cl_2 (5 mL) and the reaction was heated at reflux for 24 h. Removal of solvent under vacuum gave an oil which was triturated with hexane (3 × 10 mL) to afford a red-orange solid which was dissolved in a CH_2Cl_2:Et_2O (5:5 mL) solution and stored at 0 °C for 18 h. The resulting precipitate was collected by suction filtration to afford **4c** (0.10 g, 77%) as an orange solid; mp 277 °C (decomposition). Spectroscopic NMR data (in $CDCl_3$): ^1H δ: 8.94 (s, $J_{H\text{-}Pt}$ = 78 Hz, 1H, C(*H*) = N), 7.94 (t, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.86 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.79 (d, $J_{H\text{-}H}$ = 7 Hz, 2H, Ar), 7.47 (d, $J_{H\text{-}H}$ = 7 Hz, 1H, Ar), 7.41 (d, $J_{H\text{-}H}$ = 7 Hz, 2H, Ar), 3.15 (s, 3H, CH_3), and 1.33 (s, 12H, $O_2C_2(CH_3)_4$). $^{11}B\{^1H\}$ δ: 31.2 (br). $^{13}C\{^1H\}$ δ: 169.4, 167.4, 157.6, 149.1, 138.9, 135.0, 131.7, 131 (br, *C*-B), 127.4, 123.3, 84.2, 28.0, and 25.0. IR (cm^{-1}) (Nujol): 2925, 2856, 1581 (C=N), 1463, 1377, 1358, 1336, 1270, 1173, 1134, 1092, 1016, 935, 845, 787, 729, and 656. Anal. calcd. (%) for $C_{19}H_{23}N_2BCl_2O_2Pt$, C 38.79, H 3.95, N 4.76; found: C 38.70, H 3.99, N 4.76.

X-ray crystallography

Crystals of **3b** and **4a** were grown from saturated CH_2Cl_2 solutions at 5 °C. Single crystals were coated with Paratone-N oil, mounted using a glass fibre, and frozen in the cold stream of the goniometer. A hemisphere of data was collected on a Bruker AXS P4/SMART 1000 diffractometer using ω and θ scans with a scan width of 0.3° and 30 s exposure times. The detector distances were 4 (**3b**) and 5 cm (**4a**). The data were reduced (33) and corrected for absorption (34). The structures were solved by direct methods and refined by full-matrix least squares on F^2 (35). All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located in Fourier difference maps and refined isotropically (**3b**) or included in calculated positions and refined using a riding model (**4a**).

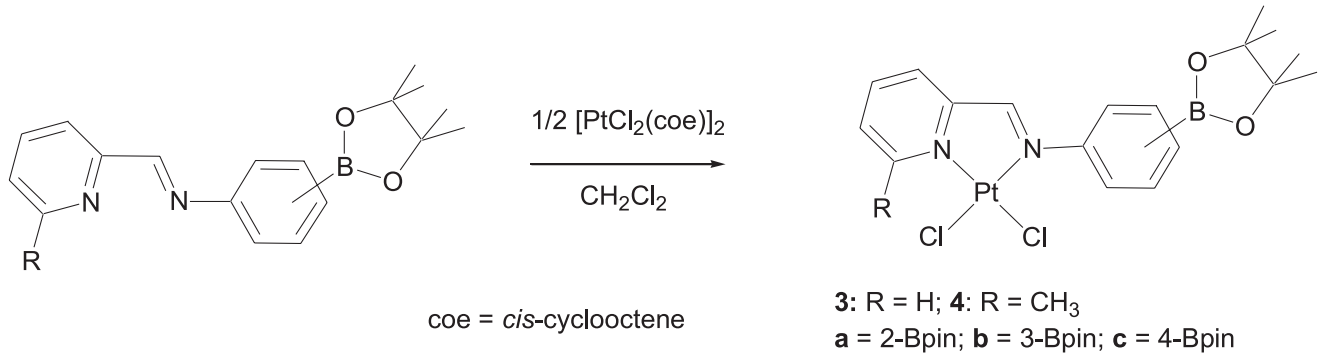
Cell culture

OV2008 (human ovarian carcinoma) and the analogous cisplatin-resistant cell line C13 were a generous gift from Dr. Barbara C. Vanderhyden of the Centre for Cancer Therapeutics, Ottawa Regional Cancer Centre, Ont. Both cell lines were cultured in complete RPMI-1640 medium with L-glutamine, supplemented with 5% fetal bovine serum, penicillin (50 units/mL), and streptomycin (50 mg/mL). Cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C and were subcultured twice weekly using trypsin-EDTA.

Cell growth inhibition assay

Cells were seeded in 96-well plates at a concentration of 0.1–1.0 × 10^4 cells/well in 200 µL of complete media and incubated for 24 h at 37 °C in a 5% CO_2 atmosphere to allow for cell adhesion. Stock solutions (15 mmol/L) of the compounds made in DMSO were filter sterilized, then diluted to 10 mmol/L in phosphate buffered saline (PBS, 0.02 mol/L phosphate, 0.11 mol/L NaCl, pH 7.0, sterile). The 10 mmol/L solutions were diluted to 50 µmol/L and 1.4 mmol/L in complete media for treatment against OV2008 and C13 cell lines, respectively, where 20 µL of compound solutions were added to 180 µL of fresh medium in wells to give final concentrations of 5 µmol/L against

Fig. 2. Synthesis of platinum complexes **3–4**.



OV2008 and 140 μmol/L for C13. All assays were performed in two independent sets of quadruplicate tests. Control groups containing no drug were run in each assay, along with standards of cisplatin and a previously studied *cis*-dichloro(pyridin-2-ylcarboxaldimine)platinum(II) complex (**2**).

Following 48 h of exposure, each well was carefully rinsed with 200 μL of PBS solution. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (50 μL, 1 mg/mL doubly distilled (dd)H_2O) along with 200 μL of fresh, complete media were added to each well, and plates were incubated for 45 min. Following incubation, the media was removed and the purple formazan precipitate in each well was solubilized in 200 μL of DMSO. Absorbances were measured using a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, Calif.) at 570 nm. Reduction of cell proliferation was determined as a fraction of the absorbance values for each drug treatment (T) by the mean absorbance of the no-drug control (C), and calculated using the expression 1 – T/C × 100%. Data from the separate trials were combined using a *t*-test ($P < 0.05$).

Results and discussion

Ligands

Aniline derivatives containing boronate ester groups were used, in place of the related boronic acids, in an attempt to increase the solubility of the ligands and the subsequent platinum complexes in organic solvents, and to prevent unwanted formation of anhydrides often seen in reactions with the acids (36, 37). Addition of these primary amines to 2-pyridinecarboxaldehydes gave the corresponding pyridinecarboxaldimine ligands (N–N_{Bpin}) in high yield (38–41). All new ligands were characterized by multinuclear NMR spectroscopy. For example, a singlet at ca. δ 8.4 ppm is observed in the ^{1}H NMR spectra for the imine methine peak and a broad peak at ca. δ 30 ppm in the ^{11}B NMR spectra for these ligands is indicative of a three coordinate boron atom. This latter result suggests that no appreciable inter- or intramolecular interaction between the Lewis-acidic boron and the basic imine or pyridine functionality is occurring in solution (42).

Platinum complexes

We have found that platinum complexes containing boronate ester appendages ($PtCl_2$(N–N_{Bpin}), Fig. 2) could be readily prepared by addition of pyridin-2-ylcarboxaldimine ligands to CH_2Cl_2 solutions of $[PtCl_2(coe)]_2$ (43).

All new platinum complexes have been characterized by a number of physical methods, including multinuclear NMR spectroscopy. A significant downfield shift in the ^{1}H NMR is observed for the imine proton upon coordination of the ligand to the metal center. For instance, the singlet at δ 8.36 ppm for the free 2-C_5H_4NCH=N[2-$C_6H_4BO_2C_2Me_4$] ligand shifts to 9.35 ppm in complex **3a**. Platinum satellites are also observed for this resonance ($J_{H\text{-}Pt}$ = 92 Hz) upon complexation of the ligand to the metal. Similar trends are observed for the pyridine hydrogen alpha to the nitrogen atom as the chemical shift changes from δ 8.50 to 9.50 ppm ($J_{H\text{-}Pt}$ = 62 Hz). Two distinct Bpin resonances are observed in both the ^{1}H (δ: 1.21 and 1.15 ppm) and ^{13}C{^{1}H} (δ: 25.4 and 25.1 ppm) NMR spectra for complex **3a**, arising from inequivalent methyl groups (Fig. 3). It is therefore possible that two of the Bpin methyl groups are lying over the plane of the platinum atom in a manner similar to that observed for AMD473.

Although we were unable to obtain single crystals of **3a** to verify if an appropriate conformational prerequisite for the shielding effect was occurring, complex **3b** has been characterized by an X-ray diffraction study (Fig. 4). Crystallographic data are given in Table 1.[3] The nitrogen–platinum bonds of 2.023(2) Å (pyridine) and 2.023(3) Å (imine) are similar to those reported in other platinum systems (37–39). Slightly longer Pt—N bond distances are observed in related five coordinate Pt(II) (37, 38) and Pt(IV) complexes (42). The imine C(7)—N(8) distance of 1.298(3) Å is in the range of accepted carbon–nitrogen double bonds (43). Interestingly, the angle between the aromatic ring on the imine nitrogen and the N(1)-Pt-N(8) plane is 108.1°, a trend that is observed in related diimine systems (44). Indeed, this structural feature has been used to create active and selective catalyst precursors for the polymerization of alpha olefins, where ortho substitution of the aniline group with bulky groups is believed to shield and stabilize the metal center.

[3] Supplementary material may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada. The material can also be obtained electronically at http://www.nrc.ca/cisti/irm/unpub_e.shtml. Crystallographic information has also been deposited with the Cambridge Crystallographic Data Centre (CCDC 239618 and 239619). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

Fig. 3. Representative ^{1}H NMR spectrum of compound **3a** in DMSO-d_6, showing an expansion of the aromatic (Ar) region.

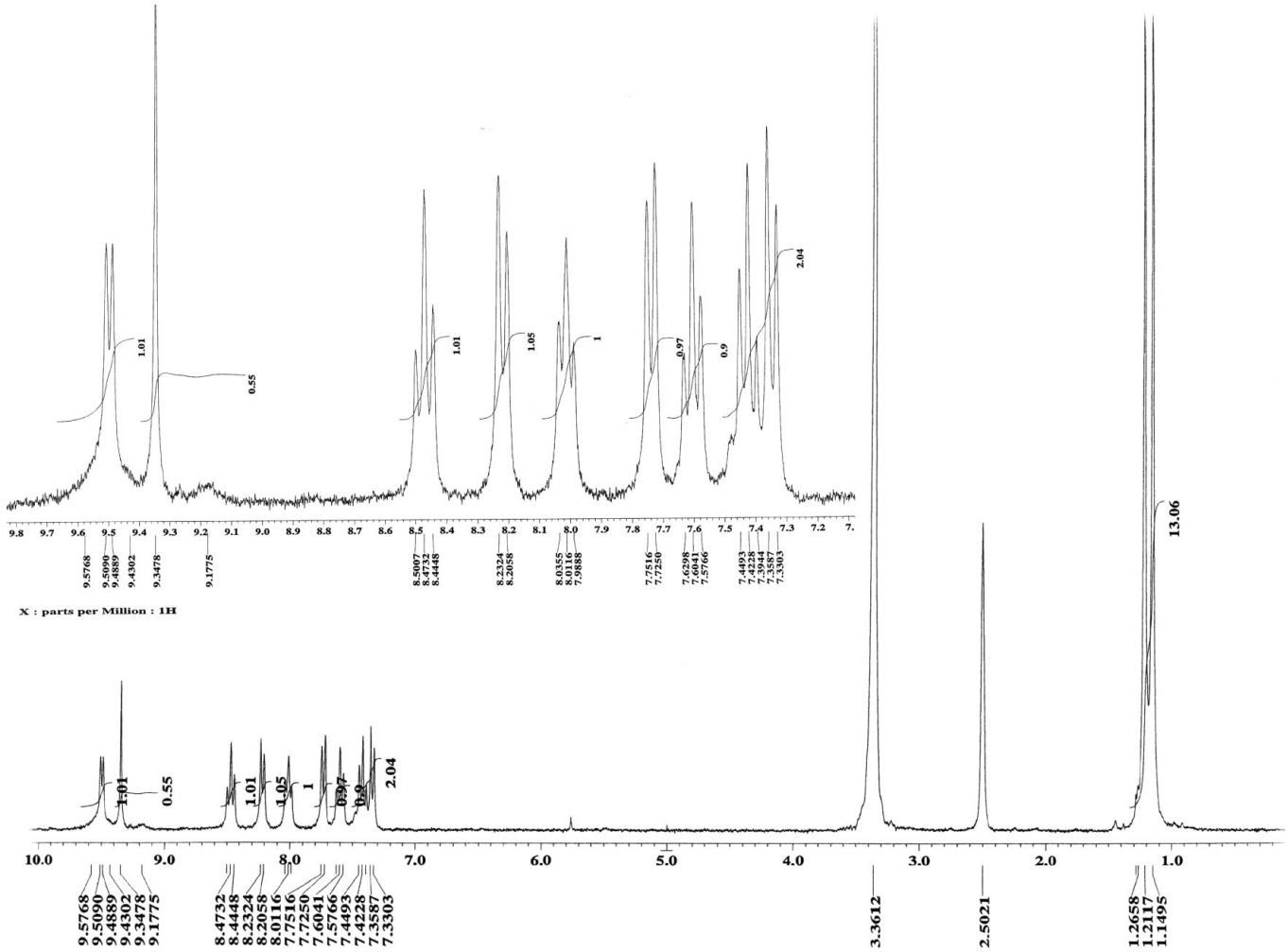


Fig. 4. A view of complex **3b**, with displacement ellipsoids drawn at the 30% probability level. H atoms have been omitted. Selected bond distances and angles; Pt—N(8) 2.023(3), Pt—N(1) 2.023(2), Pt—Cl(2) 2.2844(8), Pt—Cl(1) 2.3011(7), C(7)—N(8) 1.298(3), B—O(15) 1.374(4), B—O(18) 1.376(4), and C(11)—B 1.564(4) Å; N(8)-Pt-N(1) 80.17(9), N(8)-Pt-Cl(2) 95.36(7), N(1)-Pt-Cl(2) 175.43(7), N(8)-Pt-Cl(1) 175.04(6), N(1)-Pt-Cl(1) 94.88(7), Cl(2)-Pt-Cl(1) 89.59(3), O(15)-B-O(18) 113.7(3), O(15)-B-C(11) 121.7(3), and O(18)-B-C(11) 124.5(3)°.

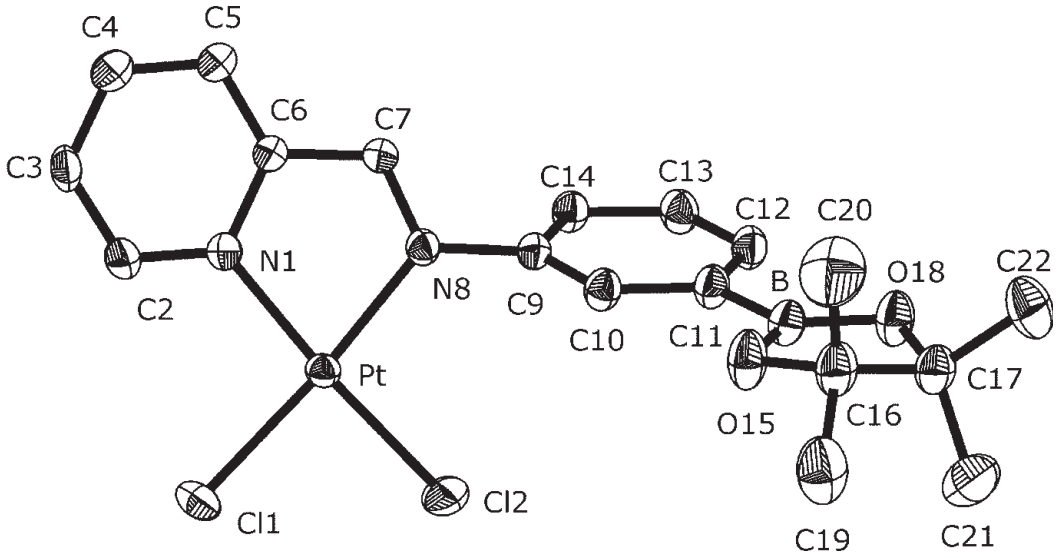


Table 1. Crystallographic data collection parameters for **3b** and **4a**.

Complex	**3b**	**4a**
Chemical formula	$C_{18}H_{21}BCl_2N_2O_2Pt$	$C_{19}H_{23}BCl_2N_2O_2Pt$
Formula weight	574.17	588.19
Crystal system	Monoclinic	Orthorhombic
Space group	P2(1)/c	P2(1)2(1)2(1)
a (Å)	14.8642(8)	10.8778(5)
b (Å)	9.8220(5)	13.3995(7)
c (Å)	15.5384(9)	15.0437(8)
β (°)	109.936(1)	90
V (Å^3)	2132.6(2)	2192.73(19)
Z	4	4
$\rho_{calcd.}$ (mg m^{-3})	1.788	1.782
Crystal dimensions (mm)	0.075 × 0.3 × 0.375	0.05 × 0.1 × 0.45
Temperature (K)	173(1)	198(1)
Radiation	Mo Kα (λ = 0.71073 Å)	Mo Kα (λ = 0.71073 Å)
μ (mm^{-1})	6.844	6.658
Total reflections	22 717	14 427
Total unique reflections	7634	4878
Observed reflections ($F_o > 4\sigma(F_o)$)	6653	4772
No. of variables	249	244
R_{int}	0.0368	0.0179
Theta range (°)	2.50 to 32.50	2.04 to 27.50
Largest difference peak/hole, e (Å^{-3})	4.777/–3.473	0.769/–0.462
S (Goodness-of-fit) on F^2	1.048	0.869
R_1[a] ($l > 2\sigma(l)$)	0.0330	0.0132
wR_2[b] (all data)	0.0903	0.0317

[a] $R_1 = \Sigma(|F_o| - |F_c|) / \Sigma|F_o|$.

[b] $wR_2 = (\Sigma[w(F_o^2 - F_c^2)^2] / \Sigma[F_o^4])^{1/2}$, where $w = 1/[\sigma^2(F_o^2) + (0.0637 \cdot P)^2]$ (**3b**) and $w = 1/[\sigma^2(F_o^2)]$ (**4a**), where $P = (\max(F_o^2, 0) + 2 \cdot F_c^2)/3$.

The boronate ester group in **3b** is roughly coplanar with the aromatic group in order to maximize the donation from the ring *p*π electrons to the empty *p* orbital on boron. No significant interaction of the bulky pinacol group is observed, however, with the metal center.

We carried out an X-ray diffraction study on **4a** to see if the boronate ester group in the ortho position could effectively crowd the platinum atom in a fashion similar to the methyl group of AMD473. The molecular structure of **4a** is shown in Fig. 5 and its crystallographic data is summarized in Table 1. While similar trends observed for **3b** are found in **4a**, inequivalent platinum nitrogen distances are observed, Pt—N(9) 1.9936(19) Å (imine) and Pt—N(1) 2.057(2) Å (pyridine). The longer Pt—N(1) bond reflects the large strain due to the presence of the methyl group in the ortho position to the binding atom in the pyridine ring. Bond angles in **4a** are also similar to those found in **3b** and the twist angle to the phenyl ring in **4a** is 64.7°. Unfortunately, however, there appears to be no significant interaction of the Bpin group with the platinum center. For instance, two of the methyl groups of the Bpin moiety lie over the Pt with long Pt—C(20) and Pt—C(21) distances of 5.133 and 6.023 Å, and the Pt—O and Pt—B distances are 4.158 and 4.483 Å, respectively, and are too far away for any significant interaction. These results suggest that the ortho Bpin groups in these complexes may not shield the platinum atom as effectively as the methyl group in AMD473.

Cytotoxic activity

We have tested the cytotoxic activity of all new platinum complexes against both cisplatin-sensitive and cisplatin-resistant human ovarian cells (Table 2), OV2008 and C13, respectively, at concentrations corresponding to cisplatin's IC_{50} value against either cell line (45–49). While no activity was observed against cisplatin-sensitive cells OV2008, compounds **3a** (with the Bpin group in the ortho position, Entry 3), **3b** and **4b** (with the Bpin group in the meta position, Entries 4 and 7, respectively) showed only minimal activity against cisplatin-resistant cell line C13. Interestingly, the analogous picoline complex **4a** showed diminished activity as compared with **3a**. In general, the presence of the Bpin groups in these complexes seems to greatly reduce their activity, compared to the non-borylated analogue **2**. It is possible that the Lewis-acidic boron atom in these complexes is competing with, and concomitantly deactivating the platinum centre, by coordinating to the nitrogen donors in biomolecules. Further in vitro studies are therefore needed to address the structure activity relationships in these complexes in order to design a potent drug candidate.

Conclusion

Pyridinecarboxaldimines derived from the condensation of 2-pyridinecaboxaldehydes and aminoboronate esters $H_2NC_6H_4Bpin$ have been prepared in high yields and used as

Fig. 5. A view of complex **4a**, with displacement ellipsoids drawn at the 30% probability level. H atoms have been omitted. Selected bond distances and angles; Pt—N(9) 1.9936(19), Pt—N(1) 2.057(2), Pt—Cl(2) 2.2830(7), Pt—Cl(1) 2.2981(6), C(8)—N(9) 1.291(3), B—O(16) 1.3704, B—O(19) 1.366(4), and C(11)—B 1.555(4) Å; N(9)-Pt-N(1) 80.10(8), N(9)-Pt-Cl(2) 92.51(6), N(1)-Pt-Cl(2) 172.45(6), N(9)-Pt-Cl(1) 174.05(6), N(1)-Pt-Cl(1) 100.01(6), Cl(2)-Pt-Cl(1) 87.18(2), O(19)-B-O(16) 113.0(3), O(19)-B-C(11) 121.4(3), and O(16)-B-C(11) 125.6(2)°.

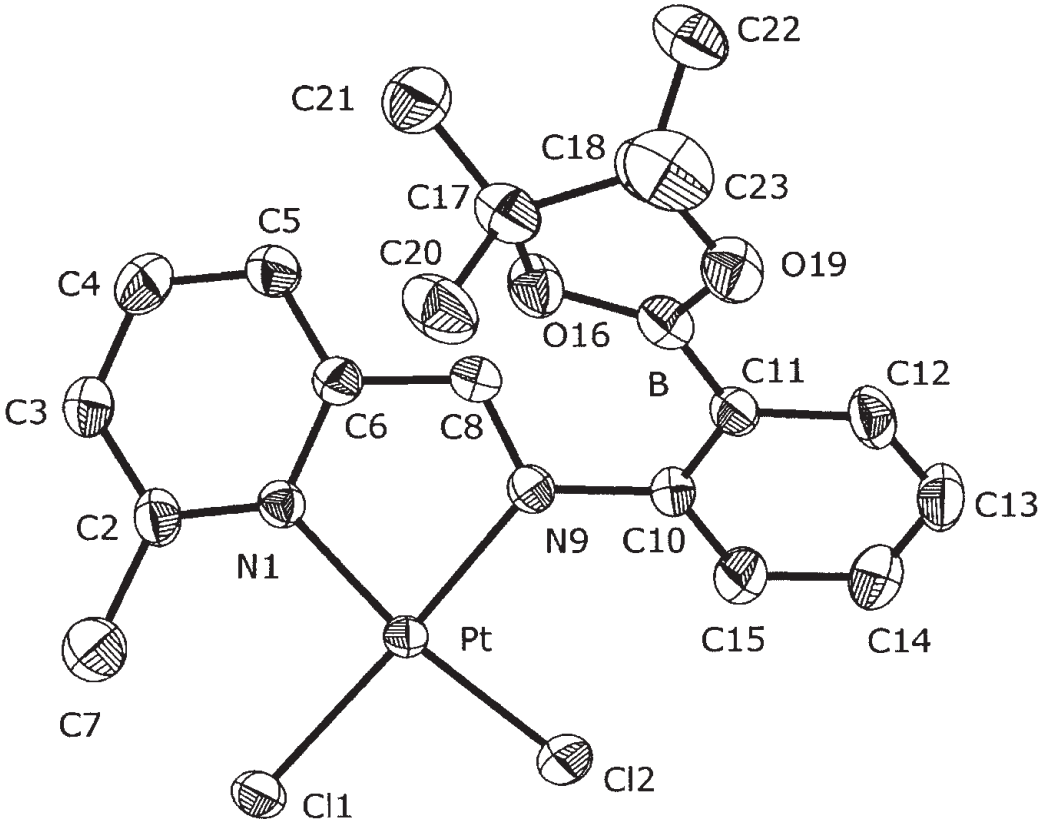


Table 2. Percent reduction of cell proliferation of cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) cancer cells to compounds at 5 and 140 μmol/L, respectively (n = 8; ± SD).

Entry	Compound	OV2008	C13
1	Cisplatin	44±11	41±8
2	**2**	21±3	37±8
3	**3a**	0	56±10
4	**3b**	0	39±10
5	**3c**	0	0
6	**4a**	0	12±8
7	**4b**	0	35±9
8	**4c**	0	0

ligands for platinum. The resulting metal complexes have shown negligible cytotoxicity against both cisplatin-sensitive and cisplatin-resistant human ovarian cells, OV2008 and C13, respectively, where the addition of a Lewis-acidic boron moiety appears to deactivate the metal complexes ability to coordinate to DNA.

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