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ARTICLE

Synthesis, characterization, and bioactivities of platinum(II) complexes bearing pyridinecarboxaldimines containing aliphatic groups

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Abstract: Condensation of 2-pyridinecarboxaldehyde with six primary amines containing aliphatic groups gave the corresponding pyridinecarboxaldimines (N-N′R). Addition of these ligands to $[PtCl_2(coe)]_2$ (coe = *cis*-cyclooctene) gave complexes of the type *cis*-$PtCl_2$(N-N′R) (**1**: R = propyl, **2**: R = hexyl, **3**: R = octyl, **4**: R = nonyl, **5**: R = hexadecyl, and **6**: R = octadecyl) in moderate yields. The molecular structure of the hexyl derivative (**2**) has been confirmed by an X-ray diffraction study. Crystals of **2** were triclinic with $a = 8.6858(19)$ Å, $b = 8.7567(19)$ Å, $c = 9.5080(19)$ Å, $\alpha = 76.546(3)°$, $\beta = 87.151(3)°$, and $\gamma = 78.586(3)°$ in the space group $P\bar{1}$. All platinum complexes show considerable anti-bacterial and anti-mycobacterial properties.

Key words: aliphatic amines, anti-microbial, anti-mycobacterial, pyridinecarboxaldimines, platinum.

Résumé : La condensation du pyridine-2-carboxaldéhyde avec six amines primaires contenant des groupes aliphatiques conduit à la formation des pyridinecarboxaldimines correspondantes (N-N′-R). L'addition de ces ligands au $[Pt(Cl_2)(coe)]_2$ (coe = *cis*-cyclooctène) conduit à la formation de complexes de type *cis*-$PtCl_2$(N-N′R) (**1**: R = propyle, **2**: R = hexyle, **3**: R = octyle, **4**: R = nonyle, **5**: R = hexadécyle et **6**: R = octadécyle), avec des rendements moyens. On a pu confirmer la structure moléculaire du dérivé hexyle (**2**) par des études de diffraction des rayons X. Les cristaux du composé **2** sont tricliniques, dans le groupe d'espace $P\bar{1}$, avec $a = 8,6858(19)$ Å, $b = 8,7567(19)$ Å, $c = 9,5080(19)$ Å, $\acute{a} = 76,546(3)°$, $\hat{a} = 87,151(3)°$ et $\tilde{a} = 78,586(3)°$. Tous les complexes de platine présentent des propriétés antibactériennes et antimycobactériennes importantes.

Mots-clés : amines aliphatiques, antimicrobien, antimicybactérien, pyridinecarboxaldimines, platine.

Introduction

Cisplatin, *cis*-$[PtCl_2(NH_3)_2]$, and related platinum-based complexes are currently used as anti-cancer agents against testicular and ovarian malignancies.[1] The primary mechanism of action in these platinum drugs is believed to arise from the metal's interaction with DNA, where the platinum is believed to bind preferentially with the N-7 positions of the purine bases. There are severe limitations to platinum therapy, however, such as neural and kidney toxicity as well as intrinsic and acquired resistance of tumor cells to the drugs. These complications have provided incentive for further research into the development of platinum-based complexes with increased solubility and enhanced specificity towards cancer cells. Previous studies have shown that *cis*-amminedichloro(2-methylpyridine)platinum(II) (ZD0473) (Fig. 1*a*) has considerable cytotoxicity in cisplatin-resistant cell lines.[2]

We have been making ZD0473 analogues by replacing the NH_3 group with a pendant imine group. Previous studies have shown that the platinum complex derived from aniline (Fig. 1*b*) has shown considerable activity against the hormone-independent human mammary carcinoma cell line MDA-MB 231.[3] Varying the aniline functionality allows us to design compounds with a wide range of physical and chemical properties. 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.[1] Although the anti-cancer chemistry of cisplatin derivatives has been studied extensively, the use of platinum compounds to treat other diseases and illness is an area that has received much less attention.[4] We report herein our results on the synthesis and initial biological testing of *cis*-dichloro(pyridin-2-ylcarboxaldimine)platinum(II) compounds containing a variety of aliphatic groups, which we propose will improve lipophilicities and aid in drug delivery.

Experimental

General procedures and methods

Reagents and solvents used were obtained from Aldrich Chemicals. Potassium tetrachloroplatinate was purchased from Precious Metals Online Ltd. $[PtCl_2(\eta^2\text{-coe})]_2$[5] was prepared as previously reported. NMR spectra were recorded on a JEOL JNM-GSX270 FT NMR spectrometer. ^{1}H NMR chemical shifts are reported in ppm and are referenced to residual protons in deuterated solvent at 270 MHz. ^{13}C NMR chemical shifts are referenced to solvent carbon resonances as internal standards at 68 MHz. Multiplicities are reported as singlet (s), doublet (d), triplet (t), quintet (quint), sext (sextet), multiplet (m), and overlapping (ov). The infrared spectra were obtained using a Mattson Genesis FT-IR spectrometer and are reported in cm^{-1}. Melting points were measured uncorrected with a Mel-Temp apparatus. Elemental analyses for carbon, hydrogen, and nitrogen were carried out at

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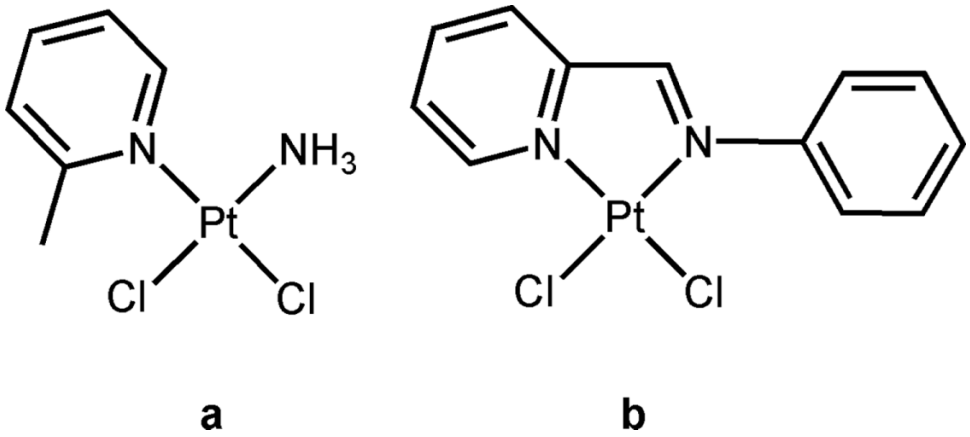
Fig. 1. Structure of (*a*) ZD0473 and (*b*) dichloro-(*E*)-*N*-(pyridin-2-ylmethylene)aniline-platinum(II).



Guelph Chemical Laboratories Ltd. (Guelph, Ontario). Antimycobacterial susceptibility tests were performed using modified Middlebrook 7H9 broth base (BBL MGIT; Becton Dickinson, Mississauga, Ontario) in non-tissue culture treated, low-binding, black 96-well microtitre plates sealed with polyester films (50 μm). Fluorometric readings (in relative fluorescence units) were recorded using a Molecular Devices Gemini EM dual-scanning microplate spectrofluorometer with a 530 nm excitation filter and a 590 nm emission filter operating in top-scan mode and are the mean values of 30 reads per well. Antibiotic susceptibility tests were performed using BBL Mueller Hinton II cation adjusted broth and Difco Sabouraud Dextrose broth (Becton Dickinson, Mississauga, Ontario) for bacterial and fungal cultures respectively in non-tissue culture treated, clear 96-well microtitre plates. Optical densities (OD) were measured using a Molecular Devices Emax microplate reader with a 600 nm filter.

Synthesis

General synthesis of iminopyridine ligands

The iminopyridines **L1-6** were prepared by modification of a known synthesis.[6] A CH_2Cl_2 (10 mL) solution of the appropriate amine (1 equivalent) was added to a CH_2Cl_2 (15 mL) solution of 2-pyridinecarboxaldehyde. Activated 4 Å molecular sieves (10 g) were added and the reaction mixture was allowed to stand at RT for 2 days. Following the removal of the molecular sieves by filtration, solvent was removed under vacuum to afford the desired iminopyridines as orange–red oils.

(E)-N-(Pyridine-2-ylmethylene)hexadecane-1-amine (L5)

NMR spectroscopic data (in $CDCl_3$): ^{1}H δ: 8.62 (d, J_{HH} = 4.7 Hz, 1H, Ar), 8.35 (s, 1H, *H*C = N), 7.96 (d, J_{HH} = 7.9 Hz, 1H, Ar), 7.72 (ov ddd, J_{HH} = 7.9, 4.7, 1.5 Hz, 1H, Ar), 7.28 (m, 1H, Ar), 3.65 (t, J_{HH} = 6.9 Hz, 2H, NC*H*$_2$), 1.70 (m, 2H, –C*H*$_2$–), 1.30–1.23 (ov m, 26H, –(C*H*$_2$)$_{13}$–), 0.85 (t, J_{HH} = 7.4 Hz, 3H, CH_2CH_3); ^{13}C{^{1}H} δ: 161.7, 154.8, 149.4, 136.5, 124.6, 121.2, 61.7, 32.0, 30.8, 29.8 (7C), 29.7, 29.5, 29.4, 27.4, 22.7, 14.2. IR (Nujol): 1650 ($\nu_{C=N}$).

General synthesis of platinum complexes

A CH_2Cl_2 (5 mL) solution of the appropriate iminopyridine ligand (0.80 mmol) was added dropwise to a stirred CH_2Cl_2 (5 mL) solution of $[PtCl_2(\eta^2\text{-coe})]_2$ (300 mg, 0.40 mmol). The reactions were allowed to proceed at RT for 18 h at which point hexane (5 mL) was added and the solutions stored at 5 °C. The resulting orange precipitates were collected by suction filtration to afford the desired platinum compounds.

PtCl$_2$(N,N′-*propyl*) (1)

Yield: 84%, mp 208–210 °C. NMR spectroscopic data (in $CDCl_3$): ^{1}H δ: 9.71 (d, J_{HH} = 5.7 Hz, J_{HPt} = 37.1 Hz, 1H, Ar), 8.65 (s, J_{HPt} = 96.7 Hz, 1H, *H*C = N), 8.15 (ov ddd, J_{HH} = 7.7, 7.7, 1.2 Hz, 1H, Ar), 7.80 (d, J_{HH} = 7.7 Hz, 1H, Ar), 7.63 (ov ddd, J_{HH} = 7.7, 5.7, 1.2 Hz, 1H, Ar), 4.15 (t, J_{HH} = 7.4 Hz, J_{HPt} = 38.6 Hz, 2H, NC*H*$_2$), 2.01 (sext, J_{HH} = 7.4 Hz, 2H, NCH_2CH_2), 0.96 (t, J_{HH} = 7.4 Hz, 3H, CH_2CH_3); ^{13}C{^{1}H} δ: 166.6, 159.3, 143.3, 138.1, 128.5, 128.3, 65.5, 43.4, 24.5. IR (Nujol): 2930 (s), 2856 (s), 1631 (m, $\nu_{C=N}$), 1601 (m), 1461 (m), 1378 (m), 1301 (m), 1237 (m), 1050 (m). Anal. calcd. for $C_9H_{12}N_2Cl_2Pt$ (414.31) (%): C 26.09, H 2.93, N 6.76; found: C 26.25, H 2.88, N 6.49.

PtCl$_2$(N,N′-*hexyl*) (2)

Yield: 86%, mp 149–150 °C. NMR spectroscopic data (in $CDCl_3$): ^{1}H δ: 9.65 (dd, J_{HH} = 4.9, 1.5 Hz, J_{HPt} = 36.1 Hz, 1H, Ar), 8.66 (s, J_{HPt} = 95.7 Hz, 1H, *H*C = N), 8.15 (ov ddd, J_{HH} = 7.7, 7.7, 1.5 Hz, 1H, Ar), 7.82 (d, J_{HH} = 7.7 Hz, 1H, Ar), 7.66 (ov ddd, J_{HH} = 7.7, 4.9, 1.5 Hz, 1H, Ar), 4.15 (t, J_{HH} = 7.2 Hz, J_{HPt} = 38.6 Hz, 2H, NC*H*$_2$), 1.95 (quint, J_{HH} = 7.2 Hz, 2H, NCH_2CH_2), 1.36–1.24 (ov m, 6H, –(C*H*$_2$)$_3$–), 0.86 (t, J_{HH} = 7.2 Hz, 3H, CH_2CH_3); ^{13}C{^{1}H} δ: 166.7, 157.0, 150.3, 139.4, 128.3, 126.8, 61.4, 31.3, 31.2, 26.2, 22.6, 14.0. IR (Nujol): 2940 (s), 2857 (s), 1623 (m, $\nu_{C=N}$), 1597 (m), 1460 (m), 1379 (m), 1297 (m), 1240 (m), 1114 (m). Anal. calcd. for $C_{12}H_{18}N_2Cl_2Pt$ (456.27) (%): C 31.59, H 3.98, N 6.14; found: C 31.77, H 4.12, N 5.87.

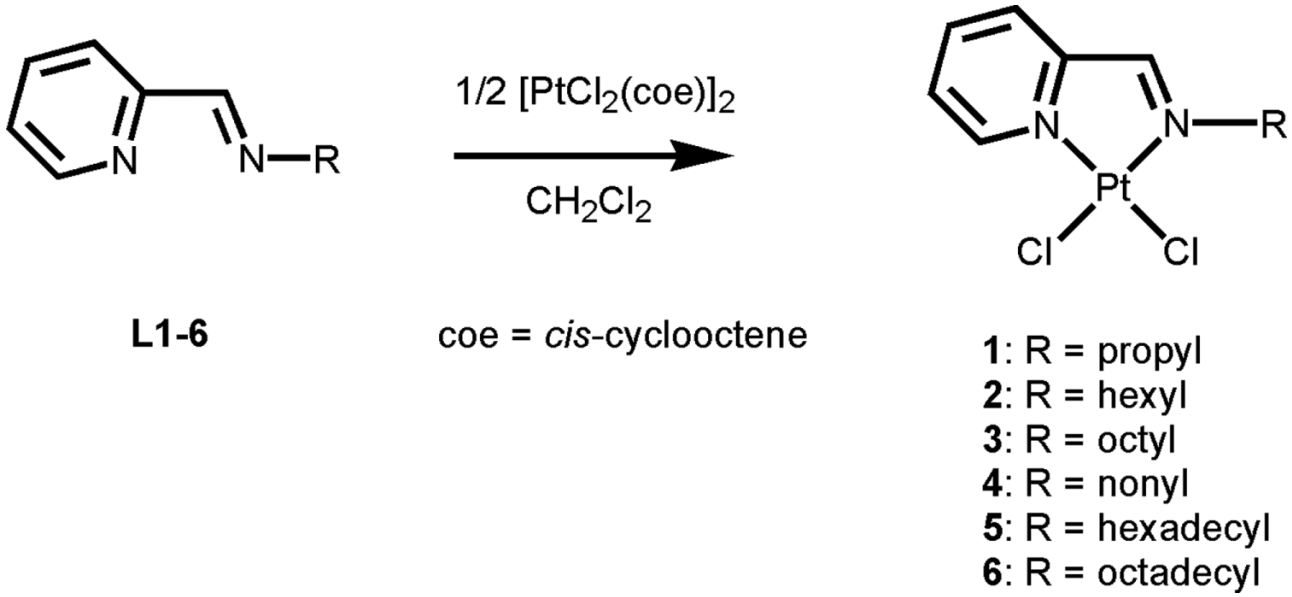
PtCl$_2$(N,N′-*octyl*) (3)

Yield: 82%, mp 126–127 °C. NMR spectroscopic data (in $CDCl_3$): ^{1}H δ: 9.49 (d, J_{HH} = 5.3 Hz, J_{HPt} = 32.3 Hz, 1H, Ar), 8.76 (s, J_{HPt} = 96.6 Hz, 1H, *H*C = N), 8.14 (ov ddd, J_{HH} = 7.9, 7.9, 1.3 Hz, 1H, Ar), 7.93 (d, J_{HH} = 7.9 Hz, 1H, Ar), 7.63 (ov ddd, J_{HH} = 7.9, 5.3, 1.3 Hz, 1H, Ar), 4.10 (t, J_{HH} = 7.3 Hz, J_{HPt} = 36.3 Hz, 2H, NC*H*$_2$), 1.90 (quint, J_{HH} = 7.3 Hz, 2H, NCH_2CH_2), 1.29–1.23 (ov m, 10 H, –(C*H*$_2$)$_5$–), 0.84 (t, J_{HH} = 6.9 Hz, 3H, CH_2CH_3); ^{13}C{^{1}H} δ: 167.7, 157.2, 149.8, 139.6, 128.2, 127.5, 61.2, 31.8, 31.1, 29.2 (2C), 26.6, 22.7, 14.2. IR (Nujol): 2926 (s), 2856 (s), 1624 (m, $\nu_{C=N}$), 1597 (m), 1459 (m), 1378 (m), 1299 (m), 1239 (m). Anal. calcd. for $C_{14}H_{22}N_2Cl_2Pt$ (484.46) (%): C 34.71, H 4.59, N 5.78; found: C 34.44, H 4.55, N 5.71.

PtCl$_2$(N,N′-*nonyl*) (4)

Yield: 91%, mp 126–128 °C. NMR spectroscopic data (in $CDCl_3$): ^{1}H δ: 9.43 (d, J_{HH} = 5.4 Hz, J_{HPt} = 31.6 Hz, 1H, Ar), 8.88 (s, J_{HPt} = 93.7 Hz, 1H, *H*C = N), 8.14 (ov ddd, J_{HH} = 7.7, 7.7, 1.2 Hz, 1H, Ar), 8.03 (d, J_{HH} = 7.7 Hz, 1H, Ar), 7.63 (ov ddd, J_{HH} = 7.7, 5.4, 1.2 Hz, 1H, Ar), 4.09 (t, J_{HH} = 6.8 Hz, J_{HPt} = 38.8 Hz, 2H, NC*H*$_2$), 1.89 (quint, J_{HH} = 6.8 Hz, 2H, NCH_2CH_2), 1.27–1.21 (ov m, 12H, –(C*H*$_2$)$_6$–), 0.83 (t, J_{HH} = 6.2 Hz, 3H, CH_2CH_3); ^{13}C{^{1}H} δ: 168.3, 157.3, 149.6, 139.8, 128.2, 128.0, 61.0, 31.9, 31.1, 29.5, 29.3 (2C), 26.6, 22.7, 14.2. IR (Nujol): 2923 (s), 2853 (s), 1625 (m, $\nu_{C=N}$), 1598 (m), 1460 (m), 1377 (m), 1295 (m), 1240 (m). Anal. calcd. for $C_{15}H_{24}N_2Cl_2Pt$ (498.49) (%): C 36.14, H 4.86, N 5.62; found: C 36.36, H 5.03, N 5.38.

Fig. 2. Synthesis of platinum complexes **1–6**.



PtCl$_2$(N,N′-hexadecyl) (5)

Yield: 92%, mp 137–140 °C. NMR spectroscopic data (in $CDCl_3$): ^{1}H δ: ^{1}H δ: 9.67 (d, J_{HH} = 6.4 Hz, J_{HPt} = 38.3 Hz, 1H, Ar), 8.65 (s, J_{HPt} = 94.5 Hz, 1H, *H*C = N), 8.14 (ov ddd, J_{HH} = 7.7, 7.7, 1.5 Hz, 1H, Ar), 7.81 (d, J_{HH} = 7.7 Hz, 1H, Ar), 7.66 (ov ddd, J_{HH} = 7.7, 6.4, 1.5 Hz, 1H, Ar), 4.16 (t, J_{HH} = 7.4 Hz, J_{HPt} = 37.8 Hz, 2H, NC*H*$_2$), 1.95 (quint, J_{HH} = 7.4 Hz, 2H, NCH$_2$C*H*$_2$), 1.32–1.23 (ov m, 26H, –(C*H*$_2$)$_{13}$–), 0.86 (t, J_{HH} = 6.9 Hz, 3H, CH$_2$C*H*$_3$); ^{13}C{^{1}H} (50 °C) δ: 167.7, 157.3, 149.8, 139.5, 128.1, 127.5, 61.1, 31.9, 31.1, 29.7 (4C), 29.6, 29.5, 29.4, 29.3, 29.2, 26.6, 23.8, 22.6, 14.0. IR (Nujol): 2922 (s), 2852 (s), 1624 (m, $\nu_{C=N}$), 1587 (m), 1469 (m), 1377 (m), 1299 (m), 1238 (m). Anal. calcd. for $C_{22}H_{38}N_2Cl_2Pt$ (596.70) (%): C 44.28, H 6.43, N 4.70; found: C 44.57, H 6.18, N 4.54.

PtCl$_2$(N,N′-octadecyl) (6)

Yield: 90%, mp 139–142 °C. NMR spectroscopic data (in $CDCl_3$): ^{1}H δ: 9.72 (d, J_{HH} = 6.2 Hz, J_{HPt} = 39.1 Hz, 1H, Ar), 8.64 (s, J_{HPt} = 98.4 Hz, 1H, *H*C = N), 8.14 (ov ddd, J_{HH} = 7.7, 7.7, 1.5 Hz, 1H, Ar), 7.78 (d, J_{HH} = 7.7 Hz, 1H, Ar), 7.67 (ov ddd, J_{HH} = 7.7, 6.2, 1.5 Hz, 1H, Ar), 4.18 (t, J_{HH} = 7.4 Hz, J_{HPt} = 38.0 Hz, 2H, NC*H*$_2$), 1.97 (quint, J_{HH} = 7.4 Hz, 2H, NCH$_2$C*H*$_2$), 1.32–1.23 (ov m, 30H, –(C*H*$_2$)$_{15}$–), 0.86 (t, J_{HH} = 7.0 Hz, 3H, CH$_2$C*H*$_3$); ^{13}C{^{1}H} (50 °C) δ: 167.4, 157.3, 149.9, 139.4, 128.1, 127.3, 61.1, 31.9, 31.1, 29.7 (7C), 29.6, 29.5, 29.4, 29.3, 29.2, 26.6, 22.7, 14.0. IR (Nujol): 2924 (s), 2851 (s), 1624 (m, $\nu_{C=N}$), 1597 (m), 1468 (m), 1377 (m), 1300 (m), 1241 (m). Anal. calcd. for $C_{24}H_{42}N_2Cl_2Pt$ (624.76) (%): C 46.14, H 6.79, N 4.48; found: C 45.88, H 6.62, N 4.77.

X-ray crystallography

Crystals of **2** were grown from a saturated solution of chloroform at 5 °C. Single crystals were coated with Paratone-N oil, mounted using a polyimide MicroMount, and frozen in the cold nitrogen 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 10 s exposure times. The detector distance was 5 cm. The data were reduced (SAINT)[7] and corrected for absorption (SADABS).[8] The structure was solved by direct methods and refined by full-matrix least squares on F^2 (SHELXTL).[9] All non-hydrogen atoms were refined using anisotropic displacement parameters. Hydrogen atoms were included in calculated positions and refined using a riding model. Crystallographic information has also been deposited with the Cambridge Crystallographic Data Centre (CCDC 882438). Copies of the data can be obtained free of charge from 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 e-mail: deposit@ccdc.cam.ac.uk).

Biological testing

Artemia *toxicity bioassay*

Dehydrated *Artemia salina* (brine shrimp) eggs were obtained from Ward's Scientific and hatched following the manufacturer's guidelines. Thirty nauplii were then transferred to a test tube containing 5 mL of stock salt solution with a final concentration of either 10, 100, or 1000 ppm of a test compound or the positive (cisplatin) or negative (distilled water) controls. The test tubes were incubated at 30 °C for 24 h after which the number of surviving brine shrimp was counted. Triplicates tests were performed for each compound and control. The LC_{50} values were calculated from the linear trendline $\log_{10}$ of concentration of compound and number of surviving brine shrimp after 24 h.

Anti-mycobacterial susceptibility screening assay

Anti-mycobacterial activity against *Mycobacterium tuberculosis* strain H37Ra (ATCC 25177) was evaluated using a microplate resazurin assay modified from Collins and Franzblau.[10] Stock solutions (5.0 mg/mL) of test compounds and rifampin (10 μg/mL, positive control) were prepared with sterile-filtered DMSO and stored at 4 °C. Antibiotic and test compound solutions were used within 1 month and 1 week of preparation, respectively. Immediately prior to use, stock solutions of test compounds and rifampin (40 μL) were diluted with modified Middlebrook 7H9 broth (960 μL) and the resulting solutions (100 μL) were transferred to non-peripheral wells of a 96-well microtitre plate and inoculated with suspensions of *M. tuberculosis* (100 μL, cell density 2.0 × 10^6 cells/mL). To reduce evaporation from the plates, sterile water (200 μL) was added to perimeter wells. In addition to the rifampin positive controls, negative controls (4% DMSO in modified Middlebrook 7H9 broth (100 μL) inoculated with suspensions of *M. tuberculosis* (100 μL)) and blanks (2% DMSO in modified Middlebrook 7H9 broth (200 μL) and test solutions (100 μL) with modified Middlebrook 7H9 broth (100 μL)) were included in each plate. All controls and test compounds were tested in triplicate. Plates were incubated (37 °C, 5% CO_2) for 3 days in a humid environment before a solution of resazurin (62.5 μg/mL) in 5% aqueous Tween 80 (50 μL) was added to all wells. Plates were then incubated for a further 24 h, sealed with an adhesive polyester film, and mycobacterial

Fig. 3. Molecular structure of **2** with ellipsoids drawn at the 50% probability level and hydrogen atoms omitted for clarity. Selected bond distances (Å): Pt(1)–N(2) 2.003(6), Pt(1)–N(1) 2.031(6), Pt(1)–Cl(2) 2.2833(18), Pt(1)–Cl(1) 2.3001(19), N(1)–C(5) 1.327(10), N(1)–C(1) 1.348(10), N(2)–C(6) 1.278(9), N(2)–C(7) 1.483(9). Selected bond angles (°): N(2)–Pt(1)–N(1) 80.6(2), N(2)–Pt(1)–Cl(2) 94.72(18), N(1)–Pt(1)–Cl(2) 175.35(18), N(2)–Pt(1)–Cl(1) 175.64(17), N(1)–Pt(1)–Cl(1) 95.01(18), Cl(2)–Pt(1)–Cl(1) 89.62(7), C(5)–N(1)–C(1) 119.3(6), C(6)–N(2)–C(7) 121.3(6).

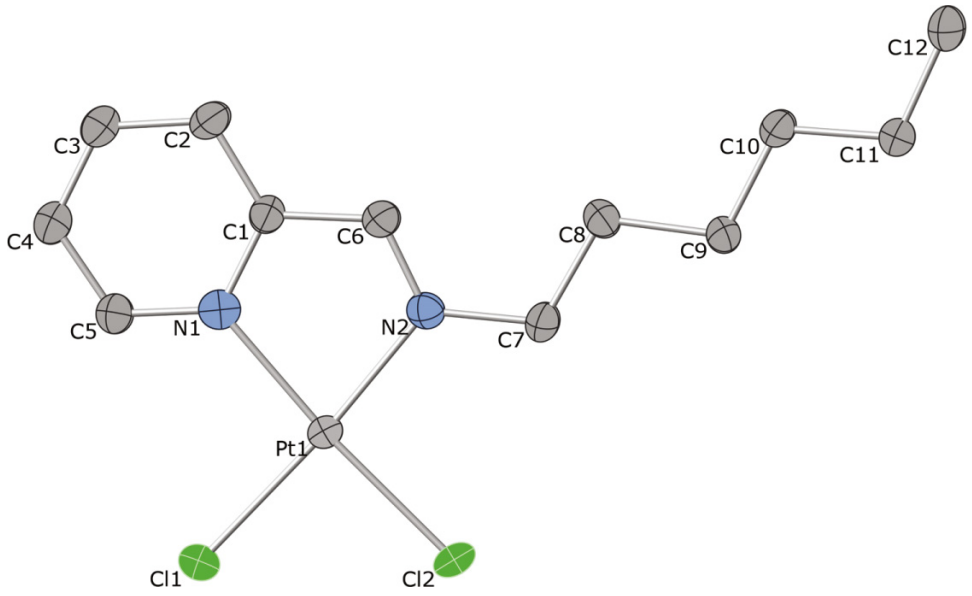


Table 1. Crystallographic data collection parameters for complex 2.

Complex	**2**
Chemical formula	$C_{12}H_{18}Cl_2N_2Pt$
Formula mass	456.27
Crystal dimensions (mm³)	0.30×0.10×0.05
Crystal system	Triclinic
Space group	*P*1
Z	2
a (Å)	8.6858 (19)
b (Å)	8.7567 (19)
c (Å)	9.5080 (19)
α (°)	76.546 (3)
β (°)	87.151 (3)
γ (°)	78.586 (3)
Volume (Å³)	689.4 (3)
d_{calcd} (mg/m³)	2.198
T (K)	198 (1)
Radiation	MoKα (λ = 0.71073 Å)
μ (mm⁻¹)	10.544
Total reflections collected	4518
Independent reflections	2949
No. of variables	155
θ (°)	2.20–27.50
GoF on F^2	1.063
R_1 ($I > 2\sigma(I)$)	0.0408
wR_2 (all data)	0.1073
Largest diff. peak and hole (Å)	4.250 and –4.030

Note: $R_1 = \Sigma||F_o| - |F_c||/\Sigma |F_o|$. $wR_2 = (\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[F_o^4])^{1/2}$, where $w = 1/[\sigma^2(F_o^2) + (0.0803P)^2]$ and $P = (\max(F_o^2, 0) + 2F_c^2)/3$.

growth assessed fluorometrically at 37 °C. Fluorescence values were corrected for any background fluorescence of the media and test compounds by subtracting the mean fluorescence readings of the appropriate blanks from the mean fluorescence readings of the control and test compound wells. The percent inhibition of mycobacterial growth was then defined as [1 – (mean test or positive control fluorescence/mean negative control fluorescence)] × 100.

Antibiotic susceptibility screening assay

Anti-bacterial activity against *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 10145) and anti-fungal activity against *Candida albicans* (ATCC 14053) were evaluated using a microbroth dilution antibiotic susceptibility assay modified from McCulloch et al.[11] Stock solutions of test compounds (5.0 mg/mL) were prepared with sterile-filtered DMSO, stored at 4 °C, and used within 1 week of preparation. Immediately prior to use, stock solutions (40 μL) were diluted with the appropriate nutrient broth (960 μL) and the resulting test solutions (100 μL) were transferred to non-peripheral wells of a 96-well microtitre plate in triplicate. Wells were then inoculated with suspensions of the appropriate microbial strain (100 μL, cell density 1.0 × 10⁶ CFU/mL), and to reduce evaporation from the plates, sterile water (200 μL) was added to perimeter wells. Positive controls consisted of one column containing a twofold serial dilution of antibiotic (50–1.56 μg/mL, 100 μL per well, kanamycin for *S. aureus* and *P. aeruginosa* and nystatin for *C. albicans*) as 4% DMSO solutions in the appropriate nutrient broth inoculated with suspensions of the appropriate pathogen (100 μL). Additionally, each plate contained six untreated controls (4% DMSO in the appropriate nutrient broth (100 μL) inoculated with suspensions of the appropriate pathogen (100 μL)) and six un-inoculated blanks (200 μL of 2% DMSO in the appropriate nutrient broth). The optical densities of the wells were measured before and after a 24 h incubation period (37 °C), and the change in OD (ΔOD) was calculated by subtracting the initial OD from the final OD of corresponding wells. ΔOD values were corrected for background absorbance of the media by subtracting the mean ΔOD readings of the blanks from the mean ΔOD readings of the control and test

Table 2. *Artemia* toxicity data.

Compound	LC_{50} (ppm) (*Artemia* assay)[a]
1	32.1
2	4.33
3	4.96
4	5.16
5	3.77
6	3.51
Cisplatin	5.17

[a]Due to the compounds' insolubility in water, DMSO was used as a vehicle.

Table 3. Anti-microbial and anti-mycobacterial testing of platinum compounds 1–6.

	Candida albicans		*Staphylococcus aureus*		*Mycobacterium tuberculosis*	
Compound	IC_{50} (μg/mL)[a]	MIC (μg/mL)[b]	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)
1	Inactive[c]	Inactive	58.7	100	1.65	75.00
2	Inactive	Inactive	61.2	100	1.91	25.00
3	81.5	100	25.7	75	1.52	12.50
4	49.2	100	13.9	75	0.70	6.25
5	Inactive	Inactive	Inactive	Inactive	1.78	12.50
6	Inactive	Inactive	40.2	75	51.3	>200.00[c]

[a]IC_{50} values estimated by probit analysis.
[b]MIC is defined as the lowest concentration that showed over 90% inhibition.
[c]Less than 50% inhibition in initial screening (at 100 μg/mL) is considered to be inactive.

compound wells. The percent inhibition of bacterial or fungal growth was then defined as [1 – (mean test or positive control ΔOD/mean negative control ΔOD)] × 100.

Determination of minimum inhibitory concentrations (MIC) and median lethal concentrations (IC_{50})

MIC and IC_{50} values were determined for test compounds that exhibited >50% growth inhibition at a concentration of 100 μg/mL in the initial screening assays. Assays were performed as described previously on twofold serial dilutions of active compounds in triplicate. All compounds were tested at a minimum of 10 and maximum of 20 concentrations obtained from two dilution series (400.0–0.781 and 300.0–0.586 μg/mL). The MIC of a compound was considered to be the lowest assay concentration at which it inhibited mycobacterial growth by more than a mean value of 90%.[11] For most of the compounds, reliable estimates of their absolute IC_{50} values could not be obtained through four-parameter logistic regression of the microbial growth data (in relative fluorescence units or OD units).[12] Therefore, IC_{50} values were obtained by probit analysis[13,14] performed by fitting mean percent inhibition values calculated from the growth data to the probit model by the maximum likelihood method[15] using SPSS Statistics 19 (IBM).

Results and discussion

Synthesis and structure

The addition of a primary amine to commercially available 2-pyridinecarboxaldehyde to afford the corresponding pyridine-2-ylcarboxaldimine ligand is a well-known route to a versatile class of bidentate ligands.[16–19] Variation of the amine allows for the facile design of ligands and metal complexes with different physical and chemical properties. The ligands **L1-6** were prepared via a simple addition of the primary amine to 2-pyridinecarboxaldehyde. Although **L1-4** and **L6** have all been made previously,[6] the hexadecyl derivative **L5** has not yet been reported. The corresponding platinum complexes **1–6** (Fig. 2) were prepared by addition of the pyridinecarboxaldimine ligands to CH_2Cl_2 solutions of $[PtCl_2(\eta^2\text{-coe})]_2$ (coe = *cis*-cyclooctene).[20]

Complexes **1–6** contain long chain side groups that are of particular interest, as a number of biologically relevant natural products, such as capsaicin,[21] also possess a hydrocarbon tail. Using lipophilic groups to deliver platinum complexes is a rapidly growing area of interest.[22–25] As such, we have decided to look at the synthesis and initial bioactivities of platinum complexes containing long chain aliphatic hydrocarbons bound to an iminopyridine ligand. In this study, complexes **1–6** 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 sp^2 proton upon coordination of the ligand to the metal center. For instance, the singlet at δ 8.35 ppm for the free ligand derived from 2-pyridinecarboxaldehyde and hexylamine shifts to 8.65 ppm in complex **1**. Platinum satellites are also observed for this resonance (J_{HPt} = 97 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 8.62 to 9.71 ppm (J_{HPt} = 37 Hz). Similar trends are observed for all platinum complexes **1–6**.

Complex **2** has also been characterized by an X-ray diffraction study (Fig. 3) with crystallographic data given in Table 1. The nitrogen–platinum bonds of 2.003(6) Å (imine) and 2.031(6) Å (pyridine) are similar to those reported in other iminopyridine platinum systems.[26–28] The imine C(6)–N(2) distance is 1.278(9) Å and in the range of accepted carbon–nitrogen double bonds.[29] The N(2)–Pt(1)–N(1) angle is 80.6(2)° and is considerably less than 90°, as is often observed in related complexes owing to the bidentate nature of the iminopyridine ligand. The platinum chloride distances are Pt(1)–Cl(2) 2.2833(18) Å and Pt(1)–Cl(1) 2.3001(19) Å, with a typical angle between these three atoms of Cl(2)–Pt(1)–Cl(1) 89.62(7)°. Interestingly, compound **2** forms π-stacked dimers around an inversion center with a "normal" platinum–platinum distance of 3.4 Å.

Bioactivities

Before carrying out time-consuming and expensive anti-cancer studies, we decided to conduct a preliminary evaluation on compounds **1–6** to see if these new platinum species displayed any biological activity. Previous studies have shown that bioactive compounds are often toxic to brine shrimp (*A. salina*) larvae and that LC_{50} values can be directly correlated with these toxicity studies.[30–33] Indeed, the brine shrimp lethality assay has been used to prescreen compounds for anti-cancer properties and good correlations have been found between the in vivo and in vitro tests.[34,35] We have found that the LC_{50} data suggest that the propyl derivative **1** is not overly toxic; however, the other compounds were on par, if not more toxic, than cisplatin (see Table 2). Furthermore, it generally appears that increasing the length of the hydrocarbon tail increases the toxicity and thus, potentially, the biological activity. This increase in activity could be due to improved cell penetration due to greater lipophilicity.

Encouraged by these initial results, we decided to investigate any potential anti-microbial properties of compounds **1–6**. Although none of the analogues showed significant activity against the Gram-negative bacterium *P. aeruginosa* in our initial antibiotic screening (growth inhibition ranged from 5% to 25% at 100 μg/mL), some compounds did show weak anti-fungal activity and moderate activity against the Gram-positive bacterium *S. aureus* (see Table 3). Of particular interest, however, is the anti-mycobacterial activity exhibited by the complexes. With the exception of the octadecyl analogue **6**, the analogues showed good activity against *M. tuberculosis* H37Ra and compound **4** in particular exhibited activity that is comparable with some currently used tuberculosis drugs. The octyl and nonyl derivatives **3** and **4** were consistently the most active in our antibiotic assays, whilst the low solubilities of the hexadecyl and octadecyl compounds (**5** and **6**) were probably responsible for their reduced activity in the assays. These results are remarkable in that only a handful of platinum complexes

have previously been examined for anti-mycobacterial activity.[36–40] Our results suggest that lipophilic platinum complexes show considerable promise for the treatment of tuberculosis.

The preliminary nature of this work meant that only a small library of analogues was tested; however, the observed biological activity of these compounds, especially their anti-mycobacterial, warrants further in-depth investigation and the synthesis of more complexes is currently underway.

Conclusion

Pyridinecarboxaldimines derived from the condensation of 2-pyridinecaboxaldehyde and aliphatic amines have been prepared in high yields and used as ligands for platinum. The resulting metal complexes have been examined for their potential antimicrobial properties and these complexes have shown incredible promise as lead compounds for tuberculosis drug development. Future work in this area is needed to design a drug candidate based on this structural motif and additional studies in this area are currently in progress, the results of which will be disclosed in due course.

Supplementary material

Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjc-2012-0371. CCDC 882438 contains the X-ray data in CIF format for this manuscript. These data can be obtained, free of charge, via http://www.ccdc.cam.ac.uk/products/csd/request or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 33603 or e-mail: deposit@ccdc.cam.ac.uk).

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