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Synthesis, characterization, and antimicrobial activities of palladium Schiff base complexes derived from aminosalicylic acids

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Abstract Six Schiff base compounds have been prepared from the condensation of *o*-vanillin, 2,3-dihydroxybenzaldehyde and 2,3,4-trihydroxybenzaldehyde with 4-aminosalicylic acid and 5-aminosalicylic acid (5-ASA). Addition of these Schiff bases to $[Pd(OAc)_2]$ afforded the corresponding bis(salicylaldiminato)palladium(II) complexes in moderate to excellent yields. All new palladium complexes have been characterized fully using standard spectroscopic methods, elemental analyses and a single-crystal X-ray diffraction study in the case of **2e**, the palladium complex containing Schiff base ligands derived from 5-ASA and 2,3-dihydroxybenzaldehyde. All derivatives of 5-ASA were examined for potential antimicrobial activities against two species of fungi, *Aspergillus niger* and *Saccharomyces cerevisiae*, as well as two species of bacteria, *Bacillus cereus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative).

Introduction

Although metals and their coordination complexes have been recognized for their therapeutic properties for many years, it was not until the serendipitous discovery that *cis*-diamminedichloroplatinum(II) (cisplatin, or *cis*-DDP; Fig. 1) inhibited cellular division in *Escherichia coli* that research into designing new metal complexes truly emerged as an important area of bioinorganic and pharmaceutical chemistry [1–5]. Unfortunately, cisplatin, although effective for treating various cancers, has limited efficacy due to numerous side effects arising from the compound's poor solubility in physiological media and for its lack of selectivity for cancerous cells. To overcome these problems associated with cisplatin, a significant amount of research over the past few decades has focused on generating and testing second and third generation platinum-based drugs based upon altering the nature of the ligands bound to the metal center. Notable examples include carboplatin and *cis*-$[PtCl_2(1,4\text{-DACH})]$ (DACH = diaminocyclohexane) (Fig. 1). Unfortunately, significant advances in platinum-based chemotherapy have yet to be realized and, as such, there has also been a considerable amount of effort directed toward examining the bioactivities of other metal complexes for various diseases and ailments [1].

Although once overlooked for their bioactivity, complexes of palladium have emerged in recent years as promising antimicrobial and anticancer candidates [6–25]. The readers are encouraged to read an excellent review by Hadjiliadis [19] summarizing the field of bioactive palladium complexes. As part of our study aimed at generating palladium compounds for their antimicrobial properties, we have recently focussed on the synthesis and testing of palladium(II) complexes containing Schiff base ligands. Schiff bases are easily prepared from any number of salicylaldehyde derivatives with simple primary amines. Variation of both the starting salicylaldehyde and the primary amine provides a synthetic strategy that readily allows for fine-tuning of the physicochemical properties of the Schiff base ligand and hence the corresponding metal

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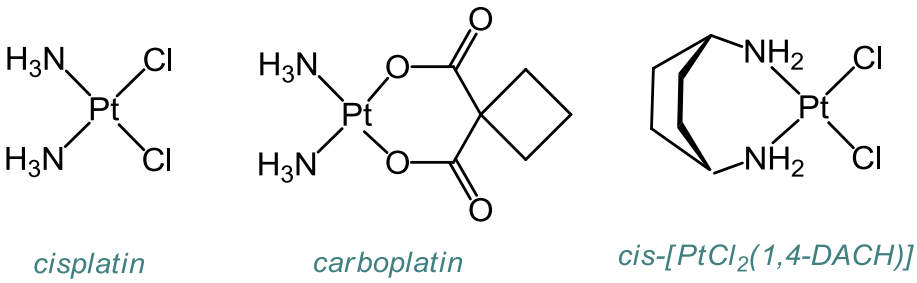


Fig. 1 Cisplatin, carboplatin, and *cis*-[$PtCl_2$(1,4-DACH)]

complex. In a previous report, we have disclosed our initial findings on designing two palladium Schiff base complexes derived from 5-ASA (5-aminosalicylic acid; Fig. 2) [26]. 5-ASA has traditionally been used in the treatment of inflammatory bowel disease, ulcerated colitis and Crohn's disease [27]. The isomeric analogue 4-ASA also has significant bioactivities and has been used in the treatment of tuberculosis [28, 29]. In this study, we have expanded our initial work and prepared a number of hydrophilic Schiff base derivatives containing both 4- and 5-ASA appendages, along with their corresponding palladium(II) complexes, and investigated their preliminary antimicrobial activities.

Experimental section

Materials and methods

Reagents and solvents used were obtained from Aldrich Chemicals. Compounds **1b** [30], **1c** [30], and **1d** [31] have been previously reported, and additional data are presented below. NMR spectra were recorded on a JEOL JNM-GSX400 FT NMR spectrometer (^{1}H: 400 MHz and ^{13}C: 100 MHz). Chemical shifts (δ) are reported in ppm (relative to residual solvent peaks). Multiplicities are reported as singlet (s), doublet (d), triplet (t), multiplet (m), broad (br), and overlapping (ov) with coupling constants (J) reported in Hz. FT-IR spectra were obtained with a Thermo Fisher Scientific Nicolet iS5 FT-IR spectrometer in ATR mode and are reported in cm^{-1}. Decomposition and melting points were measured uncorrected with a Stuart SMP30 apparatus. Elemental analyses for carbon, hydrogen, and nitrogen were carried out at Guelph Chemical Laboratories (Guelph, Ontario).

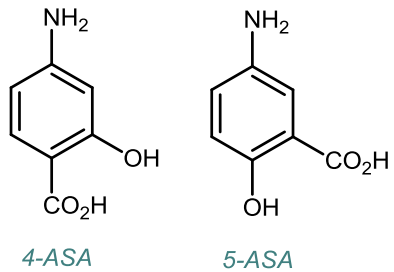


Fig. 2 4-ASA and 5-ASA

General procedure for ligand synthesis

To a stirred colorless MeOH (25 mL) solution of the appropriate aminosalicylic acid (1.00 g, 6.53 mmol) was added a pale brown MeOH (25 mL) solution of the desired amine (6.53 mmol). The reaction mixture was heated at reflux for 1 h, and upon cooling to room temperature, the resulting precipitate was collected by suction filtration and washed with cold MeOH (3 × 10 mL) to afford the desired Schiff base. All ligands were spectroscopically pure and used as obtained for the synthesis of the corresponding palladium complexes.

(E)-2-Hydroxy-4-((2-hydroxy-3-methoxybenzylidene) amino)benzoic acid (**1a**) Bright orange solid. Yield: 1.76 g (94%); m.p.: 169 °C. IR: 3444 (br, ν_{OH}), 2842 (w), 1663 (m), 1601 (s, $\nu_{C=N}$), 1451 (m), 1353 (m), 1253 (m), 1219 (s), 1153 (m), 1095 (w), 965 (m), 793 (s), 738 (s). Compound **1a** decomposes rapidly in solution negating the possibility of obtaining solution NMR data.

(E)-4-((2,3-dihydroxybenzylidene)amino)-2-hydroxybenzoic acid (**1b**) Burgundy solid. Yield: 1.64 g (92%); m.p.: 156–157 °C. Compound **1b** decomposes rapidly in solution negating the possibility of obtaining solution NMR data.

(E)-2-hydroxy-4-((2,3,4-trihydroxybenzylidene)amino benzoic acid (**1c**) Orange solid. Yield: 1.78 g (94%); m.p.: 178–179 °C. Compound **1c** decomposes rapidly in solution negating the possibility of obtaining solution NMR data.

(E)-2-hydroxy-5-((2-hydroxy-3-methoxybenzylidene)amino)benzoic acid (**1d**) Orange solid. Yield: 1.69 g (90%); m.p.: 218–220 °C. ^{1}H NMR (DMSO-d_6) δ: 13.16 (v br s, 1H, CO_2H), 12.31 (br s, 1H, O*H*), 8.93 (s, 1H, C*H*=N), 7.79 (d, J = 2.3 Hz, 1H, Ar), 7.63 (dd, J = 8.4, 2.3 Hz, 1H, Ar), 7.19 (dd, J = 7.6, 1.5 Hz, 1H, Ar), 7.06 (d, J = 8.4 Hz, 1H, Ar), 7.01 (d, J = 8.4 Hz, 1H, Ar), 6.86 (ov dd, J = 8.4, 7.6 Hz, 1H, Ar), 3.77 (s, 3H, OCH_3). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 172.1, 162.6,

160.7, 150.8, 148.3, 139.7, 129.3, 124.3, 123.1, 119.7, 119.1, 118.7, 115.7, 114.1, 56.3. IR: 3053 (br), 2834 (w), 1653 (m), 1617 (m, $\nu_{C=N}$), 1495 (m), 1354 (m), 1221 (s), 999 (m), 911 (m), 838 (m), 730 (s).

(E)-5-((2,3-Dihydroxybenzylidene)amino)-2-hydroxybenzoic acid (**1e**) Orange solid. Yield: 1.62 g (91%); decomposes above 250 °C. ^{1}H NMR (DMSO-d_6) δ: 13.09 (v br s, 1H, CO_2H), 9.16 (br s, 1H, O*H*), 8.90 (s, 1H, C*H*=N), 7.78 (d, $J = 2.3$ Hz, 1H, Ar), 7.63 (dd, $J = 8.4$, 2.3 Hz, 1H, Ar), 7.06 (dd, $J = 7.6$, 1.5 Hz, 1H, Ar), 7.02 (d, $J = 8.4$ Hz, 1H, Ar), 6.89 (dd, $J = 7.6$, 1.5 Hz, 1H, Ar), 6.74 (app t, $J = 7.6$ Hz, 1H, Ar). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 172.1, 163.1, 160.6, 149.5, 146.1, 139.8, 129.4, 123.2, 123.0, 120.0, 119.3 (2C), 118.7, 114.0. IR: 3324 (br, ν_{OH}), 3062 (w), 1659 (w), 1622 (m, $\nu_{C=N}$), 1493 (m), 1355 (m), 1271 (w), 1209 (s), 1005 (w), 846 (w), 732 (m).

(E)-2-Hydroxy-5-((2,3,4-trihydroxybenzylidene)amino)benzoic acid (**1f**) Yellow solid. Yield: 1.62 g (86%); decomposes above 250 °C. ^{1}H NMR (DMSO-d_6) δ: 12.98 (v br s, 1H, CO_2H), 9.70 (br s, 1H, O*H*), 8.75 (s, 1H, C*H*=N), 8.47 (br s, 1H, O*H*), 7.72 (d, $J = 3.1$ Hz, 1H, Ar), 7.56 (dd, $J = 8.4$, 3.1 Hz, 1H, Ar), 6.98 (d, $J = 8.4$ Hz, 1H, Ar), 6.92 (d, $J = 8.4$ Hz, 1H, Ar), 6.38 (d, $J = 8.4$ Hz, 1H, Ar). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 172.1, 162.5, 160.1, 151.4, 150.7, 139.8, 132.8, 129.0, 124.5, 122.5, 118.6, 114.1, 112.9, 108.2. IR: 3437 (br, ν_{OH}), 3083 (w), 1606 (m, $\nu_{C=N}$), 1494 (m), 1439 (w), 1216 (s), 1150 (s), 981 (w), 772 (w), 700 (m).

Synthesis of metal complexes

*Synthesis of **2a*** To a stirred EtOH (20 mL) suspension of **1a** (525 mg, 1.83 mmol) was added $Pd(OAc)_2$ (200 mg, 0.89 mmol) as a solid. The reaction mixture was gently heated at 60 °C for 2 h at which point an orange solid was collected by suction filtration. The solid was washed with EtOH (2 × 10 mL) and hexane (20 mL) to afford **2a** as a pale orange solid. Yield: 523 mg (86%); decomposes at 225 °C. ^{1}H NMR (DMSO-d_6) δ: 13.87 (v br s, 2H, CO_2H), 11.45 (v br s, 2H, O*H*), 8.00 (s, 2H, C*H*=N), 7.75 (d, $J = 8.4$ Hz, 2H, Ar), 6.98 (d, $J = 7.6$ Hz, 2H, Ar), 6.88–6.85 (ov m, 4H, Ar), 6.64 (d, $J = 6.9$ Hz, 2H, Ar), 6.38 (ov dd, $J = 8.4$, 7.6 Hz, 2H, Ar), 3.24 (s, 6H, OCH_3). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 172.4, 163.8, 161.8, 155.7, 155.5, 150.5, 130.6, 126.7, 119.7, 116.8, 115.2, 114.5, 113.7, 111.3, 55.1. IR: 3533 (br, ν_{OH}), 3436 (br, ν_{OH}), 2942 (w), 1660 (m), 1594 (m, $\nu_{C=N}$), 1427 (s), 1353 (m), 1295 (m), 1210 (s), 1145 (m), 986 (w), 864 (m), 780 (m), 734 (s). Anal. calcd. for $C_{30}H_{24}N_2O_{10}Pd$ (678.94) (%): C 53.07, H 3.56, N 4.13; found: C 52.79, H 3.42, N 4.43.

*Synthesis of **2b*** To a stirred THF (30 mL) solution of $Pd(OAc)_2$ (250 mg, 1.11 mmol) was added **1b** (609 mg, 2.23 mmol) as a solid. The reaction was gently heated at 60 °C for 2 h at which point an orange solid was collected by suction filtration. The solid was washed with EtOH (2 × 10 mL), THF (2 × 5 mL) and hexane (20 mL) to afford **2b** as an orange solid. Yield: 613 mg (86%); decomposes at 320 °C. ^{1}H NMR (DMSO-d_6) δ: 14.11 (v br s, 2H, CO_2H), 11.55 (v br s, 2H, O*H*), 8.13 (s, 2H, C*H*=N), 7.85 (d, $J = 8.4$ Hz, 2H, Ar), 7.06 (d, $J = 1.5$ Hz, 2H, Ar), 7.01 (dd, $J = 8.7$, 2.3 Hz, 2H, Ar), 6.97 (dd, $J = 8.2$, 1.5 Hz, 2H, Ar), 6.70 (dd, $J = 7.8$, 2.3 Hz, 2H, Ar), 6.43 (app t, $J = 7.8$ Hz, 2H, Ar), 5.03 (s, 2H, O*H*). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 171.7, 164.4, 161.9, 155.0, 151.9, 146.5, 130.9, 125.6, 118.8, 116.4, 116.3, 115.9, 113.3, 112.0. IR: 3409 (br, ν_{OH}), 3075 (w), 2980 (w), 2875 (w), 1662 (m), 1597 (m, $\nu_{C=N}$), 1547 (m), 1450 (s), 1320 (m), 1232 (s), 1198 (s), 1148 (s), 1043 (m), 985 (m), 772 (m), 781 (m), 738 (s), 700 (m). Anal. calcd. for $C_{28}H_{20}N_2O_{10}Pd$ (650.89) (%): C 51.67, H 3.10, N 4.30; found: C 51.54, H 3.28, N 4.19.

*Synthesis of **2c*** To a stirred EtOH (20 mL) suspension of **1c** (258 mg, 0.89 mmol) was added $Pd(OAc)_2$ (100 mg, 0.45 mmol) as a solid. The reaction mixture was gently heated at 60 °C for 1 h at which point an orange–brown solid was collected by suction filtration. The solid was washed with EtOH (2 × 5 mL) and CH_2Cl_2 (20 mL) to afford **2c** as an orange–brown solid. Yield: 150 mg (49%); m.p.: 290–292 °C. ^{1}H NMR (DMSO-d_6) δ: 13.97 (v br s, 2H, CO_2H), 11.57 (br s, 2H, O*H*), 9.67 (s, 2H, O*H*), 7.87 (s, 2H, C*H*=N), 7.84 (d, $J = 8.4$ Hz, 2H, Ar), 7.01–6.97 (ov m, 4H, Ar), 6.84 (d, $J = 8.4$ Hz, 2H, Ar), 6.12 (d, $J = 8.4$ Hz, 2H, Ar), 4.48 (s, 2H, O*H*). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 172.1, 163.1, 162.2, 155.9, 152.9, 148.9, 132.9, 131.0, 126.6, 117.0, 113.6, 113.1, 111.8, 108.3. IR: 3366 (br, ν_{OH}), 3102 (br, ν_{OH}), 1672 (m), 1594 (m, $\nu_{C=N}$), 1553 (s), 1495 (m), 1455 (m), 1408 (m), 1280 (m), 1215 (s), 1098 (s), 971 (m), 767 (m), 696 (m). Anal. calcd. for $C_{28}H_{20}N_2O_{12}Pd.CH_2Cl_2$ (767.91) (%): C 45.36, H 2.89, N 3.65; found: C 45.33, H 2.36, N 3.75.

*Synthesis of **2d*** To a stirred EtOH (20 mL) suspension of **1d** (525 mg, 1.83 mmol) was added $Pd(OAc)_2$ (200 mg, 0.89 mmol) as a solid. The reaction mixture was gently heated at 60 °C for 2 h at which point an orange solid was collected by suction filtration. The solid was washed with EtOH (2 × 10 mL) and hexane (2 × 10 mL) to afford **2d** as an orange solid. Yield: 580 mg (96%); m.p.: 290–291 °C. ^{1}H NMR (DMSO-d_6) δ: 13.88 (v br s, 2H, CO_2H), 11.35 (v br s, 2H, O*H*), 8.02 (s, 2H, C*H*=N), 7.63 (d, $J = 2.3$ Hz, 2H, Ar), 7.45 (dd, $J = 8.4$, 2.3 Hz, 2H, Ar), 6.97 (d, $J = 6.9$ Hz, 2H, Ar), 6.92 (d, $J = 8.4$ Hz, 2H, Ar), 6.63 (d, $J = 6.9$ Hz, 2H, Ar), 6.38 (app t, $J = 7.6$ Hz, 2H, Ar), 3.27 (s, 6H, OCH_3). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 172.5, 164.4, 160.1, 155.7, 150.9, 141.1, 132.8, 126.7, 126.5, 119.9, 117.2, 115.0, 114.5, 112.7, 55.3. IR: 3043 (br,

ν_{OH}), 2946 (w), 1664 (m), 1599 (m, $\nu_{C=N}$), 1424 (s), 1287 (m), 1248 (s), 1207 (s), 1078 (m), 990 (m), 734 (s). Anal. calcd. for $C_{30}H_{24}N_2O_{10}Pd$ (678.94) (%): C 53.07, H 3.56, N 4.13; found: C 52.92, H 3.38, N 4.39.

*Synthesis of **2e*** To a stirred EtOH (20 mL) suspension of **1e** (500 mg, 1.83 mmol) was added $Pd(OAc)_2$ (200 mg, 0.89 mmol) as a solid. The reaction mixture was gently heated at 60 °C for 2 h at which point an orange solid was collected by suction filtration. The solid was washed with EtOH (2 × 10 mL) and Et_2O (2 × 10 mL) to afford **2e** as an orange solid. Yield: 551 mg (95%); m.p.: 260–262 °C. ^{1}H NMR (DMSO-d_6) δ: 14.10 (v br s, 2H, CO_2H), 11.43 (v br s, 2H, O*H*), 8.12 (s, 2H, C*H*=N), 7.76 (d, J = 2.3 Hz, 2H, Ar), 7.61 (dd, J = 8.4, 2.3 Hz, 2H, Ar), 7.04 (d, J = 8.4 Hz, 2H, Ar), 6.95 (d, J = 7.6 Hz, 2H, Ar), 6.68 (d, J = 6.9 Hz, 2H, Ar), 6.41 (app t, J = 7.6 Hz, 2H, Ar), 5.03 (s, 2H, O*H*). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 171.7, 165.1, 160.2, 152.0, 146.9, 140.5, 132.5, 125.7 (2C), 119.1, 117.7, 116.3, 115.9, 113.3. IR: 3418 (br, ν_{OH}), 3098 (w), 1694 (m), 1597 (m, $\nu_{C=N}$), 1552 (m), 1458 (s), 1425 (m), 1316 (s), 1206 (m), 1182 (s), 827 (m), 725 (s), 679 (m). Anal. calcd. for $C_{28}H_{20}N_2O_{10}Pd$ (650.89) (%): C 51.67, H 3.10, N 4.30; found: C 51.88, H 3.13, N 4.58.

*Synthesis of **2f*** To a stirred EtOH (20 mL) suspension of **1f** (528 mg, 1.83 mmol) was added $Pd(OAc)_2$ (200 mg, 0.89 mmol) as a solid. The reaction mixture was gently heated at 60 °C for 1 h at which point an orange–brown solid was collected by suction filtration. The solid was washed with EtOH (2 × 10 mL) and Et_2O (20 mL) to afford **2f** as an orange–brown solid. Yield: 500 mg (82%); m.p.: 277–279 °C. ^{1}H NMR (DMSO-d_6) δ: 13.69 (v br s, 2H, CO_2H), 11.43 (br s, 2H, O*H*), 9.57 (s, 2H, O*H*), 7.88 (s, 2H, C*H*=N), 7.72 (s, 2H, Ar), 7.58 (d, J = 7.6 Hz, 2H, Ar), 7.01 (d, J = 8.4 Hz, 2H, Ar), 6.82 (d, J = 9.2 Hz, 2H, Ar), 6.11 (d, J = 9.2 Hz, 2H, Ar), 4.44 (s, 2H, O*H*). ^{13}C{^{1}H} NMR (DMSO-d_6) δ: 171.8, 163.6, 159.9, 152.8, 148.6, 141.0, 133.1, 132.8, 126.3, 125.8, 117.7, 113.3, 113.1, 108.3. IR: 3477 (br, ν_{OH}), 3441 (br, ν_{OH}), 1663 (m), 1599 (m, $\nu_{C=N}$), 1560 (s), 1444 (m), 1188 (s), 1090 (w), 834 (m), 767 (m), 683 (m). Anal. calcd. for $C_{28}H_{20}N_2O_{12}Pd$ (682.88) (%): C 49.25, H 2.95, N 4.10; found: C 49.63, H 3.11, N 4.06.

Stability of ligands and palladium complexes in dimethyl sulfoxide

Solutions of ligands **1a–f** and palladium complexes **2a–f** in wet DMSO-d_6 were monitored by ^{1}H NMR spectroscopy over a period of 2 days at RT. Compounds **1a–c** and **2a–c** were found to decompose over this time period; therefore, they were not included in the bioactivity studies. While **1a–c** reacted reversibly with wet DMSO-d_6 to generate the starting aldehyde and amines, complexes **2a–c** decomposed to give a number of unidentified products.

X-ray crystallography

Crystals of **2e** were grown from a saturated solution of dimethyl sulfoxide stored at RT. Crystals were attached to the tip of a 400 μm MicroLoop with paratone-N oil. Measurements were made on a Bruker APEXII CCD equipped diffractometer (30 mA, 50 mV) using monochromated Mo–Kα radiation (λ = 0.71073 Å) at 125 K. The initial orientation and unit cell were indexed using a least-squares analysis of a random set of reflections collected from three series of 0.5° wide scans, 10 s per frame and 12 frames per series that were well distributed in reciprocal space. For data collection, four ω-scan frame series were collected with 0.5° wide scans, 60 s frames and 366 frames per series at varying φ angles (φ = 0°, 90°, 180°, 270°). The crystal to detector distance was set to 6 cm and a sphere of data was collected. Cell refinement and data reduction were performed with the Bruker SAINT software, which corrects for beam inhomogeneity, possible crystal decay, Lorentz and polarization effects. Data processing and a multiscan absorption correction were applied using the APEX2 software package [32]. The structure was solved using direct methods [33], and all non-hydrogen atoms were refined anisotropically using ShelXle [34] graphical user interface and SHELXL [35]. Hydrogen atoms were included at geometrically idealized positions and were fixed (Ar–H, CH) or in the case of methyl groups, the dihedral angle of the idealized tetrahedral CH_3 fragment was allowed to refine. In the case of O–H bonding environments, hydrogen atoms were allowed to freely refine.

Cultures

Pure cultures of *Aspergillus niger*, *Saccharomyces cerevisiae*, *Bacillus cereus*, and *Pseudomonas aeruginosa* were revived from strains maintained at −70 °C. *A. niger* was maintained on Sabouraud dextrose agar, *S. cerevisiae* was maintained on yeast malt agar, and *B. cereus* and *P. aeruginosa* were maintained on tryptic soy agar.

Inoculations

Using aseptic techniques, a small amount (1 cm^2) of agar culture was removed from a plate via scalpel and placed into a sterile tissue homogenizer tube. Approximately 3–4 mL of doubly distilled H_2O was then added followed by gentle homogenization. Homogenate (200 μL) was added to an agar plate (Sabouraud dextrose agar for fungi;

Mueller–Hinton II agar for bacteria) and spread evenly to ensure uniform growth.

Compound testing

Disks (5 mm diameter) created from filter paper (Fisherbrand® Filter paper, diameter of 15.0 cm, porosity: coarse, flow rate: fast (09-795F)) were placed equidistantly on an inoculated agar plate (diameter 9 cm) at four points. Set concentrations of compound (0, 25, 50, and 100 μg for disks 1–4, respectively) in DMSO were added to the disks and the cultures were allowed to grow over 48 h at which point caliper measurements were obtained, measuring from the center of the disk to the nearest presence of fungus. Plates were done in triplicate and mean results calculated and reported. Control plates with a known antibiotic were performed with amphotericin B (Sigma A9528) at a concentration of 100 μg for the fungal species. Control plates for *B. cereus* were performed with erythromycin (BD BBL Sensi-Disc #230793) at 15 μg and for *P. aeruginosa*, streptomycin (BD BBL Sensi-Disc #230942) at 10 μg was used. Negative controls were disks provided with DMSO but without compound.

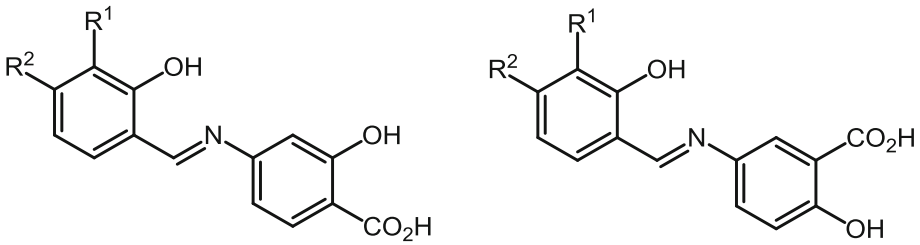
Results and discussion

Synthesis and characterization

o-Vanillin, or 2-hydroxy-3-methoxybenzaldehyde, is a natural product found in the extracts and oils of many plants. Although it only displays moderate antimicrobial properties, its use in Schiff base chemistry is well-documented [36]. We decided to use *o*-vanillin, along with the alcohol derivatives, 2,3-dihydroxybenzaldehyde and 2,3,4-trihydroxybenzaldehyde, in an attempt to increase the solubility of the resulting palladium complexes in aqueous media. Poor solubility in physiological media is a recurring problem associated with designing novel therapeutic metal complexes. Addition of the aminosalicylic acids 4-ASA and 5-ASA readily afforded the corresponding Schiff base compounds **1a–c** and **1d–f**, respectively (Fig. 3). Unfortunately, albeit stable in the solid state, compounds **1a–c** decomposed rapidly in solution (DMSO) and thus negated the possibility of obtaining solution NMR data. It is not clear at this point why these compounds are unstable with respect to decomposition and attempts to get single crystals of these compounds for X-ray diffraction studies proved unsuccessful. For the more stable Schiff bases **1d–f**, a peak for the aldehyde proton at 10 ppm disappeared upon formation of the imine and a new resonance was observed at around 9 ppm in the ^{1}H NMR spectra. Likewise, a resonance at ca. 160 ppm in the ^{13}C NMR spectra indicated the formation of the N=CH methine carbon. Generation of these Schiff base compounds was also confirmed by the diagnostic C=N stretching band in the IR spectra at ca. 1620 cm^{-1} [26].

We then decided to investigate the ligating properties of **1a–f** and were pleased to observe that all reacted readily with $Pd(OAc)_2$ in ethanol to give the corresponding bis(salicylaldiminato)palladium(II) species **2a–f** (Scheme 1) in moderate to high yields. All new metal complexes have been characterized by a number of physical methods including multinuclear NMR spectroscopy, FT-IR spectroscopy and elemental analyses, and all data are consistent with a bis-Schiff base formulation. For instance, the C=N stretch in the FT-IR spectra shifted from ca. 1620 to ca. 1595 cm^{-1} upon coordination to palladium. Likewise, as typical for these species, a significant upfield shift in the ^{1}H NMR spectra was observed for the imine methine proton, from ca. 9 to ca. 8 ppm. To unambiguously assign the solid-state structure of these complexes, we carried out a single-crystal X-ray diffraction study on the 5-ASA derivative **2e**, the molecular structure of which is shown in Fig. 4. Crystallographic data are provided in Table 1 and selected bond distances and angles are shown in Table 2. Complex **2e** crystallized in the $P2_1/c$ space group along with two molecules of DMSO. The palladium atom lies on a center of inversion and the environment around the metal center is roughly square planar. The Pd–O

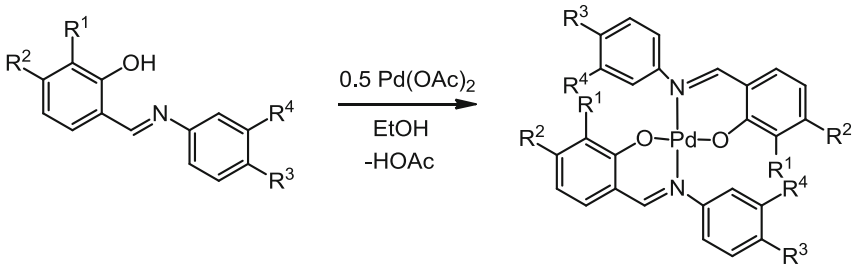
Fig. 3 Schiff base ligands **1a–f**



1a: $R^1 = OCH_3$, $R^2 = H$
1b: $R^1 = OH$, $R^2 = H$
1c: $R^1 = OH$, $R^2 = OH$

1d: $R^1 = OCH_3$, $R^2 = H$
1e: $R^1 = OH$, $R^2 = H$
1f: $R^1 = OH$, $R^2 = OH$

Scheme 1 Synthesis of palladium(II) complexes **2a–f**



2a: $R^1 = OCH_3$, $R^2 = H$, $R^3 = CO_2H$, $R^4 = OH$
2b: $R^1 = OH$, $R^2 = H$, $R^3 = CO_2H$, $R^4 = OH$
2c: $R^1 = OH$, $R^2 = OH$, $R^3 = CO_2H$, $R^4 = OH$

2d: $R^1 = OCH_3$, $R^2 = H$, $R^3 = OH$, $R^4 = CO_2H$
2e: $R^1 = OH$, $R^2 = H$, $R^3 = OH$, $R^4 = CO_2H$
2f: $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = CO_2H$

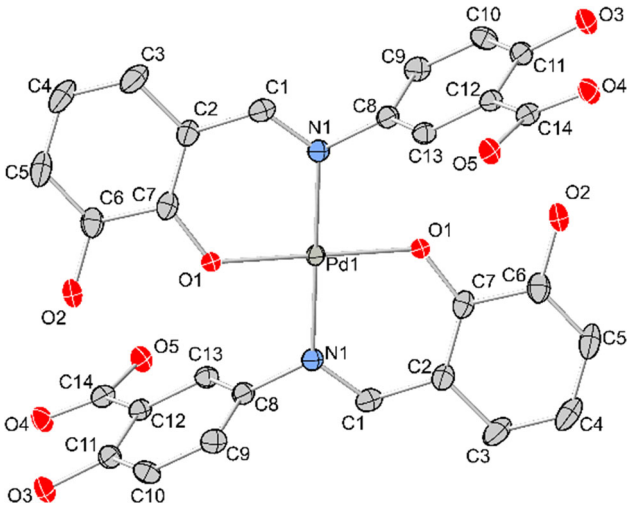


Fig. 4 Molecular structure of **2e** drawn with 50% probability ellipsoids showing atoms generated by symmetry. Hydrogen atoms and a molecule of solvent removed for clarity

and Pd–N distances of 1.9839(13) and 2.0178(16) Å, respectively, are similar to those observed in related complexes. For instance, the corresponding bis(salicylaldiminato)palladium(II) complex derived from 2-(3,4-dimethoxyphenyl)ethanamine and 2-hydroxybenzaldehyde has Pd–O and Pd–N distances of 1.984(11) and 2.020(12) Å, respectively [23]. Likewise, the C(1)–N(1) bond distance of 1.293(2) Å in **2e** is typical for Schiff base ligands bound to a metal that retain the predominant imine form containing a formal C=N double bond.

Antimicrobial testing

As mentioned previously, there has been considerable recent interest in palladium Schiff base complexes for their potential antimicrobial activities. As part of our investigation into this area, we therefore decided to examine the initial antifungal and antibacterial activities of both the Schiff base ligands **1d–f** and their corresponding palladium(II) complexes **2d–f** against two species of fungi, *Aspergillus niger* and *Saccharomyces cerevisiae*, as well as two species of bacteria, *Bacillus cereus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative).

Table 1 Crystallographic data collection parameters

Complex	**2e**
Formula	$C_{32}H_{32}N_2O_{12}PdS_2$
Molecular weight	807.11
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	18.1231(18)
b (Å)	5.8076(6)
c (Å)	17.2638(17)
α (°)	90
β (°)	115.0830(10)
γ (°)	90
V (Å^3)	1645.7(3)
Z	2
$\rho_{calc.}$ (Mg m^{-3})	1.629
Crystal size (mm^3)	0.118 × 0.066 × 0.040
Temp (K)	125 (2)
Radiation	Mo–K_α (λ = 0.71073 Å)
μ (mm^{-1})	0.758
Total reflections	12875
Total unique reflections	4022
No. of variables	234
θ range (°)	2.363–28.415
Largest difference peak/hole (e/Å^{-3})	0.408 and −0.317
S (goodness of fit) on F^2	1.016
$R1$[a] ($I > 2\ s(I)$)	0.0262
w$R2$[b] (all data)	0.0630

[a] $R1 = \sum||F_o|-|F_c||/\sum|F_o|$

[b] $wR2 = (\sum[w(F_o^2 - F_c^2)^2]/\sum[wF_o^4])^{1/2}$, where $w = 1/[\sigma^2(F_o^2) + (0.0276P)^2 + (0.9316P]$, where $P = (\max(F_o^2, 0) + 2.F_c^2)/3$

Table 2 Selected bond distances (Å) and angles (°)

Pd(1)–O(1)	1.9839(13)
Pd(1)–N(1)	2.0178(16)
O(1)–C(7)	1.316(2)
O(2)–C(6)	1.363(3)
O(3)–C(11)	1.356(2)
O(4)–C(14)	1.230(2)
O(5)–C(14)	1.307(2)
N(1)–C(1)	1.293(2)
N(1)–C(8)	1.447(2)
C(1)–C(2)	1.441(3)
O(1)#1–Pd(1)–N(1)#1	91.77(6)
O(1)–Pd(1)–N(1)#1	88.23(6)
O(1)#1–Pd(1)–N(1)	88.23(6)
O(1)–Pd(1)–N(1)	91.77(6)
C(7)–O(1)–Pd(1)	123.83(13)
C(1)–N(1)–C(8)	116.87(16)
C(1)–N(1)–Pd(1)	123.74(13)
C(8)–N(1)–Pd(1)	119.32(12)

Unfortunately, we were unable to examine the antimicrobial properties of the 4-ASA derivatives as they were not stable under the test conditions. The results from this study are provided in Table 3 and shown in Fig. 5 using known controls and Na_2PdCl_4 as a metal control. As expected, the simple palladium salt Na_2PdCl_4 displayed no appreciable activities. The most promising results were with Schiff base **1e** and palladium complexes **2e** and **2f**, which showed considerable activity against *Saccharomyces cerevisiae*. Schiff bases **1d** and **1e** and palladium complex **2d** displayed only moderate activities against *Aspergillus niger*. Although **1e** and **2d** displayed weak activities against the Gram-positive bacterium *Bacillus* cereus, no compound tested showed any activity against the Gram-negative bacterium *Pseudomonas aeruginosa*. Unfortunately, poor solubilities and stabilities preclude both the Schiff bases and the corresponding bis(salicylaldiminato)-palladium(II) complexes from being potential candidates as practical antimicrobial agents.

Conclusion

We have prepared six Schiff base compounds from the condensation of *o*-vanillin, 2,3-dihydroxybenzaldehyde and 2,3,4-trihydroxybenzaldehyde with 4-aminosalicylic acid (4-ASA) and 5-aminosalicylic acid (5-ASA). Although a significant amount of research has focussed on generating derivatives of 5-ASA, much less is known about the analogous chemistry of isomeric 4-ASA. Addition of the generated Schiff bases to $[Pd(OAc)_2]$ afforded the corresponding bis(salicylaldiminato)palladium(II) complexes in excellent yields. All new palladium complexes have been characterized fully using standard spectroscopic methods, elemental analyses, and a single-crystal X-ray diffraction study in the case of **2e**, the palladium complex containing Schiff base ligands derived from 5-ASA and 2,3-dihydroxybenzaldehyde. All derivatives of 5-ASA were examined for potential antimicrobial activities against two species of fungi, *Aspergillus niger* and *Saccharomyces cerevisiae*, as well as two species of bacteria, *Bacillus cereus* (Gram-positive) and *Pseudomonas aeruginosa*

Table 3 Antimicrobial activity of compounds **1d–f** and **2d–f**

Compound	*Aspergillus niger*		*Saccharomyces cerevisiae*		*Bacillus cereus*		*Pseudomonas aeruginosa*	
	Dose (μg disk^{-1})	Clear zone (mm ± SD)[a]	Dose (μg disk^{-1})	Clear zone (mm ± SD)	Dose (μg disk^{-1})	Clear zone (mm ± SD)	Dose (μg disk^{-1})	Clear zone (mm ± SD)
Na_2PdCl_4	100	Inactive	100	Inactive	100	Inactive	100	Inactive
1d	100	4.5 ± 0.7	100	Inactive	100	Inactive	100	Inactive
1e	100	4.9 ± 0.7	100	12.7 ± 0.2	100	2.3 ± 0.2	100	Inactive
1f	100	Inactive	100	Inactive	100	Inactive	100	Inactive
2d	100	3.6 ± 0.3	100	Inactive	100	2.1 ± 0.1	100	Inactive
2e	100	Inactive	100	7.3 ± 1.6	100	Inactive	100	Inactive
2f	100	Inactive	100	9.2 ± 0.8	100	Inactive	100	Inactive
Amphotericin B	100	9.4 ± 0.7	100	8.8 ± 1.8	–	–	–	–
Erythromycin	–	–	–	–	15	4.9 ± 0.8	–	–
Streptomycin	–	–	–	–	–	–	10	3.2 ± 0.4

[a] Clear zone measured from center of disk to end of cell-free region

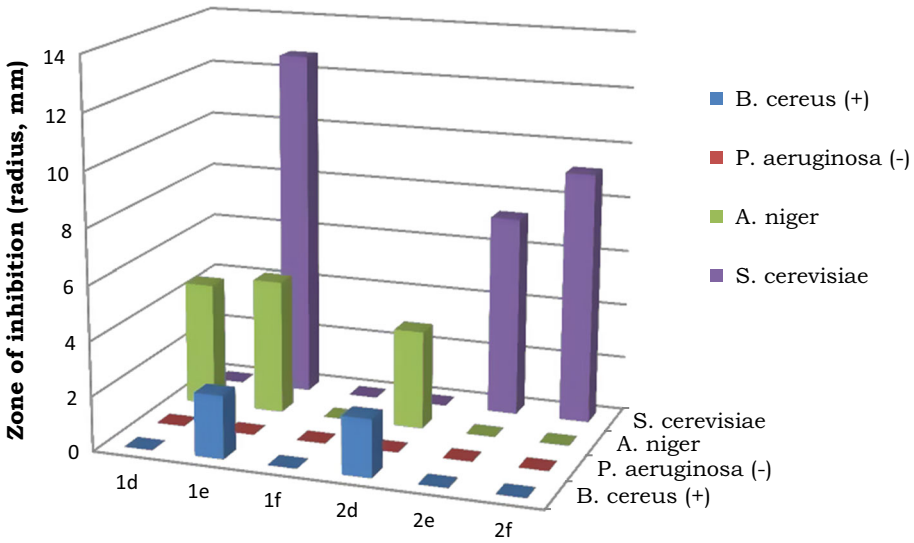


Fig. 5 Comparison of antimicrobial activity of ligands and palladium complexes (100 μg)

(Gram-negative). Problems associated with stability and solubility with the 4-ASA derivatives negated biological testing of these species.

Supplemental material

Full supplemental crystallographic data in CIF format have been deposited with the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 UK (fax: + 44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk) and are available on request, quoting deposition number 1528913).

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