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Syntheses and biological activity of platinum(II) and palladium(II) complexes with phenyl-oxadiazole-ethylenediamine ligands

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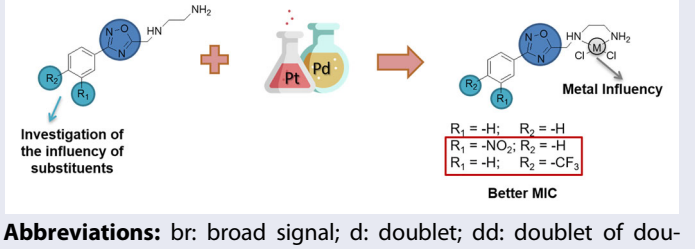
ABSTRACT

This work describes the synthesis, characterization, cytotoxicity and antibacterial activity of three ligands derived from phenyl-oxadiazole-ethylenediamine and their platinum(II) and palladium(II) complexes. These compounds were characterized by elemental analysis, and Raman, IR, and NMR spectroscopies. We have prepared complexes with different substituents on the aromatic ring to provide influence in the antibacterial activity and cytotoxicity. The antibacterial activity was evaluated against Gram-positive bacteria *Staphylococcus aureus* (ATCC 25213), *Staphylococcus epidermidis* (ATCC 12228) and Gram-negative bacteria *Escherichia coli* (ATCC 11229) and *Pseudomonas aeruginosa* (ATCC 27853). The cytotoxicity was evaluated in two different tumor cell lines: mouse metastatic mammary adenocarcinoma (4T1), and murine colon cancer cells (CT26WT), and a non-tumor cell Baby Hamster Kidney (BHK-21). Based on the results obtained for the antibacterial and the cytotoxic activity it is possible to conclude that the presence of metal and groups NO_2 and CF_3 contributed to antibacterial activity with low cytotoxicity.



Abbreviations: br: broad signal; d: doublet; dd: doublet of doublets; DMSO: dimethyl sulfoxide; MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMR: Nuclear Magnetic Resonance; RPMI: Roswell Park Memorial Institute Medium

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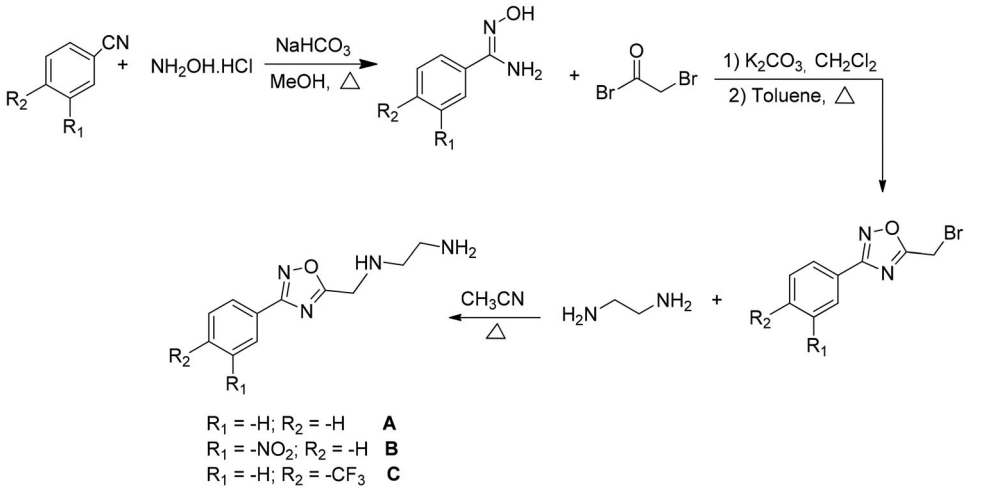
1. Introduction

Pseudomonas aeruginosa remains an important putative pathogen specially in nosocomial infections related to high mortality rates in immunocompromised individual of about 30% [1]. According to the World Health Organization these Gram-negative bacteria along the Gram-positive *Staphylococcus aureus* belong to a catalogue of twelve concerned families of bacteria considered the greatest threat to human health [2]. Besides that, as antimicrobial chemotherapy is often necessary to overcome bacterial infections, the emergence of antibiotic-resistant bacteria was considered a significant health challenge worldwide [3]. Thus, these facts may provide a clear picture of the magnitude of the antimicrobial resistance phenomenon and the necessity of new compounds that can act against bacterial infection and overcome resistance.

In this context, an important class of compounds is oxadiazoles, which are five-membered heterocycles and can be found in some different isomeric forms, being the 1,2,4- and 1,3,4- are the most used in medicinal chemistry [4]. The oxadiazole-derived compounds have presented large biological properties such as antiviral, antibacterial, antioxidant, anti-inflammatory, antifungal and anticancer activity [5–9]. The isomeric form used in this work is 1,2,4-oxadiazole and the purpose of use is to favor the biological activity with the metal coordination.

The discovery of the antitumor properties of cisplatin (*cis*-diamminedichloridoplatinum) by Rosenberg in the 1960s while researching the effect of electric field in the bacteria growth boosted the research for drugs based on platinum for cancer treatment [10–12]. Besides that, other metal compounds are used in medicine as the compound of gold(I), Ridaura® (auranofin) for rheumatoid arthritis and the complexes of Sb(V) Pentostam® (sodium stibogluconate) and Glucantime® (N-methyl glucamine antimoniate) for leishmaniasis treatment [13]. Gold(I) and platinum(II) complexes with ligands containing oxadiazole were reported and these compounds have presented good activity against tumor cells, bacteria and *Leishmania* parasites [14–18]. In the case of bacterial infections, the use of Ag(I) can be mentioned with Silvadene® that is a water-miscible cream containing silver sulfadiazine. This compound, when in contact with a wound, releases silver ions that able to kill bacteria such as *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) [19]. Additionally, with respect to the research of metal complexes, in a recent paper DuChane and collaborators demonstrated the potent activity of a class of iridium piano-stool complexes with diamine ligands against *S. aureus* and methicillin-resistant of *S. aureus* [20]. Another work reported the analysis of the antibacterial and antifungal activity of about 1000 metal complexes, highlighted 30 compounds containing as metal Mn, Co, Zn, Ru, Ag, Eu, Ir and Pt, that presented activity against Gram-positive and/or Gram negative bacteria, and activities down to nanomolar range in MRSA [21]. Thereby, we decided to contribute with new platinum(II) complexes.

Based on the similarities between Pt(II) and Pd(II) we also decided to synthesize palladium complexes [22]. However, the palladium is much more labile than platinum analogs and this fact limits the research of compounds based on this metal, whereas the possibility of rapid hydrolysis in the organism can convert the palladium complexes into inactive species unable to attain the biological targets [23]. The alternative found to overcome this inconvenience is synthesis of chelates, which can make the



Scheme 1. Synthesis of ligands.

complexes thermodynamically stable [24], which is the objective of this work. To promote a chelate coordination we have included the ethylenediamine moiety to the phenyl-oxadiazole derivative.

Throughout this work we have reported the synthesis, characterization and evaluation of cytotoxic and antibacterial activity of platinum(II) and palladium(II) complexes compared to their ligands phenyl-oxadiazole-ethylenediamine containing different substituents on the aromatic ring to provide the influence in their biological activity.

2. Experimental

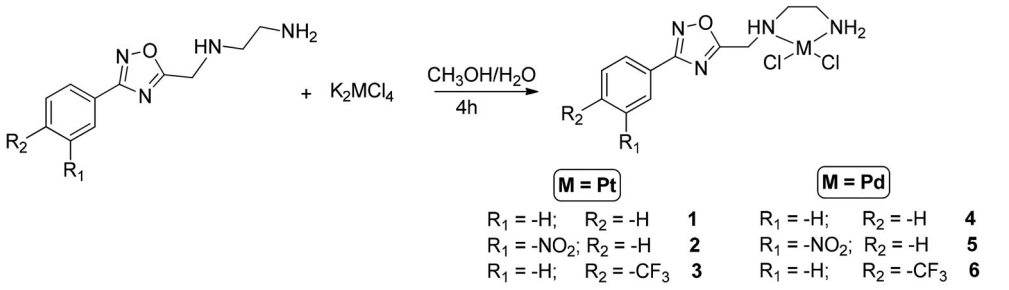
2.1. Materials and methods

All reagents were obtained from commercial sources and used without purification. Brain Heart Infusion agar (BHIA), Mueller-Hinton broth (MHB) and Mueller Hinton agar medium (AMH) were from Himedia Laboratories, Mumbai, India.

IR spectra were obtained with a Bruker Alfa FT-IR Spectrometer in KBr pellets. Raman spectra were obtained on a Bruker RFS 100 FT-Raman. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz) and ^{195}Pt (107.5 MHz) spectra were obtained in DMSO-d_6 on a Bruker Avance 500 MHz spectrometer. UV absorption spectra were obtained in aqueous solution (1% DMSO) on an UV-1800 Spectrophotometer Shimadzu. Finally, elemental analyses were performed at the University of São Paulo (USP), Brazil.

2.2. Synthesis of ligands A–C (Scheme 1)

The synthesis of oxadiazoles was taken by the reaction of the corresponding benzonitrile (8 mmol) and sodium bicarbonate (12.8 mmol) with hydroxylamine hydrochloride (12 mmol) in 15 mL of methanol. The reaction remained stirring for 6 h in reflux. After cooling, the solvent was removed under reduced pressure and the mixture was purified by extraction with ethyl acetate and water, and then with brine solution, affording the amidoxime.



Scheme 2. Synthesis of complexes.

In the second stage, a solution of bromacetyl bromide (8.1 mmol) in 5 mL of dichloromethane was slowly added to a solution of the respective amidoxime (6 mmol) and potassium carbonate (6 mmol) in 15 mL of dichloromethane. The reaction was stirred for 3 h at room temperature. The solvent was removed, and the mixture was extracted with ethyl acetate/water and washed with brine solution. The solvent was removed and it was added toluene, and the mixture remained heating at 100 °C for 21 h. The reaction was cooled and the solvent removed affording the desired oxadiazole precursors.

A solution of the respective oxadiazole (3 mmol) in 5 mL of acetonitrile was slowly added to a solution of ethylenediamine (8.1 mmol) and carbonate of potassium (4.5 mmol) in 5 mL of acetonitrile. The reaction was allowed to stir for 1 h in reflux. The reaction was cooled, and the solvent was removed under reduced pressure. The mixture was extracted with dichloromethane and water, and brine solution. Then, the crude mixture was purified by column chromatography employing dichloromethane and methanol as eluent, affording the desired compounds. Yields: (**A**) 52%, (**B**) 49%, (**C**) 53%.

A. IR (KBr), ν_{max} (cm^{-1}): 3349, 3294, 3063, 2928, 2852, 1444, 1070; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.61 (m, 4H, C**H**$_2$-NH and C**H**$_2$-NH$_2$); 4.06 (s, 2H, C**H**$_2$-oxadiazole); 7.56 (t, 3H, J = 7.5 Hz, **Ph**), 8.02 (m, 2H, **Ph**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 41.2 (**C**-NH$_2$); 44.2(**C**-NH); 51.7 (HN-**C**-oxadiazole); 126.3 (**C**-Ph); 127.0; 129.3; 131.5 (H**C**-Ph); 167.4 (N-**C**-N); 179.6 (N-**C**-O).

B. IR (KBr), ν_{max} (cm^{-1}): 3362, 3304, 3090, 2939, 2861, 1516,1446, 1346, 1075; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.61 (m, 4H, C**H**$_2$-NH and C**H**$_2$-NH$_2$); 4.11 (s, 2H, C**H$_2$**-oxadiazole); 7.86 (t, 1H, J = 8 Hz, **Ph**), 8.43 (m, 3H, **Ph**), 8.70 (m, 1H, **Ph**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 41.2 (**C**-NH$_2$); 44.3 (**C**-NH); 51.8 (HN-**C**-oxadiazole); 121.5; 126.1 (H**C**-Ph); 127.7 (**C**-Ph); 131.3; 133.1 (H**C**-Ph), 148.3 (**C**-NO$_2$); 166.1 (N-**C**-N); 180.4 (N-**C**-O).

C. IR (KBr), ν_{max} (cm^{-1}): 3357, 2940, 2868,1418, 1326, 1066; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.61 (m, 4H, C**H**$_2$-NH and C**H**$_2$-NH$_2$); 4.09 (s, 2H, C**H$_2$**-oxadiazole); 7.92 (d, 2H, J = 8 Hz, **Ph**), 8.21 (d, 2H, J = 8 Hz, **Ph**), 8.70 (m, 1H, **Ph**); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 41.2 (**C**-NH$_2$); 44.2 (**C**-NH); 51.8 (HN-**C**-oxadiazole); 120.6 (q, ^{1}J = 271.5 Hz, **C**-F$_3$); 126.3; 127.9 (H**C**-Ph); 130.1 (**C**-Ph); 130.9 (q, ^{2}J = 31,5 Hz, **C**-**CF$_3$**); 166.5 (N-**C**-N); 180.2 (N-**C**-O).

2.3. Synthesis of complexes 1–6 (Scheme 2)

The complexes were obtained by slow addition of solution of the respective ligand (0.3 mmol) in methanol (1 mL) to a 2.5 mL aqueous solution of K_2PtCl_4 (0.3 mmol) (for **1–3**) or K_2PdCl_4 (0.3 mmol) (for **4–6**). The reaction was kept protected from light under stirring for 4 h at room temperature. The solid obtained was filtered off, washed with water, methanol and dichloromethane. Yields: (**1**) 60%, (**2**) 76%, (**3**) 68%, (**4**) 73%, (**5**) 70%, (**6**) 56%.

1. IR (KBr), ν_{max} (cm^{-1}): 3288, 3184, 3093, 2961, 2877, 1594, 1445, 1344, 1073; Raman (cm^{-1}): 3074, 2966, 2893, 1608, 1527, 1475, 1446, 1159, 1005, 966, 577, 332, 160, 114; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.36 (m, 2H, C**H$_2$**-NH$_2$); 2.69 (m, 2H, C**H$_2$**-NH); 4.35 (dd, 1H, J = 15.5 Hz, J = 9 Hz C**H$_2$**-oxadiazole); 4.75 (dd, 1H, C**H$_2$**-oxadiazole); 5.42 (m, 2H, N**H$_2$**); 7.18 (br, 1H, N**H**); 7.59; 7.60; 7.61 (t, 3H, J = 5 Hz, Ph); 8.03; 8.05 (d, 2H, J = 10 Hz, Ph); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 46.6 (**C**-NH2); 46.6 (**C**-NH); 56.5 (HN-**C**-oxadiazole); 125.9 (Ph-oxadiazole); 127.1; 129.37; 131.8 (**C**-Ph); 167.6 (N-**C**-N); 174.9 (N-**C**-O). ^{195}Pt NMR (107.5 MHz, DMSO-d_6), δ (ppm): -2354. Anal. Calc. for $C_{11}H_{14}N_4OPtCl_2$ (%): C, 27.27; H, 2.89; N, 11.57. Found: C, 27.55; H, 2.82; N, 11.26.
2. IR (KBr), ν_{max} (cm^{-1}): 3267, 3199, 3118, 2959, 2890, 1519, 1446, 1349, 1076; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.36 (m, 2H, C**H$_2$**-NH$_2$); 2.68 (m, 2H, C**H$_2$**-NH); 4.40 (dd, 1H, J = 15.5 Hz, J = 9 Hz C**H$_2$**-oxadiazole); 4.79 (dd, 1H, C**H$_2$**-oxadiazole); 5.44 (m, 2H, N**H$_2$**); 7.25 (br, 1H, N**H**); 7.91 (m, 1H, Ph); 8.46 (m, 2H, Ph); 8.73 (m, 1H, Ph); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 46.6 (**C**-NH$_2$); 46.8 (**C**-NH); 56.6 (HN-**C**-oxadiazole); 121.7; 126.4 (H**C**-Ph); 127.4 (**C**-Ph); 131.4; 133.2 (H**C**-Ph); 148.4 (**C**-NO$_2$); 166.3 (N=**C**-N); 175.7 (N-**C**-O). ^{195}Pt NMR (107.5 MHz, DMSO-d_6), δ (ppm): -2355. Anal. Calc. for $C_{11}H_{13}N_5O_3PtCl_2$ (%): C, 24.95; H, 2.46; N, 13.23.Found: C, 24.54; H, 2.40; N, 12.41.
3. IR (KBr), ν_{max} (cm^{-1}): 3303, 3182, 3091, 2957, 2884, 1418, 1065; Raman (cm^{-1}): 1624, 1542, 1488, 968, 778, 634, 569, 328; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.35 (m, 2H, C**H$_2$**-NH$_2$); 2.70 (m, 2H, C**H$_2$**-NH); 4.38 (dd, J = 15.6 Hz and J = 9.6 Hz, 1H, C**H$_2$**-oxadiazole); 4.78 (dd, 1H, J = 2 Hz and J = 15.6 Hz, C**H$_2$**-oxadiazole); 5.44 (m, 2H, N**H$_2$**); 7.23 (br, 1H, N**H**); 7.97 (d, J = 8 Hz, 2H, Ph), 8.25 (d, J = 8 Hz, 2H, Ph); ^{195}Pt NMR (107.5 MHz, DMSO-d_6), δ (ppm): -2357. Anal. Calc. for $C_{12}H_{13}N_4OF_3PtCl_2$ (%): C, 26.09; H, 2.35; N, 10.14. Found: C, 26.01; H, 2.41; N, 9.76.
4. IR (KBr), ν_{max} (cm^{-1}): 3183, 3101, 2968, 1445, 1070, 1028; Raman (cm^{-1}): 1607, 1527, 1000, 962, 550, 337, 303; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.46 (m, 2H, C**H$_2$**-NH$_2$); 2.76 (m, 2H, C**H$_2$**-NH); 4.38 (dd, J = 9 Hz and J = 16 Hz, 1H, C**H$_2$**-oxadiazole); 4.57 (dd, J = 2 Hz and J = 9 Hz, 1H, C**H$_2$**-oxadiazole); 4.97 (m, 2H, N**H$_2$**); 6.78 (br, 1H, N**H**); 7.61 (m, 3H, Ph); 8.05 (m, 2H, Ph); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 44.7 (**C**-NH$_2$); 45.7(**C**-NH); 54.4 (HN-**C**-oxadiazole); 125.9 (**C**-Ph); 127.1; 129.3; 131.8 (H**C**-Ph); 167.5 (N-**C**-N); 175.0 (N-**C**-O). Anal. Calc. for $C_{11}H_{14}N_4OPtCl_2$ (%): C, 33.42; H, 3.54; N, 14.18. Found: C, 33.56; H, 3.58; N, 13.57.
5. IR (KBr), ν_{max} (cm^{-1}): 3264, 3198, 3107, 2945, 2889, 1517, 1444, 1349, 1079, 1038; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.48 (m, 2H, C**H$_2$**-NH$_2$); 2.75 (m, 2H, C**H$_2$**-NH); 4.42 (dd, J = 16 Hz and J = 9 Hz, 1H, C**H$_2$**-oxadiazole); 4.62 (dd, 1H, J = 16 Hz

and J = 2 Hz, C**H$_2$**-oxadiazole); 4.98 (m, 2H, N**H$_2$**); 6.81 (br, 1H, N**H**); 7.91 (m, 1H, Ph); 8.48 (m, 2H, Ph), 8.74 (m, 1H, Ph); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 44.7 (**C**-NH$_2$); 45.8 (**C**-NH); 54.5 (HN-**C**-oxadiazole); 121.6; 126.4 (H**C**-Ph); 127.4 (**C**-Ph); 131.3; 133.2 (H**C**-Ph); 148.3 (**C**-NO$_2$); 166.2 (N=**C**-N); 175.8 (O-**C**=N). Anal. Calc. for $C_{11}H_{13}N_5O_3PdCl_2$ (%): C, 30.00; H, 2.95; N, 15.91. Found: C, 30.59; H, 2.99; N, 15.41.

6. IR (KBr), ν_{max} (cm^{-1}): 3311, 3186, 3103, 2955, 2890, 1418, 1324, 1065; Raman (cm^{-1}): 3089, 2956, 2891, 1626, 1543, 1489, 1122, 968, 781, 550, 316, 285, 158; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.47 (m, 2H, C**H$_2$**-NH$_2$); 2.77 (m, 2H, C**H$_2$**-NH); 4.40 (dd, J = 16 Hz and J = 9 Hz, 1H, C**H$_2$**-oxadiazole); 4.59 (dd, 1H, J = 16 Hz and J = 2 Hz, C**H$_2$**-oxadiazole); 4.97 (m, 2H, N**H$_2$**); 6.80 (br, 1H, N**H**); 7.96 (d, J = 8 Hz, 2H, Ph), 8.27 (d, J = 8 Hz, 2H, Ph); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 44.8 (**C**-NH$_2$); 45.9 (**C**-NH); 54.5 (HN-**C**-oxadiazole); 120.6; 122.8; 124.9; 127.1 (**C**-F$_3$, q, ^{1}J = 270.9 Hz); 126.3; 126.4; 128.1 (H**C**-Ph); 129.8 (**C**-Ph); 131.2 (**C**-CF**$_3$**, q, ^{2}J = 31,5 Hz); 166.7 (N-**C**-N); 175.6 (N-**C**-O). Anal. Calc. For $C_{12}H_{13}N_4OF_3PdCl_2$ (%): C, 31.10; H, 2.81; N, 12.09. Found: C, 30.66; H, 2.80; N, 11.58.

2.4. Cytotoxicity assay

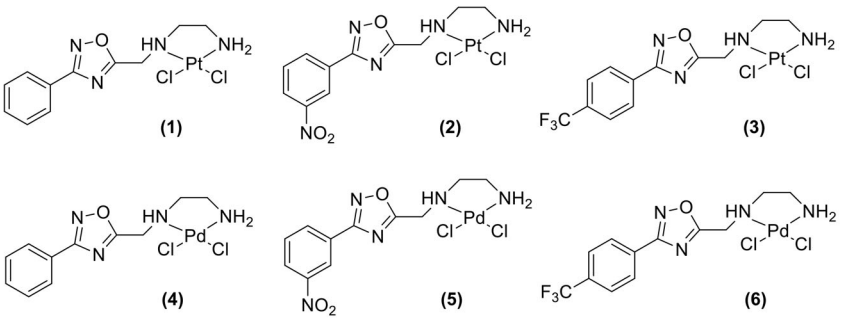
Cell viability was determined on the non-tumor cell BHK-21—Baby Hamster Kidney and the tumor cell lines: CT26-WT–murine colon cancer cells and 4T1–mouse metastatic mammary adenocarcinoma. The cells were harvested and seeded in RPMI 1640 culture medium, supplemented with fetal bovine serum (FBS) 10%, at densities ranging as the cell line (0.5×10^3 and 2×10^3 cells/100 μL/well) of 96-well plates and were appropriately incubated at 37 °C in humidified atmosphere of 5% CO_2 for 24 h to complete adherence. In the wells were distributed 100 μL of decreasing concentrations (100 to 0.1 μM) of compounds. The experiments were done in quadruplicate with culture medium as negative control and cisplatin as positive control against these cell lines.

After exposure to the compounds for 72 h, the cells were incubated with MTT (5 μg/10 μL/well) for 4 h. Next, all the supernatant liquid was removed, 100 μL of DMSO/well was added, and cell viability determined by measuring absorbance at 492 nm in a microplate spectrophotometer. The IC_{50} was calculated using GraphPad Prism 5.0 software.

2.5. Antimicrobial assay

Antibacterial activities of ligands and complexes were evaluated on strains of bacteria *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228 (Gram-positive); *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 27853 (Gram-negative) employing Chloramphenicol as positive control and Mueller-Hinton Broth (MHB–Himedia Laboratories, Mumbai, India) and bacterium as negative control.

For such an assessment it used the susceptibility test broth microdilution methodology according to CLSI guidelines [25]. Bacteria strains were routinely cultured at 37 °C for 24 h on Brain Heart Infusion agar (BHIA—Himedia Laboratories, Mumbai, India) and diluted in saline solution 0.9% (10^8 CFU/mL, according to McFarland



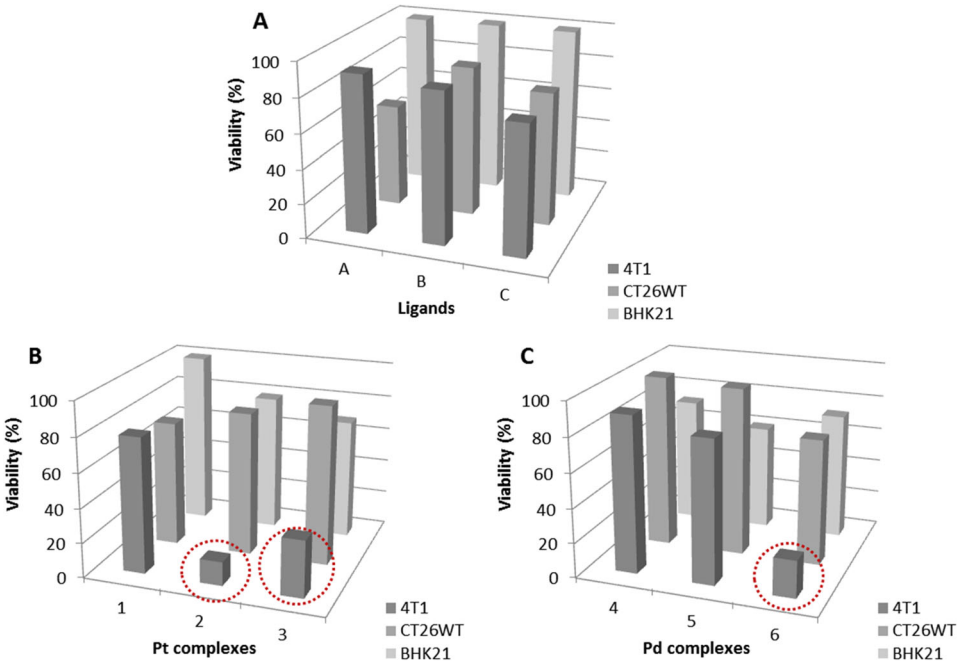


Figure 1. Viability of cells (4T1, CT26WT and BHK21) exposed during 72 h to the highest concentration tested (100 μM) of the compounds. (A) Ligands **A** and **B**, (B) Pt complexes **1–3** and (C) Pd complexes **4–6**.

turbidity standards). From stock solution (in DMSO) serial dilutions were performed from 2 mg/mL in Mueller-Hinton broth (MHB—Himedia Laboratories, Mumbai, India) until reaching final concentrations between 256.0 and 2.0 μg/mL. The compounds with inoculum were incubated at 37 °C for 24 h and the minimum inhibitory concentration (MIC) was calculated as the lowest concentration that prevented visible growth of the strain. All tests were performed in duplicate.

2.6. Bactericidal and bacteriostatic activity

The minimal bactericidal concentrations (MBC) were determined by transferring culture aliquots with sterile swabs to Petri dishes containing Mueller Hinton agar medium

Table 1. Cytotoxicity, expressed as IC_{50} (μM), of complexes obtained by MTT assay against 4T1, CT26WT and BHK21 cell lines. IC_{50} is defined as the drug required concentration to inhibit 50% of cellular growth.

	IC_{50} (μM)[a]		
Compound	4T1	CT26-WT	BHK-21
2	9.8 ± 2	>100	>100
3	58.4 ± 7	>100	>100
6	51.7 ± 5	>100	>100
Cisplatin[b]	6.2 ± 1	6 ± 1	8 ± 2

[a]Data are shown as the mean values of two independent experiments performed in triplicate with the corresponding standard deviations.
[b]Reference drug.

(AMH—Himedia Laboratories, Mumbai, India) and bacterial growth was evaluated after incubation under aerobic conditions at 37 °C for 24 h. The selected concentrations were related to the MIC, considering two dilutions above and two dilutions below. The MBC was determined as the lowest dilution that did not allow growth of the bacteria. To the compounds for which bacterial growth was observed for all dilutions around MIC, the bacteriostatic effect was attributed.

3. Results and discussion

3.1. Structures and spectroscopic characterization

The ligands were obtained by a reaction in three steps (Scheme 1). Initially, it was taken to obtain the amidoxime, in the second step the amidoxime reacts with bromoacetyl bromide getting the oxadiazole which finally reacts with ethylenediamine obtaining the ligand. The ligand received proper purification in each step.

For these ligands, the experimental IR spectra showed broad absorptions assigned to NH stretch in the 3400–3300 cm^{-1} region. The 3090, 2920 and 2870 cm^{-1} were observed absorptions corresponding to CH of aromatic and symmetric and asymmetric stretch of CH of aliphatic. Other bands were observed at 1444 cm^{-1} ν(CN) and 1070 cm^{-1} ν(CO) of oxadiazole. For ligand **B** in the 1516 and 1346 cm^{-1} region show stretch assigned to the NO_2 group and for ligand **C** at 1326 cm^{-1} it was observed one band relative to the CF_3 group. In the ^1H NMR spectra, were observed a multiplet around $\delta = 2.60$ ppm with four hydrogens assigned to the CH_2 group of ethylenediamine, at $\delta = 4.09$ ppm it was observed one singlet corresponding to the CH_2 switched on to the oxadiazole and in the region 7.56–8.70 ppm show signs it can be attributed to aromatic hydrogens. In the ^{13}C NMR spectra, signals were observed at 41.2 ppm and 44.2 ppm and can be assigned the ethylenediamine carbons linked to NH_2 and NH, respectively, and at 51.7 ppm was observed the carbon that unity ethylenediamine and the oxadiazole. For the aromatic carbons, were observed signals in the δ region 120.6–133.1 ppm. Ultimately, at 166.1 ppm (N-C-N) and 180.4 ppm (N-C-O) were observed other carbons of oxadiazole.

The complexes were obtained by reaction of the ligands with metal salt (K_2PtCl_4 or K_2PdCl_4) in methanol/water under stirring for four hours when the solid complexes (Scheme 2) could be filtered off.

Table 2. MIC - Minimum inhibitory concentration of the compounds in bacteria strains.

	MIC - Minimum inhibitory concentration—μg/mL (μM)			
	Gram-positive		Gram-negative	
Compound	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*
Ligand **A**	256.0	256.0	256.0	256.0
Ligand **B**	256.0	256.0	256.0	256.0
Ligand **C**	64.0	256.0	128.0	256.0
Complex **1**	256.0	256.0	256.0	256.0
Complex **2**	32.0	**16.0 (30.0)**	256.0	256.0
Complex **3**	32.0	**8.0 (14.4)**	256.0	256.0
Complex **4**	256.0	256.0	256.0	256.0
Complex **5**	32.0	**8.0 (18.2)**	256.0	256.0
Complex **6**	**16.0 (34.5)**	64.0	256.0	128.0
Chloramphenicol	16.0	32.0	16.0	32.0

Complexes were characterized using analytical and spectroscopic techniques. In the IR spectra of the complexes (Figures S1–S6), in comparison with respective ligand, the NH stretch was moved up to 200 cm^{-1}, and displayed around 3300–3200 cm^{-1}, indicating N-coordinating proposal for the ligand to metal. The other bands were observed without significant displacement. From the Raman spectra we notice the emergence of two bands referring to stretching metal-Cl and metal-N at 330 (Pt-Cl) and 570 (Pt-N) cm^{-1} for platinum complexes that agree with the reported for *cis*-$[Pt(NH_3)_2Cl_2]$ and *trans*-$[Pt(NH_3)_2Cl_2]$ complexes [26]. Similarly, at 316 (Pd-Cl) and 550 (Pd-N) cm^{-1} for palladium complexes such as assigned for palladium(II) complex with a ligand derived from EDTA [27]. In the ^1H NMR spectra (Figures S7–S12) important changes were observed which are strong indications of the coordination of the ligand to the metal through the nitrogen of diamine, like the hydrogens of the ethylenediamine that initially were together became separated and displaced at 2.4 ppm (CH_2-NH_2) and 2.7 ppm (CH_2-NH). The other CH_2 appeared as two double doublets at 4.4 ppm and 4.7 ppm due to the formation of a chiral center after metal coordination. Finally, the signals relative to NH_2 (5.4 ppm) and NH (7.2 ppm) were observed. The ^1H NMR spectrum of **1** is assigned in the Supplementary Information (Figure S7), and it is possible to observe the related modifications. All the signals observed in the ^1H NMR spectrum were assigned based on 2 D NMR experiment (^1H-^1H COSY—Figure S13).

Comparing the ^{13}C NMR spectra for the complexes to the free ligands (Figures S14–S19) we have observed the displacement of the assigned signals to carbons of ethylenediamine, of the CH_2 between ethylenediamine and oxadiazole ring, and the quaternary carbons of oxadiazole, which indicates the proposed coordination. Ultimately, for the ^{195}Pt NMR spectra, the complexes show only one signal at −2350 ppm which is expected for platinum coordinated to two nitrogen atoms and two chlorides, since some works (theoretical and experimental) reported signals in this region for complexes which presented the same coordination sphere [28–31]. Varying the ligand substituents can result in variations about 100 ppm or more in the chemical shifts [32], similar to that observed previously in the cited works. The values obtained from elemental analysis agreed with the molecular formula proposed. The complexes synthesized correspond to a 16-electron configuration with a square planar geometry.

Prior to biological studies, the compounds were submitted to UV-vis spectra determination at physiological conditions (buffer solution, 1% DMSO, pH 7.2 and 36 °C)

until 72 h to assess the stability. All compounds did not show any interference during this time exposure. Representative spectrum of **1** is presented in the Supplementary Material (Figure S20).

3.2. Cytotoxicity

The ligands were unable to inhibit cell viability at the concentrations used (up to 100 μM). Also, for complexes, the IC_{50} values (the concentration to inhibit 50% of cell viability) were not determined since they were not able to inhibit the cell growth satisfactorily, except for **2**, **3** and **6** for 4T1 cells. Based on that, the results of cell viability after 72 h of exposure to the compounds in their higher concentration (100 μM) are shown in Figure 1. The IC_{50} calculated to these compounds and compared to cisplatin are presented in Table 1. Only **2** shows IC_{50} 9.8 ± 2 μM closed to cisplatin (6.2 ± 1 μM); this result is very interesting since complex **2** (platinum complex) did not exhibit activity in non-tumor cell (BHK-21) been selective to mammary carcinoma cell (4T1). Complexes **3** and **6** presented low cytotoxicity in 4T1 (IC_{50} around 50 μM) without inhibition of cell viability of non-tumor cell line, which indicates some selectivity. In a similar way, the literature shows a work that describes the cytotoxicity of metal complexes with ligands derived of oxadiazole where it is possible to observe that coordination of the ligand to the metal improves the antitumor activity [16]. Other literature of gold(I) complexes with oxadiazole derivatives as ligands showed better activity of the complex when compared with the ligand (about eighty times) and were better and more selective than cisplatin in B16-F10 tumor cell line (murine melanoma) [17]. In the case of platinum(II), one complex derived of oxadiazole showed good activity (similar to cisplatin) and better cytotoxicity when compared with free ligands [14].

With respect to the ligands, it was reported that fifty-six synthetic compounds derived of oxadiazole, being that 1,2,4-oxadiazole derived did not show significant results for the cell lines tested [6]. The best results for these derivatives were for two compounds with another diamine, the piperazine N-alkylated with a long carbon chain. The 1,3,4-oxadiazoles showed better activity when compared with 1,2,4-oxadiazole, and some derived of 1,3,4- were capable to induce apoptosis in the tumor-cell tested [6]. Thereby, how it was observed that the coordination improves the cytotoxic activity, the ligands derived from oxadiazole are a promising class to study. In our case, the compounds are not active enough to be promising as anticancer agents. On the other way, the antibacterial activity evaluation is of special interest for compounds that do not show cytotoxicity, facing the antimicrobial resistance issue.

3.3. Antibacterial activity

Based on related promising antimicrobial activity of the oxadiazoles we decided to evaluate our compounds about it [33, 34]. The antibacterial activity of the compounds was determined against four strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*). The susceptibility test broth microdilution methodology was used (Figure S21) and the MIC values obtained are given in Table 2.

Table 3. Evaluation of bactericidal (X) or bacteriostatic (S) activity of the synthesized molecules. (lethal concentration in μg/mL).

	Bacteria species			
	Gram-positive		Gram-negative	
Compound	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*
Ligand **A**	S	S	S	S
Ligand **B**	S	S	S	S
Ligand **C**	S	S	S	S
Complex **1**	S	X(256)	S	S
Complex **2**	S	X(256)	X(256)	S
Complex **3**	X(256)	X(128)	S	S
Complex **4**	S	X(256)	S	S
Complex **5**	S	X(256)	S	S
Complex **6**	X(256)	X(256)	X(256)	X(256)

Based on the MIC values, it is possible to observe that for Gram-positive strains, **2**, **3**, **5** and **6** were more active than the respective ligands. The positive control used was chloramphenicol, widely used as reference drug in prospective assays with new antimicrobial candidates. Chloramphenicol is an extended-spectrum agent with both anti-Gram-positive and anti-Gram-negative bacteria, in different clinical protocols, but with test limits well established by CLSI, which validates its uses as a quality control drug to validate MIC assays [15, 25, 35–37]. It is important to state, however, that chloramphenicol resistance has emerged, which is impacting its clinical efficacy [38]. According to our data, it was observed that **6** was as active as chloramphenicol against *S. aureus* ATCC 29213, which was in turn within the MIC limits for the test according to CLSI validating the observations. As an important bacterial group mentioned before these microorganisms figure as one of the twelve procaryotes of medical importance considered the greatest threat to human health nowadays [2]. As shown in Table 2, the relative MIC (34.5 μM) is lower than the IC_{50} (51.7 μM) for **6** in the only cell line (4T1) it presents some activity (Table 1). With respect to *S. epidermidis* strain, **2**, **3** and **5** were more active than the control chloramphenicol. The coordination and the presence of groups NO_2 and CF_3 may favor activity in Gram-positive bacteria. It was reported that compounds with NO_2 and CF_3 substituents in phenyl ring present moderate or good activity against bacteria [39, 40]. The fact of the substituents has modified the activity in agreement with the literature, where it was observed that the nature of the substituent groups and their position on aromatic group affect the MIC values [41]. It is important to mention the difference between the Gram-positive and Gram-negative strains, the former have a thick cell wall and the latter have a relatively thin layer of peptidoglycan with an external phospholipidic membrane [42]. In this way, the better activity of the tested compounds among Gram-positive representative bacteria might be related to the structural differences between Gram-negative and Gram-positive bacteria, especially considering the cell wall [43]. Similarly to what was observed in this work, Almeida and coworkers showed the synthesis of gold(I) complexes with lipophilic ligands derived from oxadiazole and these complexes exhibited excellent antibacterial activity in the same Gram-positive strains tested in our paper, including being better than the ligands [15]. Besides the use of the heterocyclic ring oxadiazole, in the structure of our compounds there are a diamine and an aromatic ring, so, in this context, we can compare with compounds

containing those unities. With respect to aromatic diamines, Vieira and coworkers describe the synthesis and the antitubercular activity of platinum(II) and palladium(II) with fluoroquinolones. In this work, the ligands bind to platinum(II) in the same way of our complexes, forming a chelate with the diamine, and most complexes presented better activity against *Mycobacterium tuberculosis* than Rifampicin [44]. The metals employed in this work, platinum and palladium, were already assessed as antibacterials and have shown similar or better activity than the standards used [45, 46]. Another point reported in the literature that is in accordance to our results was the increased activity after metal complexation [47, 48].

3.4. Bactericidal and bacteriostatic activity

The antibiotics are classified according to how they act on bacteria, being called bactericidal the antibiotics that kill bacteria and bacteriostatic those that inhibit bacterial growth [49]. Despite this, it is reported that bacteriostatic compounds can kill bacteria, but in a higher concentration than bactericidal agents [50]. Thus, we have evaluated this feature of the ligands and the complexes and classify them as bactericidal or bacteriostatic (Table 3).

There are antibiotics with bacteriostatic activity as chloramphenicol against *Staphylococcus aureus* and Gram-negative bacilli of *Enterobacteriaceae* [51, 52], but in several specific cases, in terms of chemotherapy it is needed potential antimicrobial drugs with bactericidal action such as in endocarditis [53]. According to Table 3, all ligands were bacteriostatic, in turn, considering the chemical complexes, bactericidal activity was observed for some strains. Complex **6** (palladium(II) complex with the ligand containing CF_3 as substituent) was the only compound which showed bactericidal activity against all tested bacteria strains, but only in higher concentrations. For the *S. epidermidis* strain, all the complexes were bactericidal, so the metal presence might be associated to the observed activity.

4. Conclusion

This work describes the synthesis and characterization of three ligands derived from phenyl-oxadiazole-ethylenediamine, whose difference lies in the substituent on the aromatic ring, and their platinum(II) and palladium(II) complexes. We studied the antibacterial and cytotoxicity activity for the compounds. It should be highlighted that **2** is the only compound presenting antitumor activity and selective to the carcinoma mammalian cell (4T1). The fact that the compounds, in general, do not show cytotoxicity in tumor cells or normal cells suggests that they could be used to treat bacterial infections without causing toxicity to the host, an advance to be considered in potential biological application. Complexes **2**, **3**, **5** and **6** were more active when compared with their ligands in Gram-positive strains. This fact highlights and reinforces evidence that the coordination favored the activity and the presence of -NO_2 and -CF_3 in aromatic ring also contribute to the complex activity and selectivity. Thus, although further prospective studies such as those with a broader panel of freshly isolated

bacteria are needed, we may suggest that **3**, **5** and **6** are potential candidates as antimicrobial molecules without causing cytotoxicity.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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