Synthesis, Characterization and Cytotoxic Activity of Tioconazole Coordination Compounds with Nickel(II), Palladium(II) and Platinum(II)

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Abstract. Coordination compounds of nickel(II), palladium(II) and platinum(II) with tioconazole (tcnz) were synthesized and characterized by infrared, UV-Vis-NIR, elemental analysis, molar conductivity, magnetic susceptibility, mass spectrometry, NMR spectroscopy and X-ray diffraction. Tioconazole presented a monodentate coordination mode, through the nitrogen atom of the imidazolic ring. The nickel(II) compounds stabilized octahedral geometries. For the [Ni(tcnz)₂(NO₃)₂]·H₂O compound, the nitrate anion present a bidentate coordination mode, while for the dinuclear [Ni(tcnz)₂(OAc)₂]·3H₂O compound, the acetate groups behave as bridging ligands. When different molar ratios of the corresponding nickel(II) halides were used on the reaction synthesis, three tcnz ligands in the [Ni(tcnz)₃Br₂(H₂O)], or six in [Ni(tcnz)₆]Cl₂ and [Ni(tcnz)₆]Br₂, are coordinated to the nickel(II) atom. The palladium(II) and platinum(II) compounds, [Pd(tcnz)₂Cl₂], [Pt(tcnz)₂Cl₂]·2H₂O and [Pd(tcnz)₂(OAc)₂], presented a square planar geometry. The [Ni(tcnz)₆]X₂ (X⁻ = Cl, Br) compounds stabilized 3D supramolecular arrangements through hydrogen bonding and π···π stacking interactions, between benzene rings of neighboring molecules. The in vitro cytotoxic activity of the synthesized compounds was studied in four different human carcinoma cell lines: HCT-15 (colon), HeLa (cervix-uterine), MCF-7 (breast) and PC-3 (prostate).

Keywords: Tioconazole; coordination compounds; nickel(II); palladium(II); platinum(II); intermolecular interactions; cytotoxic activity.

Resumen. Los compuestos de coordinación de níquel(II), paladio(II) y platino(II) con tioconazol (tcnz) fueron sintetizados y caracterizados por infrarrojo, UV-Vis-NIR, análisis elemental, conductividad molar, susceptibilidad magnética, espectrometría de masas, espectroscopía de RMN y difracción de rayos X. El tioconazol presenta un modo de coordinación monodentado, a través del átomo de nitrógeno del anillo de imidazol. Los compuestos de coordinación de níquel(II) estabilizan geometrías octaédricas. En el compuesto [Ni(tcnz)₂(NO₃)₂]·H₂O, el anión nitrato presenta un modo de coordinación bidentado, mientras que en el compuesto dinuclear [Ni(tcnz)₂(OAc)₂]·3H₂O, los acetatos se comportan como ligantes puente. Empleando diferentes relaciones molares en las reacciones de síntesis de los correspondientes haluros de níquel(II) se obtuvieron tres ligantes tcnz coordinados al átomo metálico en el compuesto [Ni(tcnz)₃Br₂(H₂O)], o seis en los compuestos [Ni(tcnz)₆]Cl₂ y [Ni(tcnz)₆]Br₂. Los complejos de paladio(II) y platino(II), [Pd(tcnz)₂Cl₂], [Pt(tcnz)₂Cl₂]·2H₂O y [Pd(tcnz)₂(OAc)₂], presentan una geometría cuadrada plana. Los compuestos [Ni(tcnz)₆]X₂ (X⁻ = Cl, Br) estabilizan arreglos supramoleculares en 3D, a través interacciones de puentes de hidrógeno y apilamientos π···π entre los anillos bencénicos de moléculas vecinas. La actividad citotóxica in vitro...
vitro de los compuestos sintetizados fue estudiada en cuatro diferentes líneas celulares de carcinoma humano: HCT-15 (colon) y HeLa (cervicouterino), MCF-7 (mama) and PC-3 (próstata).

Palabras clave: Tioconazol; compuestos de coordinación; níquel(II); paladio(II); platino(II); interacciones intermoleculares; actividad citotóxica.

Introduction

The discovery of active sites containing nickel centers in metalloproteins, [1-4] has stimulated the study of its coordination compounds and their biological activity, such as antitumor, anticonvulsants or antiepileptic, antibacterial and antifungal.[5,6] Additionally, palladium and platinum, also from group 10, have shown anticancer activity.[7-10] Platinum(II) compounds, as cisplatin, oxaliplatin and carboplatin, are the more effective anticancer drugs used in chemotherapy.[11,12]

Cisplatin, used for the testicular and ovarian cancer treatment, is one of the most widely used antitumor drugs in the world.[13-15] However, it has side effects such as nephrotoxicity, drug tolerance, limited solubility and intravenous administration.[14,16-19] Due to the fact that the cancer is the second leading cause of death,[16,20,21] the development of improved metal based drugs is currently of interest.

When designing new antitumor agents, the similarity in the chemistry of the PtII and PdII compounds has led to the study of palladium antitumor drugs with high activity.[22-26] Some of them are stabilized by chelates, as Schiff bases, or by voluminous ligands with monodentate nitrogen atoms,[22] which presented activity in HeLa cell line. It has been reported that trans-Pd compounds with Schiff base ligands have better activity than the cis-Pd compounds.[18,22] Additionally, a series of coordination compounds with benzylamine and PdII were studied on MCF-7 and MDA-MB-231 breast cancer cell lines, indicating an effective anticancer potential. [17]

When combining metal ions with an established biological activity of the ligands, an enhancement or a modification of their pharmacological properties has been observed, as it is the case of ticonazole, clotrimazole and miconazole, with antifungal properties, which their organometallic ruthenium(II) compounds showed antiparasite activity.[27] Transition metal coordination compounds with Schiff base derivatives, (CoII, NiII, CuII, ZnII) have proved to enhance the antimicrobial and antifungal activity of the free ligands.[28]

In a previous study, we synthesized and characterized CuII and ZnII coordination compounds with ticonazole (tcnz) and their cytotoxic activity in HCT-15 and HeLa cell lines was studied. The octahedral [Cu(tcnz)Cl2] compound showed promising activity in the HCT-15 cell line. While the tetrahedral [Zn(tcnz)Br2] compound had cytotoxic activity in HeLa cell lines.[29]

Continuing our work in this field, NiII, PtII and PdII compounds were synthesized and characterized (Fig. 1) as their biological activity as possible anticancer agents in HCT-15, HeLa, MCF-7 and PC-3 cell lines, was studied.
Fig. 1. Structures for the tioconazole coordination compounds with NiII, PdII and PtII.
Experimental

Materials and methods

All reagents and solvents were purchased and used without any further purification: tioconazole 98% (Aldrich, Co); NiCl₂·6H₂O, NiBr₂·3H₂O, Ni(NO₃)₂·6H₂O, Ni(OAc)₂·4H₂O (J.T. Baker); PdCl₂, PtCl₂ and Pd(OAc)₂ (Aldrich, Co); solvents (Merck). The complexes [PdCl₂(CH₃CN)₂] and [PtCl₂(CH₃CN)₂] were synthesized according with the reported procedure.[30] FT IR spectra in the range 4000–400 cm⁻¹ were collected in a Perkin Elmer FT-IR Spectrum 400 spectrophotometer with an universal ATR sampling accessory at 298K. Mass spectra (MS –ESI⁺) were determined in an Esquire 6000 mass spectrometer (Fig. S1-S6). Elemental analyses for carbon, hydrogen, nitrogen and sulfur were carried out with a Fisons EA 1108 analyzer. Magnetic susceptibility measurements at room temperature of powdered samples were obtained on a Johnson–Matthey D8 5HJ balance, using the Gouy method. NMR spectra were obtained at room temperature on a 300 MHz Bruker-Avance Unity spectrometer. ¹H and ¹³C {¹H} NMR spectra were obtained using DMSO-d₆ and CDCl₃ (Fig. S7-S15). Electronic spectra were measured over the range 40000–5000 cm⁻¹ by the diffuse reflectance method on a Cary-5000 Varian spectrophotometer at 298 K (Fig. S16).

Synthesis of the coordination compounds

Coordination compounds form the Ni II salts were synthesized by similar procedures. A solution of one equivalent of the corresponding transition metal salt in acetone was added to a solution of one equivalent of tioconazole in acetone, with exception of the [Ni(tcnz)₂(OAc)₂]·3H₂O 3 compound where ethanol was used as solvent. For the compounds [Ni(tcnz)₆]Cl₂ 4 and [Ni(tcnz)₆]Br₂ 5 a 1:3 ratio was used. The reaction mixture was heated under reflux with constant stirring during 24 h. Pd II and PtII compounds were synthesized using a 1:2 ratio (metal salt:tcnz) in acetone. The solvent was evaporated at RT and the products were washed with water and ethanol and dried under vacuum overnight.

Synthesis of [Ni(tcnz)₃Br₂(H₂O)] (1)

NiBr₂·3H₂O (0.07 g, 0.25 mmol) was added to a solution of tioconazole (0.1g, 0.25 mmol) in acetone (15mL), the obtained dark blue solution was left to stand at RT, a bright blue solid precipitated. UV–Vis–NIR ν(cm⁻¹): ν₁ = 8665, ν₂ = 14993 and ν₃ = 24918. FT-IR (ATR, ν cm⁻¹): ν(O-H) 3324, ν(C=N) 1589, ν(C-O-C) 1088, ν(C-S) 736. MS-ESI⁺ (m/z) 1301 [C₄₈H₃₉Cl₉Br₂N₆O₃S₃Ni⁺]. Anal. Found: C, 41.08; H, 2.51; N, 6.10; S, 5.99%. Calc. for C₄₈H₄₁Cl₉Br₂N₆O₄S₃Ni: C, 41.19; H, 2.95; N, 6.00; S, 6.87%. µeff = 3.40 BM. Yield: (0.19 g, 92%).

Synthesis of [Ni(tcnz)₂(NO₃)₂]·H₂O (2)

Ni(NO₃)₂·6H₂O (0.074 g, 0.25 mmol) was added to a solution of tioconazole (0.1g, 0.25 mmol) in acetone (15mL). A dark green solution was observed, a green solid was obtained. UV–Vis–NIR ν(cm⁻¹): ν₁ = 8936, ν₂ = 15553 and ν₃ = 25477. FT-IR (ATR, ν cm⁻¹): ν(O-H) 3344, ν(C=N) 1589, ν(as(NO₃)) 1468, ν(s(NO₃)) 1306, ν(N=O) 1235, ν(C-O-C) 1087, ν(C-S) 736. MS-ESI⁺ (m/z) 896 [C₃₂H₂₆Cl₆N₅O₅S₂Ni⁺]. Anal. Found: C, 39.36; H, 2.90; N, 8.45; S, 6.26%. Calc. for C₃₂H₂₈Cl₆N₆O₉S₂Ni: C, 39.37; H, 2.89; N, 8.61; S, 6.57%. µeff = 3.50 BM. Yield: (0.11g, 81%).

Synthesis of [Ni(tcnz)₂(OAc)₂]·3H₂O (3)

Ni(OAc)₂·4H₂O (0.064 g, 0.25 mmol) was added to a solution of tioconazole (0.1g, 0.25 mmol) in ethanol (15mL). An emerald green solution was observed, a bright green solid was precipitated. UV–Vis–NIR ν(cm⁻¹): ν₁ = 8936, ν₂ = 15417 and ν₃ = 25409. FT-IR (ATR, ν cm⁻¹): ν(O-H) 3344, ν(C=N) 1589, ν(as(COO)) 1558, ν(s(COO)) 1408, ν(C-O-C) 1084, ν(C-S) 732. MS-ESI⁺ (m/z) 1456 [C₅₄H₄₇Cl₂N₉O₉S₃Ni₂⁺]. Anal. Found: C, 42.45; H, 3.01; N, 5.70; S, 5.35%. Calc. for C₅₄H₄₈Cl₂N₉O₉S₃Ni₂: C, 42.97; H, 3.81; N, 5.57; S, 6.37%. µeff = 3.20 BM. Yield: (0.11g, 90%).

Synthesis of [Ni(tcnz)₆]Cl₂ (4)

NiCl₂·6H₂O (0.07 g, 0.30 mmol) was added to a solution of tioconazole (0.34 g, 0.90 mmol) in acetone (15mL), the obtained blue solution was left to stand at RT. After two weeks purple crystals suitable for X-ray diffraction were isolated. UV–Vis–NIR ν(cm⁻¹): ν₁ = 10880, ν₂ = 17550 and ν₃ = 24330. FT-IR
Synthesis of [Ni(tcnz)₆]Br₂ (5)

NiBr₂·3H₂O (0.07 g, 0.25 mmol) was added to a solution of tioconazole (0.3 g, 0.75 mmol) in acetone (15 mL). The obtained blue solution was left to stand at RT. After a week, purple crystals suitable for X-ray diffraction were isolated. UV–Vis–NIR ν (cm⁻¹): ν₁ = 10880, ν₂ = 17550 and ν₃ = 24330. FT-IR (ATR, ν cm⁻¹): ν(C=N) 1589, ν(C-O-C) 1079, ν(C-S) 737. Due to its insolubility in common solvents, it was not possible to obtain its MS -ESI⁺ (m/z). Anal. Found: C, 44.47; H, 3.21; N, 7.54; S, 6.59%. Calc. for C₉₆H₇₈Cl₁₈Br₂N₁₂O₆S₆Ni: C, 45.31; H, 3.09; N, 7.56; S, 6.60%. µeff = 3.01 BM. Yield: (0.25 g, 36%).

Synthesis of [Pd(tcnz)₂Cl₂] (6)

[PdCl₂(CH₃CN)₂] (0.05 g, 0.19 mmol) was added to a solution of tioconazole (0.14 g, 0.38 mmol) in acetone (15 mL) during 24 h at RT. A light yellow solid precipitated from the solution. UV –Vis –NIR ν (cm⁻¹): ν₁ = 25595. FT-IR (ATR, ν cm⁻¹): ν(C=N) 1587, ν(C-O-C) 1084, ν(C-S) 735. 1H NMR (DMSO-d₆, 300 MHz): 8.08 (2H, s, CH, Ha), 7.67 (2H, d, CH, Hf), 7.51 (2H, dd, CH, Hj), 7.43 (2H, s, CH, Hc), 7.17 (2H, s, CH, Hg), 7.11 (2H, s, CH, Hb), 6.89 (2H, d, CH, Hk), 4.97 (2H, dd, CH, He), 4.37 - 4.26 (8H, m, CH₂, Hi, d). 13C NMR (DMSO-d₆, 75 MHz assignments by HSQC): 139.79 (C1), 134.44 (C6), 134.06 (C7), 133.73 (C9), 133.20 (C13), 129.36 (C14), 128.99 (C8), 128.63 (C10), 127.98 (C15), 127.91 (C11), 126.50 (C16), 124.56 (C3), 119.92 (C2), 75.59 (C5), 63.08 (C12), 50.96 (C4). MS-ESI⁺ (m/z) 917 [C₃₂H₂₆Cl₇N₄O₂S₂Pd]⁺. Anal. Found: C, 40.84; H, 2.38; N, 5.88; S 6.73%. Calc. for C₃₂H₂₆Cl₇N₄O₂S₂Pd: C, 40.34; H, 2.75; N, 5.88; S, 6.73%. Yield: (0.12 g, 68%).

Synthesis of [Pt(tcnz)₂Cl₂]·2H₂O (7)

[PtCl₂(CH₃CN)₂] (0.05 g, 0.10 mmol) was added to a solution of tioconazole (0.082 g, 0.20 mmol) in a mixture of acetone and acetonitrile (15 mL) under reflux during 4 h at RT. A yellow solid precipitated from the solution. UV–Vis–NIR ν (cm⁻¹): ν₁ = 27024. FT-IR (ATR, ν cm⁻¹): ν(O-H) 3444, ν(C=N) 1588, ν(C-O-C) 1083, ν(C-S) 732. 1H NMR (CDCl₃, 300 MHz): 8.24 (2H, s, CH, Ha), 7.65 (2H, d, CH, Hf), 7.47 - 7.38 (4H, m, CH, Hc, h, j), 7.23 (2H, m, CH, Hg), 7.17 (2H, m, CH, Hb), 6.88 (2H, d, CH, Hk), 4.97 (2H, dd, CH, He), 4.33 % (2H, d, CH₂, Hi), 4.28 (4H, m, CH₂, Hd), 2.06 (6H, s, CH₃, Hl). 13C NMR (CDCl₃, 75 MHz assignments by HSQC): 139.84 (C1), 138.55 (C6), 135.29 (C7), 133.64 (C9), 133.42 (C13), 129.77 (C8), 129.64 (C14), 129.46 (C10), 128.40 (C11), 127.02 (C8), 126.50 (C15), 127.02 (C12), 121.37 (C2), 75.78 (C5), 63.53 (C10), 51.43 (C4). MS-ESI⁺ (m/z) 970 [C₃₂H₂₆Cl₆N₄O₄S₂Pt]⁺. Anal. Found: C, 35.72; H, 2.06; N, 5.48; S 5.17%. Calc. for C₃₂H₃₀Cl₈N₄O₄S₂Pt: C, 35.67; H, 2.81; N, 5.20; S, 5.95%. Yield: (0.07 g, 61%).

X-ray crystallographic study

Diffraction intensity patterns from single crystals of compounds 4 and 5 were collected on a SMART APEX I diffractometer (Bruker AXS) equipped with a CCD-detector and using graphite monochromated Mo Kα (λ = 0.71073 Å) radiation source. APEX2 v2012.10.0 (Bruker, 2012) package was used for data collection and data integration. Absorption corrections were applied using analytical procedure. The structures were
solved by direct methods using the package SHELXS-2012 and refined with an anisotropic approach for non-hydrogen atoms using the SHELXL-2014/7 program. All hydrogen atoms attached to C atoms were positioned geometrically as riding on their parent atoms, with C-H = 0.93–0.99 Å and Uiso(H) = -1.2 Ueq(C) for aromatic and methylene groups.[31-33] A summary for data collection and refinements is given in Table 1.

Table 1. Crystallographic data and refinement parameters of compounds [Ni(tcnz)6]Cl2 4 and [Ni(tcnz)6]Br2 5.

| Compound | 4          | 5          |
|----------|------------|------------|
| Empirical formula | C96H78Cl20Ni12O12S6 | C96H78Br2Cl18Ni12O6S6 |
| Formula weight (g mol⁻¹) | 2551.77 | 2640.69 |
| Crystal size (mm) | 0.249 x 0.217 x 0.168 mm | 0.357 x 0.240 x 0.234 mm |
| Crystal color | Purple | Purple |
| Crystal system | Trigonal | Trigonal |
| Space group | R-3 | R-3 |
| Unit cell dimensions | | |
| a (Å) | 24.6475(9) | 24.655(4) |
| b (Å) | 24.6475(9) | 24.655(4) |
| c (Å) | 15.8565(6) | 15.943(3) |
| α (°) | 90 | 90 |
| β (°) | 90 | 90 |
| γ (°) | 120 | 120 |
| V (Å³) | 8342.3(7) | 8393(3) |
| Z | 3 | 3 |
| Dcalc (g/cm³) | 1.524 | 1.567 |
| μ (mm⁻¹) | 0.831 | 1.492 |
| F(000) | 3894 | 3858 |
| Temp (K) | 298(2) | 298 |
| Completeness | 99.7% | 99.6% |
| Rint | 0.0641 | 0.0729 |
| R (I>2σ(I)) | 0.0906 | 0.0785 |
| Rw (I>2σ(I)) | 0.2764 | 0.2213 |
| S | 1.027 | 1.025 |

\[ R_{int} = \frac{\sum w(F_o^2 - F_c^2)^2}{\sum w(F_o^2)^2} = \frac{\sum w(F_o^2)^2}{\sum w(F_o^2)^2} \]

In vitro cytotoxic activity determination

Cell culture

HCT-15 (colon), HeLa (cervix uterine), MCF-7 (breast) and PC-3 (prostate) human carcinoma cell lines were acquired from ATCC (American Tissue Culture Collection) and maintained in incubation at 310 K and 5% CO₂ with RPMI (GIBCO®, Invitrogen corporation) supplemented with 10% BFS (GIBCO®, Invitrogen corporation), 1% L-glutamine and 1% penicillin/streptomycin. Experiments were performed with cells within at least 5 passages from each other. All cells were split when around 80–95% confluence was reached using 0.25% trypsin/EDTA.

In vitro growth inhibition assay

After placing 2 x 10⁴ cells/well in 96-well microplate (Costar®) with 300 IL capacity and allowed to attach incubating at 310 K for 48 h, HCT-15 (colon), HeLa (cervix-uterine), MCF-7 (breast) and PC-3 (prostate) human carcinoma cells were treated with the NiII, PdII, and PtII complexes. The metal complexes (cisplatin and tioconazole control was added to the plates to act as a positive and comparative control) were tested in 5% DMSO and in a physiological solution to 0.9% NaCl to give a 1 mM stock solution. Two rows
free of drug solution acted as the 100% cell survival control. Sonication was sometimes used to facilitate complete dissolution. Serial dilutions were carried out to give final screening, concentrations of the coordination compounds of 400, 200, 20, 2 and 0.2 μM (final concentration of DMSO of 0.5% (v/v)). Aliquots of 50 μL of these solutions were added to the wells (in triplicate) already containing 150 μL of media, so that the final concentrations were 0.01, 0.1, 1, 10, and 100 μg/mL (final concentration of DMSO of 0.125% (v/v)). The cells were exposed to the complex for 24 h, which then was removed and the cells washed with washing media followed by the addition of 200 μL of fresh RPMI media. Then the cells were incubated for 72 h of recovery time. The remaining biomass was then estimated by the sulforhodamine B assay [34-36] (SRB assay). The four screening concentrations were used in an initial test of activity. The selected complexes were then tested for half maximal inhibitory concentration (IC50) values determination. Each assay was done in triplicate. IC50 values were obtained from plots of % cell survival against log of the drug concentration.

Results and discussion

A series of coordination compounds of tioconazole with nickel(II), palladium(II) and platinum(II) were synthesized. The geometry depends of the metal ion, with nickel(II) the octahedral compounds 1-5 were obtained, for palladium and platinum(II) compounds 6-8 square planar arrangement is preferred. The structures of the coordination compounds shown in Fig. 1 are based on their spectroscopic characterization, elemental analyses, molar conductivity, magnetic susceptibility and by X-ray diffraction, when suitable crystals of the coordination compound were obtained.

Spectroscopic characterization

The IR spectra of the complexes present a characteristic band of the ν(C=N) vibration from the tioconazole ligand, which is shifted to 1586-1589 cm⁻¹, compared with free ligand (1562 cm⁻¹), indicating that the metal ion is coordinated through the imidazolic nitrogen atom. The band associated to the ν(C=S) vibration in the tcnz, at 733 cm⁻¹, remains in the same region, 733-737 cm⁻¹, as the sulphur atom does not participate as coordination site. Compound 1 presents a broad band at 3324 cm⁻¹ associated to the ν(O-H) vibration of the coordinated water molecule, while for compounds 2, 3 and 7 a broad band in the region 3128-3444 cm⁻¹ was assigned to the water molecules of crystallization. The nitrate compound 2 showed three bands associated to the NO3 group, at 1468 cm⁻¹ νas(NO3), 1306 cm⁻¹ νs(NO3) and 1235 ν(N=O) cm⁻¹, where the ∆ν(νas-νs) = 162 cm⁻¹ indicating a bidentate coordination mode. Compound 3 presents two intense bands at 1558 cm⁻¹, νas(COO), and at 1408 cm⁻¹ νs(COO), with a ∆ν(νas-νs) = 150 cm⁻¹, characteristic of a bridging coordination mode, [37-40] while for compound 8, νas(COO) was assigned at 1632 cm⁻¹ and νs(COO) at 1354 cm⁻¹, with a ∆ν(νas-νs) = 278 cm⁻¹, characteristic of a monodentate carboxylate coordinated to the metal ion.

The solid state electronic spectra (UV-Vis-NIR) for the nickel(II) compounds correspond to an octahedral geometry for the metal atom. For the [Ni(tcnz)2Br2(H2O)]1, [Ni(tcnz)2(NO3)2]H2O 2 and [Ni(tcnz)2(OAc)2]2H2O 3 compounds, the corresponding electronic transitions were observed: ν1 3T2(F)←3A2g(F) (8665, 8936 and 8970 cm⁻¹), ν2 3T1g(F)←3A2g(F) (14993, 15553 and 15417 cm⁻¹) and ν3 3T1g(F)←4A2g(F) (24918, 25477 and 25409 cm⁻¹), for 1-3 respectively. Compounds 4 and 5, [Ni(tcnz)6]X2 (X= Cl, Br), presented similar spectra, with ν1 3T2(F)←3A2g(F) at 11060-11000 cm⁻¹, ν2 3T1g(F)←3A2g(F) at 17557-17550 cm⁻¹ and ν3 3T1g(F)←4A2g(F) at 25550-25450 cm⁻¹, with a larger 10Dq (ca. 11030 cm⁻¹) than those of compounds 1-3 (ca. 8900 cm⁻¹) due to the coordination of six tcnz ligands. For compounds [Pd(tcnz)2Cl2]6, [Pt(tcnz)2Cl2]2H2O 7 and [Pd(tcnz)2(OAc)2]8 a broad band centered on 25595, 27024 and 27683 cm⁻¹ respectively, was assigned to the electronic transition 1A2g←1A1g, for these metal ions in a square planar environment, (Table 2, Fig. SI 16). [39]

1H and 13C {1H} NMR spectroscopy

The 1H, 13C and HSQC NMR data (Fig. SI 7-15) supported the coordination mode of the tioconazole to the metal ion, where chemical shifts of the signals was observed compared to free ligand, confirming the proposed trans-square planar structures for the diamagnetic coordination compounds 6-8.
The $^1$H NMR spectra of the coordination compounds showed a shift for the (C2-H) imidazole proton $H_a$, between the two nitrogen atoms to 8.08 ppm, 8.24 ppm and 7.67 ppm, the free ligand present this signal in 7.55 ppm [29], confirming the proposed coordination mode via N3 to the metal ion. The compound 8 presented one singlet in 1.94 ppm due to the acetate.

$^{13}$C NMR ($^1$H) spectra of coordination compounds exhibited sixteen signals corresponding to tioconazole, the compound 8 presented two more signals C17 in 23.66 ppm and C18 in 178.09 ppm for acetate. The C1 signal was observed in 137.81 ppm for the free ligand, the shifting in coordination compounds were 139.79, 139.84 and 138.55 ppm for 6, 7 and 8, respectively.

X-ray diffraction analysis

$[Ni(tcnz)_6]Cl_2$ 4 and $[Ni(tcnz)_6]Br_2$ 5

Crystals of $[Ni(tcnz)_6]Cl_2$ 4 and $[Ni(tcnz)_6]Br_2$ 5 were grown from a saturated acetone solution at room temperature. The compounds are isostructural and crystallized in a trigonal system with an R-3 spatial group. The unit cell consists of three molecules. In both compounds the metal ion presents an octahedral geometry, with six tioconazole molecules in the coordination sphere (Fig. 2). The N3-Ni coordination bond length is 2.12(1) Å. The molecules are highly symmetrical with N-Ni-N angles in the range of 89.14 - 90.82° for cis-positions and 179.93° for trans-positions, and. The nickel(II) atom presents a regular octahedral geometry, despite the fact that tioconazole is a bulky ligand.
Fig. 2. ORTEP diagram of the \([\text{Ni(tcnz)}]_6\)Br\(_2\) 5 compound. Displacement ellipsoids are drawn at 30% probability. H atoms were omitted for clarity.

Compounds 4 and 5 present intermolecular hydrogen bonding between the chloro atoms from the thiophen moiety and the methylene hydrogen atoms from neighboring molecules, C(9)-H(9B)···Cl(2), 2.763(4) Å (Fig. 3).

Fig. 3. Intermolecular hydrogen bonding C(9)-H(9B)···Cl(2) in compound 5, giving place to a 3D supramolecular arrangement.

The tioconazole ligands are accommodated as a propeller, occupying the six octahedral coordination sites of the nickel(II) atom, giving place to a 3D supramolecular arrangement, stabilized through intermolecular hydrogen bonding and \(\pi\)···\(\pi\) stacking interactions between the benzene rings of neighboring molecules, with a distance of \(ca.\) C(17)···\(\pi\) of 3.482 Å, from the ring centroid to the C(17) aromatic carbon, as shown in Fig. 4, [44].
Cancer cell growth inhibition

The in vitro cytotoxic activity of the coordination compounds in the human cancer cell lines; HCT-15 (colon adenocarcinoma), HeLa (breast adenocarcinoma), MCF-7 (breast) and PC-3 (prostate) was investigated, using cisplatin as reference. The IC_{50} value (µg/mL) indicates the amount of drug necessary to inhibit 50% of the growth of cancer cells, after 24 h of exposition (table 2). The free ligand was not active under these conditions.

In previous work with coordination compounds of imidazole and benzimidazole derivatives it was found that the copper(II) tetrahedral compounds, with coordinated halides, were the most active.[45-47] With the analogous ligand clotrimazole, the tetrahedral nickel(II) coordination compounds presented moderate cytotoxic activity in vitro and the octahedral complexes were not active.[47] Interestingly, for the tioconazole copper(II) octahedral compound a significant cytotoxic activity was observed.[29]

In the present work the nickel(II) octahedral compounds presented cytotoxic activity. The [Ni(tcnz)_5]Cl_2 4, without halogens in the coordination sphere, showed moderate activity against HCT-15, HeLa, and PC-3; while the [Ni(tcnz)_3(NO_3)_3]·H_2O 2 compound, presented the best activity in HeLa and MCF-7 cell lines, and the [Ni(tcnz)_3Br_2(H_2O)] 1 compound showed moderate activity in PC-3, followed by compounds 2, 4 and 5. The palladium(II) and platinum(II) compounds did not presented any significant activity, Table 2. The stability of the studied compounds was determined in DMSO solution and all of them were stable up to 24 hours (Fig. S17).
Table 3. Cell-growth inhibitory assay results. IC<sub>50</sub> value (µg/mL) for Ni<sup>II</sup>, Pd<sup>II</sup> and Pt<sup>II</sup>, tioconazole coordination compounds (1-3 and 6-8).

| Coordination compound | HCT-15 | HeLa | MCF-7 | PC-3 |
|-----------------------|--------|------|-------|------|
| 1                      | 46.35  | 12.15| 14.26 | 11.43|
| 2                      | 18.50  | 7.39 | 12.53 | 16.74|
| 3                      | 323.19 | 32.70| NA    | 31.15|
| 4                      | 14.02  | 12.33| 101.12| 11.67|
| 5                      | 303.27 | 10.12| 15.36 | 16.15|
| 6                      | NA     | 15.67| 51.27 | NA   |
| 7                      | ND     | ND   | ND    | ND   |
| 8                      | NA     | NA   | NA    | NA   |
| Cisplatin              | 8.25   | 5.55 | 1.3   | 3.83 |

ND = not determined; NA = not active

Conclusions

Coordination compounds with tioconazole and Ni<sup>II</sup>, Pd<sup>II</sup>, and Pt<sup>II</sup> were synthesized and fully characterized. The coordination compounds stabilized octahedral (1-5) and square planar (6-8) geometries, depending on the metal ion. Despite that tioconazole is a bulky ligand it can be accommodated in a propeller arrangement, occupying the six octahedral coordination sites of a nickel(II) atom. The crystallographic arrangements of compounds 4 and 5 were stabilized through hydrogen bonding and π⋯π stacking interactions.

The octahedral nickel(II) compounds showed moderate cytotoxic activity (HeLa), the IC<sub>50</sub> increased upon coordination to the metal ion when compared to the inactive free ligand, which can be related to the nature and the geometry of the metal ion, as the square planar platinum(II) and palladium(II) complexes did not presented any significant activity.

Supplementary Information

CCDC No. 1581442 for [Ni(tcnz)<sub>6</sub>Cl<sub>2</sub>] and 1581443 for [Ni(tcnz)<sub>6</sub>Br<sub>2</sub>] contains the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary Information associated with this article can be found in the online version.

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