# 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 $Ni^{II}$ compounds stabilized an octahedral geometry. In $[Ni(tcnz)_2(NO_3)_2]\cdot H_2O$ the coordinated nitrate presented a bidentate coordination mode, while for the $[Ni(tcnz)_2(OAc)_2]\cdot 3H_2O$ compound, the acetate behaves as a bridging ligand. When different molar ratios were used on the reaction synthesis, three or six ligands were coordinate to the nickel(II) atom, $[Ni(tcnz)_3Br_2(H_2O)]$, $[Ni(tcnz)_6]Cl_2$ and $[Ni(tcnz)_6]Br_2$. The palladium(II) and platinum(II) compounds, $[Pd(tcnz)_2Cl_2]$, $[Pt(tcnz)_2Cl_2]\cdot 2H_2O$ and $[Pd(tcnz)_2(OAc)_2]$, stabilized a *trans*-square planar geometry. The compounds $[Ni(tcnz)_6]X_2$ give place to 3D supramolecular arrangements through hydrogen bonding (X···H, X = Cl and Br) and π···π stacking interactions, between the six membered rings of neighbouring 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; intermolecular interactions; cytotoxic activity.

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**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 $Ni^{II}$ estabilizan una geometría octaédrica. En el compuesto $[Ni(tcnz)_2(NO_3)_2]\cdot H_2O$, el nitrato presenta un modo de coordinación bidentado, mientras que para el compuesto $[Ni(tcnz)_2(OAc)_2]\cdot 3H_2O$, el acetato se comporta como un ligante puente. Usando diferentes relaciones molares, se coordinan tres o seis ligantes al átomo de níquel(II), $[Ni(tcnz)_3Br_2(H_2O)]$, $[Ni(tcnz)_6]Cl_2$ y $[Ni(tcnz)_6]Br_2$. Los compuestos de paladio(II) y platino(II), $[Pd(tcnz)_2Cl_2]$, $[Pt(tcnz)_2Cl_2]\cdot 2H_2O$ y $[Pd(tcnz)_2(OAc)_2]$, estabilizan una geometría *trans*-cuadrada plana. Los compuestos $[Ni(tcnz)_6]X_2$ estabilizan arreglos supramoleculares en 3D, a través interacciones de puentes de hidrógeno (X···H, X = Cl y Br) y apilamientos π···π, entre los seis miembros del anillo de moléculas vecinas. La actividad citotóxica *in vitro* de los compuestos sintetizados fue estudiada en dos 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, paladio, platino; 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] $Pt^{II}$ 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 $Pt^{II}$ and $Pd^{II}$ 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 voluminous ligands with monodentate nitrogen atoms,[22] which presented activity in HeLa cell line. It has been observed that *trans*-Pd compounds with Schiff base ligands have better activity than the *cis*-Pd compounds.[18, 22] Additionally, a series of coordination compounds whit benzylamine and $Pd^{II}$ were studied on MCF-7 and MDA-MB-231 breast cancer cell lines, indicating an effective anticancer potential.[17] Palladium(II) compounds have the advantage of having fewer side effects, as well as greater lipophilicity.

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 the organometallic ruthenium(II) compounds with the antifungal activity of ticonazole, clotrimazole and miconazole, that showed antiparasite activity.[27] Research on transition metal coordination compounds with Shiff base derivatives, ($Co^{II}$, $Ni^{II}$, $Cu^{II}$, $Zn^{II}$) have proved to enhance the antimicrobial and antifungal activity of the free ligands.[28]

In a previous study, we synthesized and characterized $Cu^{II}$ and $Zn^{II}$ coordination compounds with tioconazole (tcnz) and their cytotoxic activity in HCT-15 and HeLa cell lines was studied. The octahedral $[Cu(tcnz)_4Cl_2]$ compound showed promising activity in

the HCT-15 cell line. While the tetrahedral $[Zn(tcnz)_2Br_2]$ compound had cytotoxic activity in HeLa cell lines.[29]

Continuing our work in this field, $Ni^{II}$, $Pt^{II}$ and $Pd^{II}$ compounds were synthesized and characterized, as their biological activity as possible anticancer agents in HCT-15, HeLa, MCF-7 and PC-3 cell lines, were studied.

## Experimental

### Materials and methods

All reagents and solvents were purchased and used without any further purification: tioconazole 98% (Aldrich, Co); $NiCl_2{\cdot}6H_2O$, $NiBr_2{\cdot}3H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Ni(OAc)_2{\cdot}4H_2O$ (J.T. Baker); $PdCl_2$, $PtCl_2$ and $Pd(OAc)_2$ (Aldrich, Co); solvents (Merck). The complexes $[PdCl_2(CH_3CN)_2]$ and $[PtCl_2(CH_3CN)_2]$ was 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 a 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 DG8 5HJ balance, using the Gouy method. NMR spectra were obtained at room temperature on a 300 MHz Bruker-Avance Unity spectrometer. $^1H$ and $^{13}C$ $\{^1H\}$ NMR spectra were obtained using DMSO-$d_6$ and $CDCl_3$ (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 of $Ni^{II}$ salts were synthesized by similar procedures. A solution of one mole equivalent of the corresponding transition metal salt in acetone was added to a solution of one mole equivalent of tioconazole in acetone, with exception of $Ni(OAc)_2{\cdot}4H_2O$ where ethanol was used as solvent. For the compounds $[Ni(tcnz)_6]Cl_2$ **4**

and $[Ni(tcnz)_6]Br_2$ **5** a 1:3 ratio was used. The reaction mixture was heated under reflux with constant stirring during 24 h. $Pd^{II}$ and $Pt^{II}$ 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 $[Ni(tcnz)_3Br_2(H_2O)]$ (1)**

$NiBr_2 \cdot 3H_2O$ (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 $\nu(cm^{-1})$: $\nu_1$ = 8665, $\nu_2$ = 14993 and $\nu_3$ = 24918. FT-IR (ATR, $\nu cm^{-1}$): $\nu$(O-H) 3324, $\nu$(C=N) 1589, $\nu$(C-O-C) 1088, $\nu$(C-S) 736. MS-ESI$^+$ (m/z) 1301 $[C_{48}H_{39}Cl_9BrN_6O_3S_3Ni]^+$. Anal. Found: C, 41.08; H, 2.51; N, 6.10; S, 5.99%. Calc. for $C_{48}H_{41}Cl_9Br_2N_6O_4S_3Ni$: C, 41.19; H, 2.95; N, 6.00; S, 6.87%. $\mu_{eff}$ = 3.40 BM. Yield: (0.19 g, 92%).

**Synthesis of $[Ni(tcnz)_2(NO_3)_2] \cdot H_2O$ (2)**

$Ni(NO_3)_2 \cdot 6H_2O$ (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 $\nu(cm^{-1})$: $\nu_1$ = 8936, $\nu_2$ = 15553 and $\nu_3$ = 25477. FT-IR (ATR, $\nu$ $cm^{-1}$): $\nu$(O-H) 3344, $\nu$(C=N) 1589, $\nu_{as}(NO_3)$ 1468, $\nu_s(NO_3)$ 1306, $\nu$(N=O) 1235, $\nu$(C-O-C) 1087, $\nu$(C-S) 736. MS-ESI$^+$ (m/z) 896 $[C_{32}H_{26}Cl_6N_5O_5S_2Ni]^+$. Anal. Found: C, 39.36; H, 2.90; N, 8.45; S, 6.26%. Calc. for $C_{32}H_{28}Cl_6N_6O_9S_2Ni$: C, 39.37; H, 2.89; N, 8.61; S, 6.57%. $\mu_{eff}$ = 3.50 BM. Yield: (0.11g, 81%).

**Synthesis of $[Ni(tcnz)_2(OAc)_2]_2 \cdot 3H_2O$ (3)**

$Ni(OAc)_2 \cdot 4H_2O$ (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 precipitated. UV–Vis–NIR $\nu(cm^{-1})$: $\nu_1$ = 8936, $\nu_2$ = 15417 and $\nu_3$ = 25409. FT-IR (ATR, $\nu$ $cm^{-1}$): $\nu$(O-H) 3128, $\nu$(C=N) 1586, $\nu_{as}$(COO) 1558, $\nu_s$(COO) 1408, $\nu$(C-O-C) 1084, $\nu$(C-S) 732. MS-ESI$^+$ (m/z) 1456 $[C_{54}H_{47}Cl_9N_6O_9S_3Ni_2]^+$. Anal. Found: C, 42.45; H, 3.01; N, 5.70;

S, 5.35%. Calc. for $C_{36}H_{38}Cl_6N_4O_9S_2Ni$: C, 42.97; H, 3.81; N, 5.57; S, 6.37%. $\mu_{eff}$ = 3.20 BM. Yield: (0.11g, 90%.).

**Synthesis of $[Ni(tcnz)_6]Cl_2$ (4)**

$NiCl_2{\cdot}6H_2O$ (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 $\nu(cm^{-1})$: $\nu_1$ = 10880, $\nu_2$ = 17550 and $\nu_3$ = 24330. FT-IR (ATR, $\nu$ $cm^{-1}$): $\nu$(C=N) 1589, $\nu$(C-O-C) 1078, $\nu$(C-S) 738. Due to its insolubility in common solvents it was not possible to obtain its MS-$ESI^+$ (m/z). Anal. Found: C, 7.70; H, 3.33; N, 6.58; S 7.70%. Calc. for $C_{96}H_{78}Cl_{20}N_{12}O_6S_6Ni$: C, 46.94; H, 3.20; N, 6.84; S, 7.83%. $\mu_{eff}$ = 3.14 BM. Yield: (0.24g, 33%).

**Synthesis of $[Ni(tcnz)_6]Br_2$ (5)**

$NiBr_2{\cdot}3H_2O$ (0.07 g, 0.25 mmol) was added to a solution of tioconazole (0.3 g, 0.75 mmol) in acetone (15mL), the obtained blue solution was let to stand at RT. After a week purple crystals suitable for X-ray diffraction were isolated. UV–Vis–NIR $\nu(cm^{-1})$: $\nu_1$ = 10880, $\nu_2$ = 17550 and $\nu_3$ = 24330. FT-IR (ATR, $\nu$ $cm^{-1}$): $\nu$(C=N) 1589, $\nu$(C-O-C) 1079, $\nu$(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 $C_{96}H_{78}Cl_{18}Br_2N_{12}O_6S_6Ni$: C, 45.31; H, 3.09; N, 7.56; S, 6.60%. $\mu_{eff}$ = 3.01 BM. Yield: (0.25g, 36%).

**Synthesis of $[Pd(tcnz)_2Cl_2]$ (6)**

$[PdCl_2(CH_3CN)_2]$ (0.05 g, 0.19 mmol was added to a solution of tioconazole (0.14g, 0.38 mmol) in acetone (15mL) during 24h at RT a light yellow solid precipitated from the solution. UV–Vis–NIR $\nu(cm^{-1})$: $\nu_1$=25595. FT-IR (ATR, $\nu$ $cm^{-1}$): $\nu$(C=N) 1587, $\nu$(C-O-C) 1084, $\nu$(C-S) 735. $^1$H NMR (DMSO-$d_6$, 300MHz): 8.08 (2H, s, CH, Ha), 7.67 (2H, d, CH, Hf), 7.51 (2H, dd, CH, Hh), 7.43 (2H, s, CH, Hj), 7.41 (2H, s, CH, Hc), 7.17 (2H, s, CH, Hg), 7.11 (2H, s, CH, Hb), 6.89 (2H, d, CH, Hk), 4.95 (2H, dd, CH, He), 4.37-4.26 (8H, m, $CH_2$, Hi,d).$^{13}$C NMR (DMSO-$d_6$, 75MHz 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 $[C_{32}H_{26}Cl_7N_4O_2S_2Pd]^+$. Anal. Found: C, 40.84; H, 2.38; N, 5.88; S 6.73%. Calc. for $C_{32}H_{26}Cl_7N_4O_2S_2Pd$ : C, 40.34; H, 2.75; N, 5.88; S, 6.73%. Yield: (0.12g, 68%).

**Synthesis of $[Pt(tcnz)_2Cl_2]\cdot 2H_2O$ (7)**

$[PtCl_2(CH_3CN)_2]$ (0.05 g, 0.10 mmol) was added to a solution of tioconazole (0.082g, 0.20 mmol) in mixture of acetone and acetonitrile (15 mL) under reflux during 4h at RT a yellow solid precipitated from the solution. UV–Vis–NIR ν(cm$^{-1}$): $\nu_1$=27024. FT-IR (ATR, ν cm$^{-1}$): ν(O-H) 3444, ν(C=N) 1588, ν(C-O-C) 1083, ν(C-S) 732. $^1$H NMR ($CDCl_3$, 300MHz): 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, $CH_2$, Hi), 4.28 (4H, m, $CH_2$, Hd), 2.06 (6H, s, $CH_3$, Hl).$^{13}$C NMR ($CDCl_3$, 75MHz assignments by HSQC): 139.84 (C1), 134.88 (C7), 134.36(C6), 134.16 (C9), 134.65 (C13), 129.77 (C8), 129.64 (C14), 129.46 (C10), 128.40 (C11), 128.26 (C15), 127.02 (C16) , 125.06 (C3), 121.37 (C2), 75.78 (C5), 63.53 (C12), 51.43 (C4). MS-ESI$^+$ (m/z) 970 $[C_{32}H_{26}Cl_6N_4O_2S_2Pt]^+$. Anal. Found: C, 35.72; H, 2.06; N, 5.48; S 5.17%. Calc. for $C_{32}H_{30}Cl_8N_4O_4S_2Pt$ : C, 35.67; H, 2.81; N, 5.20; S, 5.95%. Yield: (0.07g, 61%).

**Synthesis of $[Pd(tcnz)_2(OAc)_2]$ (8)**

$[Pd(OAc)_2]$ (0.026 g, 0.12 mmol) was added to a solution of tioconazole (0.1g, 0.25 mmol) in acetone (15mL) during 24h at RT a beige solid precipitated from the solution. UV–Vis–NIR ν(cm$^{-1}$): $\nu_1$=27683. %. FT-IR (ATR, ν cm$^{-1}$): ν(C=N) 1588, $\nu_{as}$(COO) 1632, $\nu_s$(COO) 1354 ν(C-O-C) 1089, ν(C-S) 733. $^1$H NMR ($CDCl_3$, 300MHz): 7.67 (2H, s, CH, Ha), 7.42 (2H, d, CH, Hf), 7.35-7.30 (4H, m, CH, Hh,j), 7.17 (2H, d, CH, Hc), 6.96 (2H, m, CH, Hg), 6.77 (2H, d, CH, Hk), 6.73 (2H, m, CH, Hb), 4.85 (2H, dd, CH, He), 4.40 (2H, d, $CH_2$, Hi), 4.24 (4H, d, $CH_2$, Hi), 4.09-3.88 (4H, m, $CH_2$, Hd), ), 1.94 (6H, s, $CH_3$, Hl).$^{13}$C $CDCl_3$, 75MHzassignments by HSQC):178.09 (C17), 138.55 (C1), 135.29 (C6), 133.64(C7), 133.42 (C9), 133.35 (C13), 129.79 (C8), 128.73 (C16) ,128.63 (C14), 128.53 (C10), 128.53

(C11), 127.71 (C15), 124.03 (C3), 119.57 (C2), 75.89 (C5), 63.75 (C12), 52.44 (C4), 23.66 (C18). MS-ESI$^+$ (m/z) 940 $[C_{34}H_{29}Cl_6N_4O_4S_2Pd]^+$. Anal. Found: C, 43.35; H, 2.81; N, 5.99; S 5.77%. Calc. for $C_{36}H_{32}Cl_6N_4O_6S_2Pd^{\cdot}$ C, 43.24; H, 3.23; N, 5.60; S, 6.41. Yield: (0.089g, 69%).

## X-ray crystallographic study

The 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.

Table 1. Crystallographic data and refinement parameters of compounds $[Ni(tcnz)_6]Cl_2$ **4** and $[Ni(tcnz)_6]Br_2$ **5**.

| **Compound** | **4** | **5** |
|---|---|---|
| Empirical formula | $C_{96}H_{78}Cl_{20}N_{12}NiO_{12}S_6$ | $C_{96}H_{78}Br_2Cl_{18}N_{12}NiO_6S_6$ |
| 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 | Colourless | 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 (Å$^3$) | 8342.3(7) | 8393(3) |
| Z | 3 | 3 |
| $D_{calc}$ (g/cm$^3$) | 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 % |
| $R_{int}$ | 0.0641 | 0.0729 |
| R (I>2σ(I)) | 0.0906 | 0.0785 |
| $R_w$ (I>2σ(I)) | 0.2764 | 0.2213 |
| S | 1.027 | 1.025 |

$$R\,\mathrm{int} = \sum \left| F_o^2 - \left\langle F_o^2 \right\rangle \right| \Big/ \sum F_o^2 \,,\; R_1 = \sum \left| |F_o| - |F_c| \right| \Big/ \sum |F_o| \,,\; wR_2 = \left[ \sum w\left(F_o^2 - F_c^2\right)^2 \Big/ \sum w\left(F_o^2\right)^2 \right]^{1/2}$$

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 A 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.

### *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% $CO_2$ 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 plating 2 x $10^4$ cells/well in 96-well microplate (Costar®) with 300 lL 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 $Ni^{II}$, $Pd^{II}$, and $Pt^{II}$ complexes. The tested metal complexes (cisplatin and tioconazole control was added to the plates to act as a positive and comparative control) were made up in 5% DMSO and saline to give a 1 mM stock solution by initial dissolution in DMSO followed by dilution with saline. 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 ($IC_{50}$) values determination. Each assay was done in triplicate. $IC_{50}$ 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) metal salts, octahedral compounds **1-5** were obtained, for palladium and platinum(II) compounds **6-8** an arrangement planar square is preferred. The proposed structures in Figure 1 are based on data obtained from the spectroscopic characterization, elemental analyses, molar conductivity and magnetic susceptibility.

## 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 at 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** present 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** broad band at 3128-3444 $cm^{-1}$ corresponds to the water molecules of crystallization. The nitrato compound **2** showed three bands corresponding to the $NO_3$ group, at 1468 $cm^{-1}$ $\nu_{as}(NO_3)$, 1306 $cm^{-1}$ $\nu_s(NO_3)$ and 1235 ν(N=O) $cm^{-1}$, where the $\Delta\nu(\nu_{as}-\nu_s)$ = 162 $cm^{-1}$ indicating a bidentate coordination mode.[37] Compound **3** presents two intense bands at 1558 $cm^{-1}$, $\nu_{as}$(COO), and at 1408 $cm^{-1}$ $\nu_s$(COO), with a $\Delta\nu(\nu_s-\nu_{as})$ = 150 $cm^{-1}$, characteristic of a bridging coordination mode,[37-40] while for compound **8**, $\nu_{as}$(COO) was assigned at 1632 $cm^{-1}$ and $\nu_s$(COO) at

1354 $cm^{-1}$, with a $\Delta\nu(\nu_s-\nu_{as})$ = 278 $cm^{-1}$, characteristic of a monodentate carboxylate bound to the metal ion.

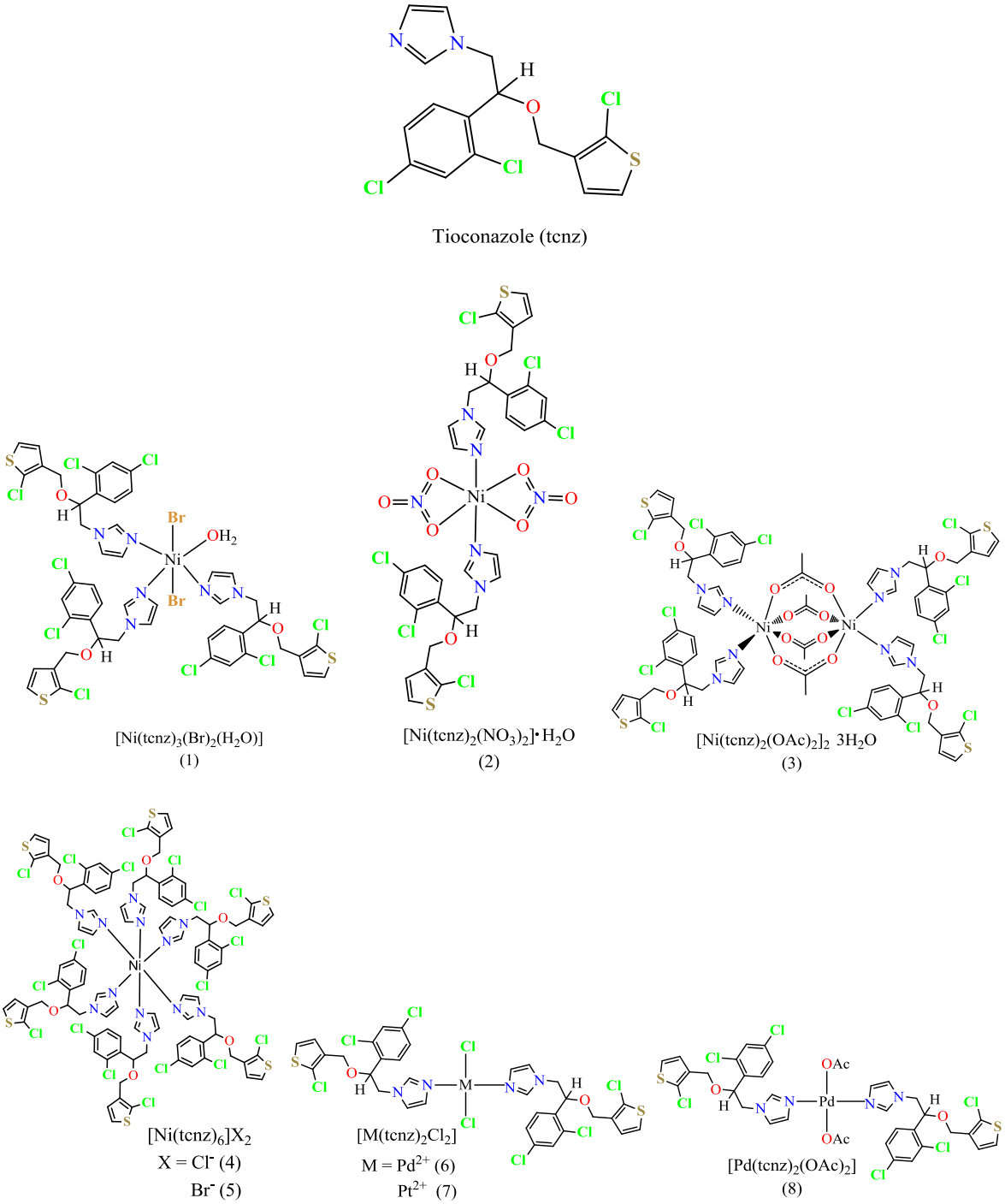


Figure 1. Structures for the tioconazole coordination compounds with $Ni^{II}$, $Pd^{II}$, and $Pt^{II}$.

The electronic spectra (UV-Vis-NIR) for the nickel(II) compounds correspond to an octahedral geometry for the metal atom. For the $[Ni(tcnz)_3Br_2(H_2O)]$ **1**, $[Ni(tcnz)_2(NO_3)_2]\cdot H_2O$ **2** and $[Ni(tcnz)_2(OAc)_2]\cdot 3H_2O$ **3** compounds, the corresponding electronic transitions $\nu_1$ $^3T_2(F)\leftarrow{}^3A_{2g}(F)$ at 8665, 8936 and 8970 $cm^{-1}$, $\nu_2$ $^3T_{1g}(F)\leftarrow{}^3A_{2g}(F)$ at 14993, 15553 and 15417 $cm^{-1}$, and $\nu_3$ $^3T_{1g}(P)\leftarrow{}^4A_{2g}(F)$ at 24918, 25477 and 25409 $cm^{-1}$ were assigned for **1-3** respectively. Compounds **4** and **5,** $[Ni(tcnz)_6]X_2$ (X= Cl, Br), presented similar spectra, with $\nu_1$ $^3T_2(F)\leftarrow{}^3A_{2g}(F)$ at 11060-11000 $cm^{-1}$, $\nu_2$ $^3T_{1g}(F)\leftarrow{}^3A_{2g}(F)$ at 17557-17550 $cm^{-1}$, and $\nu_3$ $^3T_{1g}(P)\leftarrow{}^4A_{2g}(F)$ at 25550-25450 $cm^{-1}$, with a larger 10Dq (*ca.* 11030 $cm^{-1}$) than those of compounds **1-3,** due to the coordination of six tcnz ligands. For compounds $[Pd(tcnz)_2Cl_2]$ **6**, $[Pt(tcnz)_2Cl_2]\cdot 2H_2O$ **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 $^1A_{2g}\leftarrow{}^1A_{1g}$, for these metal ions in a square planar environment, (Table 2, Fig. SI 16).

### $^1H$ and $^{13}C$ $\{^1H\}$ NMR spectroscopy

The $^1H$, $^{13}C$ 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 structures for the diamagnetic coordination compounds **6-8**.

The $^1H$ NMR spectra of the coordination compounds showed a shift for the (C2-H) imidazole proton Ha, between the two nitrogen atoms to 8.08 ppm **6**, 8.24 ppm **7** and 7.67 ppm **8**, 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 $\{^1H\}$ 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.

## Magnetic susceptibility

All the effective magnetic moments ($\mu_{eff}$) for the nickel(II) complexes are in the range 3.20–3.50 BM, these values are consistent within the expected range for the metal center in 2+ oxidation state.[41] The conductivity was measured for all compounds, showing good agreement for the neutral compounds with coordinated halides, nitrate or acetate, compounds **1**-**3** and **6**-**8**, while **4** and **5** presented a 1:2 conductivity, Table 2.

**Table 2**. Spectroscopic and magnetic data of tcnz complexes of $Ni^{II}$, $Pd^{II}$ and $Pt^{II}$.

| | Coordination compound | $\mu_{eff}$ (BM) | $\Lambda_M$[a] | $\nu_1$ | $\nu_2$ | $\nu_3$ |
|---|---|---|---|---|---|---|
| **1** | $[Ni(tcnz)_3Br_2(H_2O)]$ | 3.40 | 33.9 | 8665 | 14993 | 24918 |
| **2** | $[Ni(tcnz)_2(NO_3)_2]\cdot H_2O$ | 3.50 | 13.2 | 8936 | 15553 | 25477 |
| **3** | $[Ni(tcnz)_2(OAc)_2]\cdot 3H_2O$ | 3.20 | 10.4 | 8970 | 15417 | 25409. |
| **4** | $[Ni(tcnz)_6]Cl_2$ | 3.14 | 231.3* | 11060 | 17557 | 25550 |
| **5** | $[Ni(tcnz)_6]Br_2$ | 3.01 | 225.1* | 11000 | 17550 | 25450 |
| **6** | $[Pd(tcnz)_2Cl_2]$ | - | 4.26 | 25595 | - | - |
| **7** | $[Pt(tcnz)_2Cl_2]\cdot 2H_2O$ | - | 1.55 | 27024 | - | - |
| **8** | $[Pd(tcnz)_2(OAc)_2]$ | - | 1.71 | 27683 | - | - |

[a] $\Lambda_M$, molar conductance ($\mu S\ cm^{-1}$) for $1x10^{-3}$ M solutions in acetone (no-electrolyte = 0-99 $\mu S\ cm^{-1}$), and acetonitrile* (electrolyte 2:1 = 220-300 $\mu S\ cm^{-1}$) at 293 K.

## 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 has 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 an undistorted octahedral geometry.

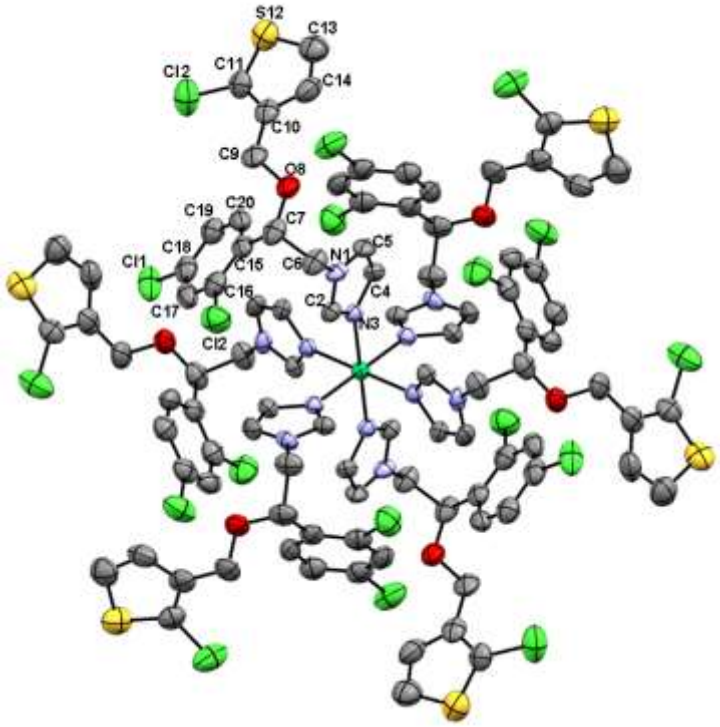


Figure 2. ORTEP diagram of the $[Ni(tcnz)_6]Br_2$ **5** compound. Displacement ellipsoids are drawn at 30% probability. H atoms were omitted for clarity.

Compounds **4** and **5** presented hydrogen bonding intermolecular interactions between the chloro atoms from the thiophen and the methylene hydrogen atoms, C(9)-H(9B)···Cl(2), 2.763(4) Å (fig. 3). On the other hand, the halide atoms stabilized a trifurcated hydrogen bonding with the methylene hydrogen atoms, C(6)-H(9A)···X (X= Cl, Br), the bond lengths and angles are in 3.219(3) Å and 108.7(4)°, respectively (fig. 4).

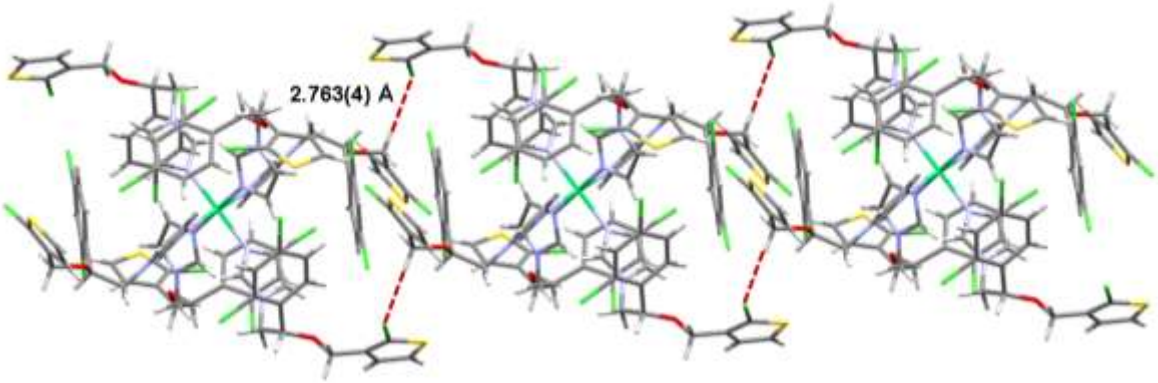


Figure 3. Intermolecular hydrogen bonding C(9)-H(9B)···Cl(2) in compound **5**, giving place to a 3D supramolecular arrangement.

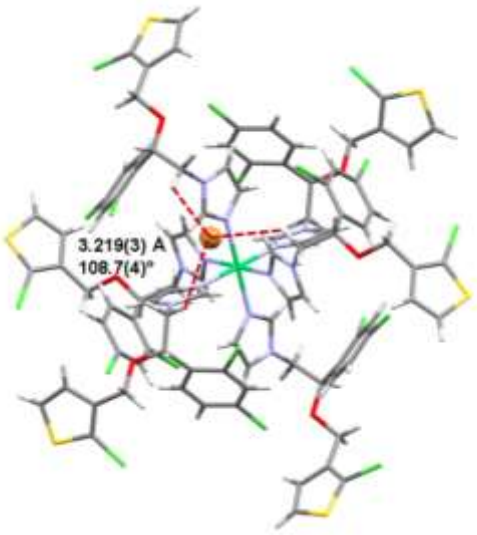


Figure 4. Trifurcated hydrogen bridging C(6)-H(9A)···Br in compound **5**.

The tioconazole can be accommodated as a propeller, occupying the six coordination sites of an octahedral nickel(II) atom, giving place to a 3D suramolecular arrangement, stabilized through parallel slipped π···π stacking interactions between the benzene rings, with a distance C(17)···π of 3.482 Å, from the ring centroid to the C(17) aromatic carbon, as shown in Fig. 5, [42].

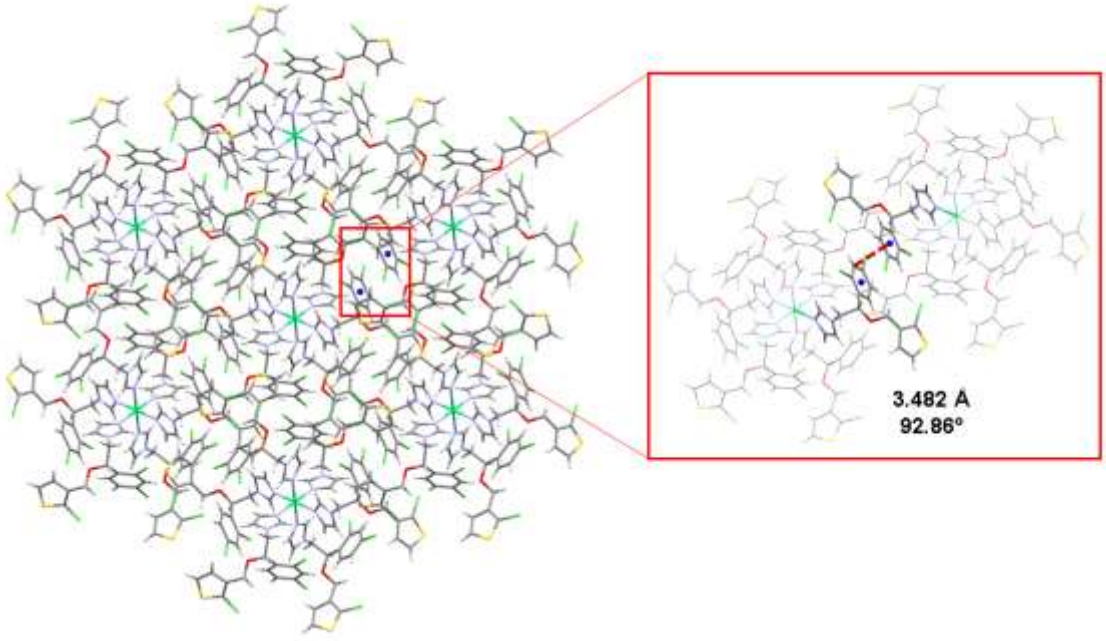


Figure 5. Supramolecular arrangement stabilized though π···π stacking in compound **5**.

**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 complexes of imidazole and benzimidazole derivatives it was found that the copper(II) tetrahedral compounds, with coordinated halides, were the most active.[43, 44] With the analogous ligand clotrimazole, the tetrahedral nickel(II) coordination compounds presented moderate cytotoxic activity *in vitro* and the octahedral complexes were not active.[45, 46] Interestingly, the tioconazole octahedral copper(II) compound showed significant cytotoxic activity.[29]

In the present work, the octahedral nickel(II) compounds showed moderate cytotoxic activity, where the $[Ni(tcnz)_6]Cl_2$ **4** was active towards three different cell lines, HCT-15, HeLa and PC-3; while the $[Ni(tcnz)_2(NO_3)_2]\cdot H_2O$ **2** compound, presented the best activity in HeLa and MCF-7 and the $[Ni(tcnz)_3Br_2(H_2O)]$ **1** compound in PC-3, followed by **4**. The palladium(II) and platinum(II) compounds did not presented any significant activity, Table 2.

**Table 3**. Cell-growth inhibitory assay results. $IC_{50}$ value (μg/mL) for $Ni^{II}$, $Pd^{II}$ and $Pt^{II}$. tioconazole coordination compounds (**1-3** and **6-8**).

| | | Human carcinoma cell line | | | |
|---|---|---|---|---|---|
| | Coordination compound | HCT-15 | HeLa | MCF-7 | PC-3 |
| **1** | $[Ni(tcnz)_3Br_2(H_2O)]$ | 46.35 | 12.15 | 14.26 | 11.43 |
| **2** | $[Ni(tcnz)_2(NO_3)_2]\cdot H_2O$ | 18.50 | 7.39 | 12.53 | 16.74 |
| **3** | $[Ni(tcnz)_2(OAc)_2]\cdot 3H_2O$ | 323.19 | 32.70 | NA | 31.15 |
| **4** | $[Ni(tcnz)_6]Cl_2$ | 14.02 | 12.33 | 101.12 | 11.67 |
| **5** | $[Ni(tcnz)_6]Br_2$ | 303.27 | 10.12 | 15.36 | 16.15 |
| **6** | $[Pd(tcnz)_2Cl_2]$ | NA | 15.67 | 51.27 | NA |
| **7** | $[Pt(tcnz)_2Cl_2]\cdot 2H_2O$ | ND | ND | ND | ND |
| **8** | $[Pd(tcnz)_2(OAc)_2]$ | NA | NA | NA | NA |
| | Cisplatin | 8.25 | 5.55 | 1.3 | 3.83 |

ND = not determined; NA = not active

## Conclusions

Coordination compounds of tioconazole with $Ni^{II}$, $Pd^{II}$, and $Pt^{II}$ were synthesized and fully characterized. The coordination compounds stabilized octahedral (**1-5)** and square planar (**6-8)** geometries, depending on the metal ion. Despite being voluminous, in excess of ligand, the tioconazole ligand can be accommodated in a propeller arrangement, occupying the six coordination sites of an octahedral 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 cytotoxic activity (HeLa), the $IC_{50}$ increased upon coordination to the metal ion when compared to the inactive free ligand, and is related to the nature and the octahedral geometry of the metal ion, as the platinum and palladium complexes did not presented any significant activity. Compound **4** merits further studies to investigate its anticancer activity.

## Supplementary information

CCDC No. 1581442 for $[Ni(tcnz)_6]Cl_2$ and 1581443 for $[Ni(tcnz)_6]Cl_2$ 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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