

Inorganica Chimica Acta 275-276 (1998) 528-540

Toward a novel metal based chemotherapy against tropical diseases 4. Synthesis and characterization of new metal-clotrimazole complexes and evaluation of their activity against *Trypanosoma cruzi*¹

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Received 19 June 1997; revised 14 August 1997; accepted 10 December 1997

Abstract

The syntheses and characterization of metal imidazole complexes showing activity against Trypanosoma cruzi, the causative agent of Chagas disease, are presented. $RuCl_2(CTZ)_2$ (2) and $RuCl_2(BTZ)_2$ (4) were prepared by reaction of $RuCl_2(NCCH_3)_4$ (1) with the appropriate ligand (CTZ, clotrimazole = 1-[(2-chlorophenyl)diphenylmethyl]-1H-imidazole; BTZ = 1-[(2-bromophenyl)diphenylmethyl]-1H-imidazole). $[Ru(bipy)(CTZ)_2](PF_0)_2$ (3) (bipy = 2.2'-bipyridy!) was obtained by reaction of 2 with bipy and NH₄PF₀ in MeCN. Reaction of [RhCl(COD)], with CTZ yielded RhCl(COD)(CTZ) (5) (COD=1.5-cyclooctadiene), while AuCl(CTZ (6). K₂[PiCl₄(CTZ)₂] (7) and [Cu(CTZ)₂]PF_n (8) were obtained by interaction of CTZ with AuCl₄•HCl, K₂PiCl₄ and [Cu(CH₄CN)₄]PF_n respectively. All the new complexes were characterized by NMR and other appropriate techniques. X-ray diffraction studies of 4.3H₂O, 5 and 6 were also carried out. The structure of 4.3H₂O consists of a distorted tetrahedral arrangement of two N atoms from the BTZ ligands and two Cl atoms around the Ru(11) ion; 4.3H₂O crystallizes in the orthorhombic space group Pnma (No. 62) with a = 12.818(5), b = 29.115(5), c = 12.040(5) Å, V = 4493.2(8) Å⁴ and Z = 4. Complex 5 displayed a square planar structure typical for Rh(1) bound to N from CTZ, Cl, and the two C=C bonds of COD; 5 crystallized in the triclinic space group (P(-1) (No. 2) with a = 12.407(3), b = 12.876(4), b = 12.876(c = 10.069(3) Å, $\alpha = 111.59(2)^\circ$, $\beta = 107.80(2)^\circ$, $\gamma = 103.28(2)^\circ$, V = 1313.4(8) Å³ and Z = 2. Complex 6 also displayed a square arrangement of N from CTZ, plus three Cl atoms around the Au(III) ion; 6 crystallized in the monoclinic space group $P2_1/n$ (No. 14) with a = 9.507(1), b = 18.280(4), c = 12.877(1) Å, $\beta = 100.59(1)^\circ$, V = 2199.7(5) Å' and Z = 4. All the new compounds were found to be active against in vitro cultures of Trypanosoma cruzi, following the trend 3=7<8<CTZ < 6<5 < 2. More detailed testing and preliminary mechanistic studies were carried out on the most active complex (2), which allowed the proposition of a simple model of action of this compound. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Chemotherapy: Tropical diseases: Troponosoma crnei: Clotrimazole complexes; Ruthenium complexes; Rhodium complexes; Gold complexes; Platinum complexes; Copper complexes

1. Introduction

Chagas disease is a parasitosis caused by *Trypanosoma* (Schizotrypanum) cruzi, a haemoflagellate protozoon transmitted to man by a cone-nosed bug known as Reduviid or Triatomid; this illness afflicts an estimated 16–18 million people in Latin America, causing approximately 50 000 deaths and 850 000 new human infections per year, and thus ranks as the third largest disease burden caused by a parasite worldwide, after malaria and schistosomiasis [1]. Particularly distressing is the fact that, in contrast to other parasitic diseases and despite recent advances in the knowledge of the biochemistry of the parasite [2], no treatment is available for the long-term prevalent form of this illness [3]; some nitrofurans (e.g. nifurtimox, Bayer, recently discontinued) and nitroimidazoles (e.g. benznidazole, Roche) are of variable

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 Dedicated to Professor Ivano Bertini.

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efficacy in short-term cases, and cause serious side effects (anorexia, vomiting, peripheral polyneuropathy, and allergic dermopathy), which limits the use of these drugs considerably [3,4].

Our strategy toward the development of new agents against *T. cruzi* finds a starting point in two separate types of observations:

(i) Azole derivatives such as clotrimazole, ketoconazole and itraconazole have been developed as chemotherapeutic agents for the treatment of fungal diseases on the basis of their properties as sterol biosynthesis inhibitors (SBIs) [5]. Such SBIs have also been found to block the proliferation of parasites such as Leishmania tropica, Leishmania mexicana and T. cruzi, by inhibiting the cytochrome P-450 dependent C-14 α -demethylation of lanosterol to ergosterol [6]; however, none of the commonly available SBIs are powerful enough to eradicate the parasite from patients or experimental animals [7]. A recent report by Urbina et al. [8] described the exceptional efficacy of D0870 (the R(+) enantiomer 2-(2,4-difluorophenyl)-1-(3-[(Z)-4-(2,2,3,3-tetrafluoroof propoxy)styryl]-1,2,4-triazol-1-yl)-propan-2-ol (ICI 195, 739) in curing short- and long-term Chagas disease in experimental animals.

(ii) The use of metal complexes as chemotherapeutic agents is well established, particularly in the cancer field for cisplatin and related compounds; the most important mechanistic models of action of these Pt-containing drugs involve binding of the metal center to DNA, generally at the N-7 position of a guanine base [9]. Due to a similarity between the metabolism of tumor cells and that of pathogenic trypanosomes, caused by an increased rate of respiration which results from an inefficient or nonfunctional mitochondrial system [10], as well as the lack of catalases and peroxidases in both systems [11], concepts analogous to those used in the development of cisplatin and other anti tumor agents might be applicable to the case of Chagas disease. In fact, some anti tumor and related metal complexes have been reported to be moderately active against African trypanosomes [10–12], but strikingly little information is available concerning activity against American trypanosomiasis [12].

In this paper we present a novel approach toward the development of a chemotherapy against Chagas disease, consisting in the modification of clotrimazole (CTZ = 1-[(2-chlorophenyl)-diphenylmethyl]-1H-imidazole, an azole-type anti fungal agent that is known to have some anti*T. cruzi*[6b]) by coordination to transition metals, in an effort to combine the SBI properties of the parental drug with the DNA binding (or other related) capacity of the central metal atom. In this context the synthesis and characterization of a number of new CTZ and related complexes containing Ru, Rh, Pt and Au are reported, together with the X-ray structures of three of these compounds. These results have been complemented with an evaluation of the biological activity of the new metal complexes against*T. cruzi*, and some initial studies on the possible mechanism of action of

these novel compounds. A preliminary account of part of this work has appeared [13].

2. Experimental

2.1. General procedures

Solvents of analytical grade were distilled from appropriate drying agents immediately prior to use. Other commercially available reagents were purified by standard procedures; CTZ, BTZ, bipy and 1,5-cyclooctadiene were used without further purification. Elemental analyses were performed by Mikroanalytisches Labor Pascher and Analytische Laboratorien Professor Dr H. Malissa and G. Reuter, GmbH, Germany. IR spectra (in KBr disks) were recorded on a Nicolet 5DCX FT spectrometer. NMR spectra were obtained on a Bruker AM-300 spectrometer; ¹H NMR shifts were recorded relative to residual ¹H resonances in the deuterated solvent and ³¹P{¹H} chemical shifts are relative to external 85% H₃PO₄ with downfield values reported as positive.

2.2. RuCl₂(MeCN)₄(1)

RuCl₃· $_{3}H_{2}O$ (5.0 g, 19.1 mmol) was dissolved in freshly distilled acetonitrile (150 ml); zinc powder previously activated by washing with 2% HCl (2.61 g, 39.9 mmol) was added to the mixture which was stirred under reflux in a nitrogen atmosphere for 2 h. After filtering the yellow-orange solution, the volume of the solvent was reduced until precipitation began and the mixture was allowed to stand overnight at -10° C, after which the yellow solid obtained was filtered, washed with diethyl ether and dried in vacuo (yield, 6.1 g, 95%). If necessary, the product may be recrystallized from a methanol/acetonitrile mixture. In our experience this synthetic method is much more convenient than the previously published procedures [14].

2.3. RuCl₂(CTZ)₂ (2)

A 2:1 mixture of CTZ (3.28 g, 9.52 mmol) and RuCl₂(CNMe)₄ (1.60 g, 4.76 mmol) was dissolved in methanol (100 ml) and allowed to react for 1 h at room temperature, after which a white solid precipitated, which was filtered off, washed with methanol and dried in vacuo (yield, 5.0 g; 61%; m.p. 260°C). Anal. Calc. for C₃₄H₃₄N₄Cl₄Ru: C, 61.3; H, 4.0; N, 6.5; Cl, 16.5; Ru, 11.7. Found: C, 61.5; H, 4.1; N, 6.7; Cl, 16.6; Ru, 11.2%. MW Calc. 861. Found 808 (cryoscopically in CHCl₃). IR (cm⁻¹) v(C = C) 1496, v(C=N) 1446. ¹H NMR (CDCl₃) CTZ: 7.87 (s, H_2), 7.15 (s, H_4) , 6.76 (s, H_5) , 6.91 (dd, ${}^{3}J = 8.36$ Hz, ${}^{4}J = 1.07$ Hz, H_{6}), 7.30 (m, H_{7} , H_{11} , H_{12} , H_{13} , H_{16} , H_{17} , H_{18}), 7.25 (m, H_8), 7.37 (dd, H_9), 7.06 (m, H_{10} , H_{14} , H_{15} , H_{19}), 7.51 (m, H_7, H_9). ¹³C{¹H} NMR (CDCl₃) CTZ: 139.3 (C₂), 125.6 $(C_4), 122.3 (C_5), 130.7 (C_6), 129.4 (C_7), 126.3 (C_8), 131.5$ (C_9) , 129.8 $(C_{10}, C_{14}, C_{15}, C_{19})$, 127.3 $(C_{11}, C_{12}, C_{13}, C_{16})$ C_{17}, C_{18} , 140.1 (C_{20}), 135.5 (C_{21}, C_{22}), 139.6 (C_{23}), 75.5 (C_{24}).

2.4. [Ru(bipy)(CTZ)2](PF6)2(3)

Complex 2 (0.56 g, 0.45 mmol) in acetonitrile (30 ml) was refluxed until complete dissolution was observed, then NH₄PF₆ (0.15 g, 0.92 mmol) was added and the mixture was stirred under reflux under nitrogen. After 1 h, the precipitated NH₄Cl was filtered off and washed with acetonitrile; bipy (0.07 g, 0.45 mmol) was added to the solution and allowed to react for 1 h at room temperature. The volume of the solvent was then reduced to about 50% under a nitrogen stream and the mixture was allowed to stand overnight at room temperature, after which the white micro crystals obtained were filtered off, washed with diethyl ether and dried in vacuo (yield 0.4 5. 71%, m.p. 230°C). Anal. Calc. for 3.2MeCN, C38H48N8F12P2Ru: C, 52.80; H, 3.64; N, 8.49. Found: C, 52.55; H, 3.96; N, 8.48%. IR $(cm^{-1}) v(C=C)$ 1600, $\nu(C_{m}N)$ 1493, $\nu(PF_6)$ 840. ¹H NMR ((CD₃)₂CO) CTZ: 8.03 (br, H_2), 7.28 (br, H_4 , H_5), 7.05 (dd, ${}^3J = 8.36$ Hz, $^{4}J = 1.07$ Hz, H_{6}), 7.51 (m, H_{7} , H_{9}), 7.41 (m, H_{9} , H_{11} , H_{12} , H_{13} , H_{16} , H_{17} , H_{18}), 7.15 (m, H_{10} , H_{14} , H_{15} , H_{19}), MeCN 2.22 (s, Me), bipy: 8.79 (d, $J = 8.1 \text{ Hz}, H_{2}$), 7.71 (d, J = 6.05Hz, $H_{3'}$), 8.35 (t, J = 8.0 Hz, $H_{4'}$), 8.22 (d, J = 4.78 Hz, $H_{5'}$). $^{13}C{^{1}H}$ NMR CTZ: 140 (C₂), 127.1 (C₄), 126.9 (C₅), 132.6 (C_6) , 129.4 (C_7) , 129.3 (C_8) , 133.2 (C_9) , 131.8 $(C_{10}), 129.2 \ (C_{11}, C_{17}, C_{18}), 128.8 \ (C_{12}), 128.7 \ (C_{13}),$ $131.7(C_{14}), 130.6(C_{15}, C_{19}), 127.7(C_{16}), 144(C_{20}), 135.9$ (C_{21}) , 134.3 (C_{22}) , 139.8 (C_{23}) , 77.3 (C_{24}) ; CNMe: 117 (CN), 1.0 (Mc); bipy: 149 ($C_{2'}$), 123.9 ($C_{3'}$), 142.7 ($C_{4'}$), 124.4 ($C_{5'}$), 150.2 ($C_{5'}$). ³⁴P{¹H} NMR: -141.3 (hept. PF_6^{-}).

2.5. RuCl₂(BTZ)₂ (4)

A 2:1 mixture of BTZ (1.0 g, 2.57 mmol) and RuCl₂(CNMe)₄ (0.43 g, 1.28 mmol) was dissolved in methanol (75 ml) and allowed to react for 1 h at room temperature after which a white solid precipitated, which was filtered off, washed with methanol, and dried in vacuo (yield 1.6 g, 65%). Crystals suitable for X-ray analysis were obtained by recrystallization from a CH₂Cl₂/methanol mixture. *Anal.* Calc. for C₄₄H₃₄N₄Cl₂Br₂Ru · 3H₂O: C, 55.60; H, 3.61; N, 5.89; Ru, 10.63. Found: C, 55.37; H, 3.80; N, 5.86; Ru, 10 40%.

2.6. RhCl(COD)(CTZ) (5)

To a solution of [RhCl(COD)]₂ (0.10 g, 0.20 mmol) in ethanol (30 ml) was added CTZ (0.14 g, 0.4 mmol), and the mixture was stirred under nitrogen at room temperature. After 1 h, the yellow precipitate was filtered off, washed with diethyl ether and dried under vacuum (yield, 0.19 g, 80%; m.p. 145°C). Anal. Calc. for RhC₃₀H₂₉N₂Cl₂: C, 60.87; H, 4.90; N, 4.73. Found: C, 60.47; H, 4.82; N, 4.73%. FAB MS M⁺ = 590, (M⁺-Cl) = 555. IR ν (C-H st)_{arom} 3120 cm⁻¹, v(C=C) 1587 cm⁻¹, v(C=N) 1490 cm⁻¹. ¹H NMR ((CD₃)₂CO) CTZ: 7.90 (br, H₂), 6.95 (br, H₄), 6.86 (br, H₅), 6.89 (br, H₆), 7.53 (m, H₇, H₉), 7.46 (m, H₈, H₁₁, H₁₂, H₁₃, H₁₆, H₁₇, H₁₈), 7.03 (m, H₁₀, H₁₄, H₁₅, H₁₉). COD: 4.12 (br, CH), 2.32 (m, CH₂), 1.73 (m, CH₂). ¹³C{¹H} NMR CTZ: 139.5 (C₂), 126.5 (C₄), 121.9 (C₅), 130.4 (C₆), 130.7 (C₇), 127.6 (C₈), 132.1 (C₉), 129.3 (C₁₀, C₁₄, C₁₅, C₁₉), 128.2 (C₁₁, C₁₂, C₁₃, C₁₆), 128.3 (C₁₇, C₁₈), 140.3 (C₂₀), 134.3 (C₂₁, C₂₂), 138.9 (C₂₃), 75.3 (C₂₄), COD: 78.4 (CH), 30.2 (CH₂).

2.7. AuCl₃(CTZ) (6)

AuCl₃·HCl·3H₂O (2.0 g, 5.0 mmol) was dissolved in methanol (5 ml), and a mixture of CTZ (1.72 g, 5.0 mmol) and triethylamine (0.5 g, 5.0 mmol) in methanol (15 ml) was added. After 3 h, the gold-yellow precipitate was filtered off and washed with methanol. The complex was redissolved in CH₂Cl₂ and washed with water; the organic phase was dried over drierite and the solvent was eliminated under vacuum. The solid thus obtained was recrystallized from toluene (yield, 1.9 g, 60%). Anal. Calc. for AuC₂₂H₁₇N₂Cl₄: C, 40.77; H, 2.64; N, 4.32; Cl, 21.87; Au, 30.38. Found: C, 41.30; H, 2.70; N, 4.30; Cl, 21.10; Au, 30.20% IR v(C-H)_{ar} 3173 cm⁻¹, v(C=C) 1512 cm⁻¹, v(C=N) 1447 cm⁻¹. ¹H NMR CTZ: 8.36 (s, H_2), 6.94 (s, H_4), 6.92 (br, H_5 , H_6), 7.45 (m, H_7), 7.41 (m, H_8 , H_{11} , H_{12} , H_{13} , H_{16} , H_{17} , H_{18}), 7.60 (br, H_9), 7.05 (m, H_{10} , H_{14} , H_{15} , H_{19}). ¹³C{¹H} NMR: $139.2(C_2), 126.6(C_4), 122.8(C_5), 131.6(C_6), 131.7(C_7),$ $128.2 (C_8), 133.4 (C_9), 130.4 (C_{10}, C_{14}, C_{15}, C_{19}), 129.4$ $(C_{11}, C_{12}, C_{13}, C_{16}), 129.3 (C_{17}, C_{16}), 139.9 (C_{20}), 136.1$ $(C_{21}, C_{22}), 138.6 (C_{23}), 78.9 (C_{24}).$

2.8. K2[PICl4(CTZ)2](7)

A 2:1 mixture of CTZ (0.33 g, 0.96 mmol) and K₂[PtCl₄] (0.20 g, 0.48 mmol) was dissolved in methanol (50 ml) and allowed to react for 24 h at room temperature; the mixture was then evaporated to dryness and the yellow solid was washed with methanol, water, methanol and diethyl ether, and dried under vacuum (yield 0.37 g, 70%, m.p. 220°C). Anal. Calc. for C44H34N4Cl6K2Pt: C, 47.8; H, 3.1; N, 5.15. Found: C, 47.6; H, 3.3; N, 5.3%. IR (cm⁻¹) v(C=C) 1601, v(C=N) 1496. ¹H NMR (CDCl₃) CTZ: 7.99 (s, H_2), 7.02 $(s, H_4), 6.87 (s, H_5), 6.99 (dd, {}^3J = 8.36 Hz, {}^4J = 1.07 Hz,$ H_6), 7.40 (m, H_7 , H_{11} , H_{12} , H_{13} , H_{16} , H_{17} , H_{18}), 7.25 (m, H_8 , 7.45 (dd, H_9), 7.17 (m, H_{10} , H_{14} , H_{15} , H_{19}). ¹³C{¹H} NMR CTZ: 139.3 (C_2), 125.6 (C_4), 122.3 (C_5), 130.7 (C_6), 129.4 (C_7), 126.3 (C_8), 131.5 (C_9), 129.8 (C_{10} , C_{14} , C_{15} , $(C_{19}), 127.3 (C_{11}, C_{12}, C_{13}, C_{16}, C_{17}, C_{18}), 140.1 (C_{20}), 135.5$ $(C_{21}, C_{22}), 139.6 (C_{23}), 75.5 (C_{24}).$

2.9. [Cu(CTZ)₂]PF₆ (8)

A 2:1 mixture of CTZ (0.37 g, 1.07 mmol) and $[Cu(CNMe)_4]PF_6$ (0.20 g, 0.54 mmol) was dissolved in

dichloromethane (50 ml) and allowed to react for 24 h at room temperature. The volume of the solvent was reduced to about 50% under a nitrogen stream, and diethyl ether was added until the solution became turbid; on cooling to -5° C for 3 h, the white product precipitated; it was filtered off and washed with diethyl ether and dried under vacuum (yield 0.38 g, 75%, m.p. 209°C). Anal. Calc. for 8-MeCN, C46H37N5Cl2PF6Cu: C, 58.8; H, 3.9; N, 7.4. Found: C, 58.1; H, 3.7; N, 6.8%. ¹H NMR (CDCl₃) CTZ: 7.86 (s, H₂), 7.15 $(s, H_4), 6.76 (s, H_5), 6.91 (dd, {}^{3}J = 8.36 Hz, {}^{4}J = 1.07 Hz,$ H_6), 7.30 (m, H_7 , H_{11} , H_{12} , H_{13} , H_{16} , H_{17} , H_{18}), 7.25 (m, H_8), 7.37 (dd, H_9), 7.06 (m, H_{10} , H_{14} , H_{15} , H_{19}), 7.51 (m, H_7, H_9). ¹³C{¹H} NMR CTZ: 139.3 (C_2), 125.6 (C_4), 122.3 $(C_5), 130.7 (C_6), 129.4 (C_7), 126.3 (C_8), 131.5 (C_9), 129.8$ $(C_{10}, C_{14}, C_{15}, C_{19}), 127.3 (C_{11}, C_{12}, C_{13}, C_{16}, C_{17}, C_{18}),$ 140.1 (C_{20}), 135.5 (C_{21} , C_{22}), 139.6 (C_{23}), 75.5 (C_{24}).

2.10. X-ray diffraction studies

2.10.1. General

The intensities of crystals of $4\cdot 3H_2O$, 5 and 6 were collected at room temperature (20°C) on Enraf-Nonius CAD-4 (CNR, Florence), Rigaku AFC-7S (IVIC, Caracas) and Philips PW 1100 (CNR, Florence) diffractometers, respectively. As a general procedure standard reflections were measured for orientation and intensity control. No decay of the specimens was noticed. Intensity data were corrected for Lorentz-polarization effects. Atomic scattering factors were those reported by Cromer and Waber [15] with anomalous dispersion correction [16]. Empirical absorption corrections for $4\cdot 3H_2O$ and 6 were based on ΔF refinement [17a], while for 5 it was based on Ψ scan measurements [17b]. The computational work was carried out on Digital Dec 5000/ 200 (CNR) (for $4\cdot 3H_3O$ and 6) or Silicon Graphics Indigo (1VIC) (for 5) workstations.

Crystallographic details are reported in Table 1. Final atomic coordinates with equivalent isotropic thermal parameters are reported in Tables 2-4.

2.10.2. Complex 4 · 3H₂O

The structure was solved by the heavy atom technique using the program SHELX76 [18]; all of the non-hydrogen atoms were found through a series of F_0 Fourier maps. Only two of the phenyl rings were treated as rigid bodies of D_{6h} symmetry (C-C = 1.39 Å) Hydrogen atoms were introduced in calculated positions. Refinement was carried out by fullmatrix least square calculations based on F_0 , initially with isotropic thermal parameters, then with anisotropic thermal parameters only for Ru, Br and Cl atoms because of the limited number of reflections available. In the final stage of the refinement three molecules of H₂O were found. Most likely the latter have freedom to move, which accounts for their high thermal parameters. The final ΔF map showed a difference peak of 0.90 e Å⁻³. Even though the quality of this structure is crystallographically poor, it is consistent and appropriate as a means of characterizing this compound, and thus its chemical significance appears to be reliable.

2.10.3. Complex 5

The structure was solved by the Patterson method and conventional Fourier techniques using teXsan [19] and refined by full-matrix least squares (SHELXL-93) [20] based on F^2 . After inclusion of anisotropic displacement parameters for all non-hydrogen atoms, a residual electron density has been interpreted as a chlorine atom of the chlorophenyl group in a second orientation of the substituent $-C(Ph)_2(Cl-Ph)$ around the N(2)-C(4) bond. The carbon atoms of this second chlorophenyl group were also found and included in the refinement. Distances and bond angles (not torsion angle) of the phenyl ring were restrained to make them equivalent (with e.s.d. of 0.01 Å) to the C(5)-C(10)phenyl ring, while the occupancy of the chlorine atom was retined to a final value of 0.788(4) for the one labeled 'a'. Atoms involved in the disorder were isotropically refined, except Cl(2a) (anisotropically refined), with the assumption of similar isotropic displacement parameters for spatially adjacent atoms (<0.8 Å). All H atoms, except those involved in disorder, were located and included in the refinement, riding on carbon atoms with common isotropic displacement parameters.

2.10.4. Complex 6

The structure was solved by using the heavy atom technique (SHELX76 [18]) and all of the non-hydrogen atoms were found through a series of F_0 Fourier maps. Hydrogen atoms were introduced at the calculated positions. Refinement was carried out by full-matrix least square calculations based on F_0 , initially with isotropic thermal parameters. In the last L.S. cycles, anisotropic parameters were used for Au and Cl atoms. A ΔF map at the end of the refinement showed two peaks of about 1.2 e Å⁻³ at very short distances from the heavy atoms; these ripples could in no way be removed.

2.11. Biological tests

2.11.1. Tests against epimastigotes of T. cruzi

An EP stock of the epimastigote form of T. (S.) cruzi (cultured as previously described) [6d] was used throughout this study. The epimastigotes were cultured in liver infusiontryptose medium supplemented with 10% calf serum at 28°C with strong or rring (120 rpm); the cultures were initiated with a cell delight of $2 \times 10^{\circ}$ epimastigotes per ml; CTZ and compounds 2–8 were added as DMSO solutions (10^{-5} M) when the cultures reached a cellular density of 10^{7} epimastigotes per ml. Parasite proliferation was followed daily by the use of an electronic particle counter (model ZBI; Coulter Electronics, Inc., Hialeah, FL) and by direct counting with a hemacytometer.

2.11.2. Tests against amastigotes of T. cruzi

Amastigotes were cultured in Vero cells maintained in MEM medium supplemented with 2% fetal calf serum in a humidified 95% air-5% CO2 atmosphere at 37°C, as previously described [6e]. Vero cells were infected with a 20:1 ratio of tissue culture derived trypomastigotes per cell for 2 h and then washed three times with phosphate-buffered saline solution to remove the non-adherent parasites. Fresh medium, with and without the tested drug, was added and the cells were incubated in an appropriate atmosphere for 120 h. The medium was changed every 48 h. At the end of the experiment the cells were fixed, stained and microscopically examined under oil immersion. Three parameters were examined: % of infected cells; number of amastigotes per infected cell, and the number of Vero cells per field of the microscope.

2.11.3. Analysis of the sterol composition of the parasites

For the study of the effect of the drug on the sterol composition of the epimastigote form of T. cruzi the total lipid contents of control and drug-treated cells were extracted and fractionated as described previously [6f,g]. The neutral lipid fraction was analyzed directly or saponified in 90% methanolic sodium hydroxide (0.3 M) for 90 min under reflux. This fraction was analyzed by GLC (isothermal separation at 273°C in a 4 m glass column packed with 3% OV-1 on CHROMOSORB 100-200 mesh, nitrogen as carrier gas at 24 ml min⁻⁺ and a flame ionization detector in a Varian 3700 gas chromatograph).

Table I

2.11.4. Analysis of the interaction of CTZ and complex 2 with DNA

Phosphate-buffered saline (PBS, 50 mM Na₂HPO₄, 140 mM NaCl, pH 7.4) solutions of complex 2 (4 ml, 10^{-5} M) and calf thymus DNA (Sigma, 4 ml, 1.52 mM; concentration determined by OD measurements in a Milton Roy spectrophotometer) were mixed and left standing for 2 h after which they were divided in two equal portions, one of which was irradiated for 2 h at 300 ± 10 nm in a photochemical reactor equipped with a Rayonet with 8 16 W fluorescent lamps. Samples of the mixtures (10 µl) were injected through a Rheodyne 7125 injection port, and analyzed by HPLC using a Waters-Millipore Deltapre 4000 instrument equipped with an optical detector (200-360 nm) and an ODS column $(4.6 \times 250 \text{ mm}, 5 \mu\text{m}, \text{Regis}, \text{Morton Grove, IL})$ according to Gasparro and co-workers [21]. The optimized solvent system used was MeOH/H₂O programmed as: 10% MeOH, 10 min/50% MeOH, 15 min/75% MeOH, 10 min/100% MeOH, 5 min. Detection was done at 220 and 260 nm. The control solutions of irradiated and non-irradiated individual components (DNA, CTZ, 2), as well as DNA/CTZ mixtures were also analyzed using the same procedure.

3. Results and discussion

3.1. Synthesis and characterization of new complexes

Clotrimazole (CTZ) reacts readily with appropriate metal salts or complexes under mild conditions to produce a range

Summary of crystal data			
Compound	4·3H2O	5	6
Formula	C44H40Cl-N4Br3O3Ru	C wH wCl N Rh	C ₂₂ H ₁₂ Cl ₄ N ₂ Au
M (g mol ⁼¹)	1004.6.	591.36	648.17
Crystal dimension (mm)	0.05×0.10×0.35	$0.34 \times 0.28 \times 0.24$	0.40×0.25×0.20
Crystal system	orthorhombic	triclinic	monoclinic
Space group	Pnma (No. 62)	P(-1) (No. 2)	$P2_1/n$ (No. 14)
a (Å)	12.818(5)	12.407(3)	9.507(1)
あ(Å)	29.115(5)	12.876(4)	18.280(4)
e (Å)	12.040(5)	10.069(3)	12.877(1)
a (°)		111.59(2)	
₿(°)		107.80(2)	100.59(1)
γ(°)		103.28(2)	
V(Å')	4493.2(8)	1313.4(6)	2199.7(5)
2	4	2	4
Acate (g em 11)	1.48	1,495	1.96
µ(Mo, Cu Ka) (cm -')	22.6.1	8.75	174.28
Radiation	Mo Ka, a = 0.71069 Á	Mo Ka, λ = 0.71069 Å	Cu Kα, λ ∞ 15418 Å
20 Range (°)	5-46	4-40	5-120
Total no. of data	3533	2300	3239
Unique data, $l > 3\sigma(l)$	1012	2146	2688
R	0.080	0.034	0.039
R. °	0.086	0.1013 *	0.043

* For 4 and 6: $R_{w} = [\sum w(|F_{w}| - |F_{v}|)^{2} / \sum w(F_{w}|^{2})^{1/2}$.

^b For 5: $wR2 = [\sum w(F_0^2 - F_0^2)^2 / \sum w(F_0^2)^2]^{1/2}; w = 1/[\sigma^2(F_0^2 + (0.0692(P))^2 + 2.29(P)]]$ where $P = (Max(F_0^2, 0) + 2F_0^2)/3.$

Table 3

Table 2 Atomic parameters for the structure of RuCl₂(BTZ)₂ (4) "

1			
Atomic paramet	ers for the struct	are of RhCl(CO	D)(CTZ) (5) *

Atom	x	<u>v</u>	2	U or U(eq)
Rul	11001(3)	2500	8763(4)	59(3) ^b
Brl	11336(2)	728(1)	8654(3)	49(2) "
Cll	10782(12)	2500	10600(9)	68(8) ^h
Cl2	12627(8)	2500	8114(11)	51(7) ^b
NI	9914(12)	1328(6)	7185(14)	11(4)
N2	10274(15)	1930(7)	8171(16)	26(5)
CI	9940(17)	948(8)	6330(21)	28(6)
C2	10571(18)	1673(8)	7346(18)	19(6)
C3	9170(20)	1364(9)	7997(21)	31(6)
C4	,9407(18)	1749(8)	8613(21)	30(6)
CI.I	41042(14)	933(5)	5764(14)	22(6)
C2,1	11711(14)	558(5)	5884(14)	38(8)
C3,1	12673(14)	\$55(3)	5341(14)	44(8)
C4,1	12965(14)	926(5)	4678(14)	42(8)
C5,1	12296(14)	1301(5)	4559(14)	45(8)
C6,1	11335(14)	1304(5)	5102(14)	36(7)
C1,2	9154(13)	1039(5)	5433(11)	18(5)
C2,2	8417(13)	1389(5)	5520(11)	35(7)
C3,2	7720(13)	1469(5)	4652(11)	40(7)
C4,2	7760(13)	1199(5)	3697(11)	44(7)
C5,2	8497(13)	848(5)	3609(11)	36(7)
C6.2	9194(13)	768(5)	4478(11)	26(6)
C1,3	9714(18)	506(8)	7025(19)	20(6)
C2,3	8977(18)	212(8)	6648(19)	24(6)
C3,3	8760(20)	-211(9)	7192(21)	36(7)
C4,3	9342(21)	-313(10)	8136(23)	40(8)
C5,3	10077(20)	- 39(10)	8496(22)	35(7)
C6,3	10290(18)	367(8)	7965(20)	23(6)
01	1939(41)	2500	5573(45)	150(19)
02	4811(43)	2500	-164(46)	156(20)
03	3216(67)	2500	3822(77)	286(37)

* Thermal parameters multiplied by 1000, coordinates by 10 000.

 $^{\rm tr}$ $U({
m eq})$ defined as one third of the trace of the orthogonalized thermal tensor.

of M-CTZ complexes (M = Ru, Rh, Cu, Pt, Au) in high yields, as summarized in Scheme 1. The new compounds are air stable in the solid state and in solution, a necessary condition for biological use. They were characterized by micro-analytical and spectroscopic methods (see Section 2), and, in three cases, by X-ray diffraction.

Selected NMR data collected in Table 5 show that CTZ binds to the metals through the unsubstituted N(3) atom, which is the best donor site of this molecule. Correspondingly, the largest ¹H and ¹³C shifts with respect to the free ligand are observed for the protons and carbons that are located α to N(3).

RuCl₂(CTZ)₂ (2), prepared by reaction of RuCl₂-(NCMe)₄ (1) with 2 equiv. CTZ in MeOH, is an interesting complex regarding both its chemical structure and its bⁱo⁴ogical properties. The proposed formulation corresponds to a highly stable diamagnetic tetracoordinated 14-electron configuration, which is very unusual for Ru(II), for which the vast majority of complexes display 18-electron octahedral coordination, or, if voluminous ligands are present, 16-electron trigonal bipyramidal or square based pyramidal coordination [22]. Twinning problems have precluded a crystal structure determination, but we have performed 2D-corre-

	x	У У	2	U(eq)/
				U(iso)
Rh	19058(4)	4363(4)	30721(5)	45(1)
Cl(1)	198(2)	-1436(2)	1343(2)	70(1)
Cl(2a)	3595(2)	-3241(2)	1777(2)	52(1)
Cl(2b) ^h	3301(9)	- 3864(8)	-4340(10)	78(3)
N(1)	3026(5)	-368(4)	2242(6)	43(1)
N(2)	3527(5)	-1535(4)	496(5)	37(1)
C(1)	2598(7)	-1330(5)	834(7)	42(2)
C(2)	4283(7)	38(5)	2818(7)	48(2)
C(3)	4611(6)	-670(5)	1766(7)	44(2)
C(4)	3346(6)	-2571(5)	-994(6)	36(2)
C(5)	2318(5)	~ 3081(5)	- 1275(6)	38(2)
C(6)	2385(6)	~4017(5)	-83(6)	43(2)
C(7)	1462(6)	~ 5031(6)	- 360(8)	54(2)
C(8)	477(6)	- 5732(6)	-1825(8)	62(2)
C(9)	394(6)	-5421(6)	- 3001(8)	65(2)
C(10)	1308(6)	-4400(5)	-2721(7)	53(2)
C(11)	4543(6)	-2758(5)	-815(6)	38(2)
C(12)	4655(8)	-3839(5)	-974(6)	44(2)
C(13)	5747(9)	-3981(6)	-875(7)	58(2)
C(14)	6738(7)	- 3072(7)	-609(7)	59(2)
C(15)	6634(8)	- 1988(6)	-478(7)	54(2)
C(16)	5545(8)	-1853(6)	-608(6)	45(2)
C(17a) "	2964(11)	-2302(8)	- 2406(9)	38(2)
C(18a) ^b	2460(9)	- 1440(7)	- 2395(9)	49(2)
C(19a) ^h	2141(9)	-1202(8)	-3702(11)	62(2)
C(20a) ^b	2334(10)	-1825(9)	- 4964(10)	63(2)
C(21a) ^h	2817(9)	-2700(8)	5043(10)	65(2)
C(22a) "	3140(9)	-2920(8)	- 3711(10)	48(2)
C(17b) ^h	2926(39)	-2061(30)	- 2208(29)	38(2)
C(18b) ^h	2465(33)	~1139(26)	- 1933(27)	48(2)
C(19b) "	2126(32)	758(25)	- 3083(31)	61(2)
C(20b) ^b	2182(34)	1324(28)	- 4497(28)	62(2)
C(21h) ^b	2578(36)	- 2254(29)	- 4816(26)	64(2)
C(22b) ⁶	2928(33)	- 2628(25)	3662(28)	48(2)
C(23)	3271(7)	2194(5)	4166(8)	60(2)
C(24)	3397(6)	1746(5)	5233(7)	57(2)
C(25)	3020(8)	2116(7)	6572(8)	73(2)
C(26)	1700(8)	2038(7)	6021(8)	83(3)
C(27)	882(7)	1112(7)	4303(8)	70(2)
C(28)	736(7)	1394(7)	3060(9)	68(2)
C(29)	1388(11)	2628(8)	3267(10)	100(3)
C(30)	2753(10)	3161(7)	4230(10)	95(3)
		· · · · · ·		

* Atomic coordinates ($\times 10^4$; $\times 10^5$ for Rh atom), isotropic and equivalent isotropic displacement parameters ($\mathring{A}^2 \times 10^5$) for 1. U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor.

^b These atoms, involved in disorder, were refined with isotropic displacement parameters.

lated ¹H and T_1 NMR studies, as well as theoretical calculations which have shown that the optimum conformation corresponds to an approximately C_{2i} arrangement of the RuCl₂N₂ core [13], a geometry that has been previously predicted by general theoretical arguments to be the most favorable situation for d^o ML₄ species [22c]. This proposed structure was confirmed through an X-ray diffraction study of the closely related complex RuCl₂(BTZ)₂ (4) prepared (as a trihydrate) by reaction of 1 with BTZ, the brominated analog of CTZ; this experimentally determined molecular structure (discussed below) is remarkably similar to the one predicted by our calculations and NMR studies [13].

 Table 4

 Atomic parameters for the structure of AuCl₃(CTZ) (6) *

Atom	x	y	2	U or U(eq)
Aul	1679(1)	5027(1)	9200(1)	22(1) ^b
CII	5290(3)	259(1)	2641(2)	42(1) ^b
C12	6035(3)	1029(1)	4931(2)	37(1) ^b
C13	7386(3)	- 1084(1)	3511(2)	46(1) ^b
C14	10575(3)	-1802(1)	5971(2)	38(1)
NI	8681(7)	-912(3)	7022(4)	21(1)
N2	7879(8)	- 297(4)	5599(5)	27(2)
CL	8921(9)	- 1496(4)	7879(5)	19(2)
C2	9545(10)	- 316(4)	7029(6)	26(2)
C3	9026(10)	82(4)	6155(6)	30(2)
C4	7709(9)	- 888(4)	6145(6)	23(2)
C1,1	8084(5)	-2201(2)	7487(4)	22(2)
C2,1	6596(5)	-2180(2)	7206(4)	27(2)
C3.1	5828(5)	-2824(2)	6940(4)	37(2)
C4,1	6549(5)	- 3490(2)	6956(4)	38(2)
C5.1	8037(5)	-3511(2)	7237(4)	37(2)
C6,1	8805(5)	-2867(2)	7503(4)	27(2)
C1,2	10539(9)	-1627(4)	8122(6)	25(2)
C2,2	11270(10)	- 1655(4)	9156(6)	31(2)
C3,2	12718(12)	1804(5)	9403(8)	44(2)
C4,2	13503(12)	- 1905(5)	8612(7)	45(2)
C5,2	12788(11)	- 1878(5)	7576(7)	38(2)
C6,2	11344(10)	- 1758(4)	7330(6)	30(2)
C1,3	8297(7)	1207(2)	8834(4)	22(2)
C2,3	8008(7)	-1713(2)	9579(4)	38(2)
C3.3	7435(7)	1478(2)	10444(4)	44(2)
C4.3	7149(7)	= 737(2)	10564(4)	49(3)
C5,3	7438(7)	= 231(2)	9819(4)	51(3)
C6,3	8011(7)	- 466(2)	8954(4)	40(2)

* Thermal parameters multiplied by 1000, coordinates by 10 000.

" U(eq) defined as one third of the trace of the orthogonalized thermal tensor.

Table 5 Selected NMR data (δ) for metal-CTZ complexes ^a

Compound	H ₂ (ppm)	C ₂ (ppm)
CTZ	7.47	138.9
2	7.87	139.2
3	8.03	140
5	7.90	139.5
6	8.36	139.2
7	7.99	139.3
8	7.87	139.2

* For solvents and conditions, see Section 2.

its therapeutic potential. Therefore, in order to improve the solubility necessary for biological testing and eventual use, we thought it would be appropriate to modify the composition of the complex in order to make it cationic; furthermore, taking into account that one of the most important mechanisms of action of metal-containing drugs is via interactions with DNA [9], it was desirable to incorporate a potential DNA-intercalating fragment such as bipy in our design. A successful synthesis of the desired complex [Ru(bipy)- $(CTZ)_{2}$ (PF₆)₂ (3) involved reaction of 2 with 2 equiv. of NH₄PF₆, followed by addition of 1 equiv. bipy in MeCN as the solvent; the spectroscopic data for 3 (see Section 2) are all in good accord with the proposed 14-electron formulation containing a Ru(II) ion, one bidentate bipy, two bound CTZ ligands and two PF6° counterions. It is reasonable to assume that complex 3 adopts an approximately $C_{2\mu}$ coordination arrangement similar to that found for 2 and 4.

In the case of rhodium, a simple bridge-splitting reaction of $[Rh(COD)Cl]_2$ with 1 equiv. CTZ leads to complex 5, which has been fully characterized by analytical and spectro-



Sebeme 1. Synthesis of new CTZ complexes: (i) $RuCl_2(NCCH_1)_4/MeOH$, r.t.; (ii) NH_4PF_6/CH_3CN , reflux, then bipy at r.t.; (iii) $[Rh(COD)Cl]_2/EtOH$, r.t.; (iv) $AuCl_3+HCl/Et_3N/MeOH$, r.t.; (v) $K_2[PtCl_4]/MeOH$, r.t.; (vi) $[Cu(NCCH_3)_4]PF_6/CH_2Cl_3$, r.t.

Complex 2 has shown very interesting properties against T. cruzi (see Section 4), but its low solubility in most common polar solvents other than DMSO imposes limitations on

scopic methods (see Section 2) as well as by an X-ray diffraction study which is described below. CTZ also adds to gold chloride to produce $AuCl_3(CTZ)$ (6), characterized by spectroscopic and X-ray methods (see discussion below), and to $K_2[PtCl_4]$ yielding octahedral $K_2[PtCl_4(CTZ)_2]$ (7). Finally, two CTZ ligands are able to displace all four acetonitriles from $[Cu(NCCH_3)_4]PF_6$ to generate the new derivative $[Cu(CTZ)_2]PF_6$ (8). We have not been able to obtain crystals of 7 or 8 suitable for X-ray studies, and therefore their characterization has been based on analytical and NMR data.

3.2. X-ray structures of complexes $4 \cdot 3H_2O$, 5 and 6

The molecular structures of compounds $4 \cdot 3H_2O$, 5 and 6 are represented in Figs. 1–3; the most relevant bond distances and angles are collected in Tables 6–8.



Fig. 1. Diagram of the molecular structure of $RuCl_2(BTZ)_2(4)$ with thermal ellipsoids drawn at the 30% probability level.



Fig. 2. Diagram of the molecular structure of RhCl(COD)(CTZ) (5) with thermal ellipsoids drawn at the 50% probability level.



Fig. 3. Diagram of the molecular structure of $AuCl_1(CTZ)$ (6) with thermal ellipsoids drawn at the 30% probability level.

Table 6

Sel	ected	bond	lengths	i (A) and	bond	l angles	(°)	for	RuCl ₂	(B	TZ)2	(4	I)
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Bond lengths			
Ru-Cl(1)	2.23(1)		
RuCl(2)	2.23(1)	C(6,3)-Br(1)	1.89(2)
Ru-N(2)	2.03(2)	N(1)-C(2)	1.33(3)
N(1)-C(1)	1.51(3)	N(1)-C(3)	1.37(3)
C(1)-C(1,1)	1.57(3)	N(2)-C(2)	1.30(3)
C(1)-C(1,2)	1.56(3)	N(2)-C(4)	1.34(3)
C(1)-C(1,3)	1.56(3)	C(3)-C(4)	1.38(4)
Bond angles			
Cl(1)-Ru(1)-Cl(2)	117.8(5)	Ru(1) - N(2) - C(4)	124.3(1)
CI(1)-Ru(1)-N(2)	106.9(6)	C(1)-N(1)-C(2)	130(2)
Cl(2)-Ru(1)-N(2)	107.8(6)	C(1)-N(1)-C(3)	124(2)
Ru(1)-N(2)-C(2)	127.1(1)		

Table 7

Selected bond lengths (Å) and bond angles (°) for RhCl(COD)(CTZ) (5)

C(1)-N(1)-Rh	123.5(5)	C(2)=N(1)=Rh	130.5(4)
N(1)-Rh-C(27)	167.0(2)	C(1) = N(1) = C(2)	105.3(5)
C(28)-Rh-C(23)	82.3(3)	C(27)-Rh- $Cl(1)$	90.7(2)
N(1)-Rh-C(23)	91.9(2)	C(23)-Rh- $Cl(1)$	166.5(2)
C(24)-Rh-C(23)	38.3(2)	C(28)RhCl(1)	90.9(2)
N(1)RhC(28)	154.6(2)	N(1)-Rh-Cl(1)	89.2(2)
C(24)-RhC(28)	97.8(3)	C(24)-Rh-Cl(1)	155.1(2)
C(24)-Rh-N(1)	92.6(2)	C(23)-Rh-C(27)	91.2(3)
Bond angles			
RhN(1)	2.106(5)		
RhCl(1)	2.375(2)	N(2)-C(4)	1.504(7)
RhC(23)	2.114(6)	N(2)-C(3)	1.377(7)
RhC(27)	2.141(7)	N(2)-C(3)	1.352(8)
RhC(28)	2.109(6)	N(1)-C(2)	1.376(7)
RhC(24)	2.099(6)	N(1)-C(1)	1.331(7)
Bond lengths			

Selected bond lengths (Å) and bond angles (°) for AuChCTZ (6)

and the second		and a second	C THE REAL PROPERTY OF THE PARTY OF THE PART
Bond lengths			
Au-Cl(1)	2.231(2)	N(1)~C(1.3)	1.55(1)
Au-Cl(2)	2.197(2)	C(6,2)-Cl(4)	1.770(8)
Au-Cl(3)	2.362(3)	N(1)-C(2)	1.36(1)
Au-N(1)	2.035(6)	N(1)-C(4)	1.322(9)
N(1)-C(1)	1.522(9)	N(2)C(3)	1.38(1)
C(1)-C(1,1)	1.549(8)	N(2)C(4)	1.32(1)
C(1)-C(1,2)	1.53(1)	C(2)-C(3)	1.35(1)
Bond angles			
Cl(1)-Au-Cl(2)	93.6(1)	Au(1)-N(2)-C(3)	125.2(5)
Cl(2)-Au-Cl(3)	176.6(1)	Cl(1)-Au-N(2)	137.9(2)
Cl(2)-Au-N(2)	91.0(2)	Cl(3) - Au - N(2)	86.0(2)
Cl(1)-Au-Cl(3)	89.3(1)	Au(1)-N(2)-C(4)	127.5(6)

3.2.1. RuCl₂(BTZ)₂•3H₂O (4•3H₂O)

This compound crystallized only with difficulty and consequently this structure is not of high quality from a crystallographic point of view. However, it is very adequate as a method of characterization of the new complex, since it confirms a very unusual example of a 14-electron tetracoordinated, diamagnetic Ru(II) complex. The coordination about the Ru atom (Fig. 1) consists of two chlorines, plus two N donor atoms from the BTZ ligands, which accommodate themselves in a distorted tetrahedral arrangement in order to avoid steric repulsions caused by the bulky phenyl and bromophenyl substituents on the BTZ imidazole ring. The Cl-Ru-Cl and Cl-Ru-N angles are 117.8(5) and 107° (average), respectively, and the Ru-N distance is 2.03(2) Å, similar to those reported for other related Ru-imidazole complexes [23-30]. All other distances and angles are normal.

3.2.2. RhCl(COD)(CTZ) (5)

This compound displays a typical square planar arrangement around Rh(I) formed by the N atom of CTZ, one Cl and the two C=C bonds of COD (Fig. 2). The structural feature of most relevance for our purposes is the Rh-N bond distance of 2.106(5) Å, which is in the appropriate range for Rh imidazole derivatives, e.g. $[Rh(CO)_2(2-Me-im)]_4$ (2.00-2.11 Å) [31], $[(COD)Rh]_2(\mu-Bi-im)$ (2.120-2.141Å) [32], cis-[Rh(CO)_2Cl(N-Me-im)] (2.072(4)Å) [33], Cp*[P(OEt)_3]Rh(\mu-Bi-im)[Rh(NBD)]_2 (2.08-2.10 Å) [34], $[(COD)Rh](\mu-Bi-bzim)[Au(PPh_3)]_2$ (Rh-N 2.08(3) and 2.11(3) Å) [35], $(Ph_3P)_2(CO)(H)$ -Ru(μ -Bi-im)Rh(COD) (Rh-N: 2.127(5) and 2.134(5)) [24]. There are no other remarkable features in this structure.

3.2.3. AuCl₃(CTZ) (6)

Complex 6 revealed a molecular structure (Fig. 4) consisting of a square planar coordination around the gold atom formed by the N atom of CTZ and the three Cl atoms. The Au=N bond distance is 2.035(6) Å, similar to those in the shorter range of observed Au=N lengths in other Au imidazole complexes, such as $[(COD)Rh](\mu-Bi-bzim)-[Au(PPh_1)]_2$ (Au=N 1.96(3) and 1.98(4) Å) [35], [AuMe₂{(py)(mim₂)COH}]' (2.098(6) and 2.113(7)) [36], [Au(gly-L-his)Cl]Cl (1.991(8) Å) [37], [Au(PPh_3)]_2(\mu-Bibzim) (2.053(9) and 2.03(2) Å) [38], and (PPh_3)Au(N7-Theophyl) (2.047(5) Å) [39]. All other structural parameters may be considered normal.

4. Biological activity

4.1. In vitro tests of complexes 2–8 against epimastigotes of T. cruzi

The effect of the new complexes on the proliferation of in vitro cultures of the epimastigote form of *T. cruzi* (equivalent to the form present in the Reduviid vector [8]) was evaluated as described in Section 2. Table 9 summarizes the results of such tests. The activities of the new metal derivatives ranged from slightly lower to considerably higher than the free parental compound CTZ, observing the trend 3=7<8<CTZ <6<5 <2.

Some interesting observations can be extracted from these data: (i) The Ru-, Rh- and Au-containing complexes herein described are capable of enhancing the antiparasitic activity



Fig. 4. Effects of CTZ and 2 on the proliferation of (a) intracellular *T. cruzi* amastigotes and (b) Vero cells. For details see Section 2.

Table 9

Effect of CTZ and M-CTZ compounds on the proliferation of the epimastigotes of *T. cruzi* *

Compound	Inhibition (%)
CTZ	58
2	100
3	50
8	62
6	60
7	50
8	54

"Complexes used as 10 " M DMSO solutions. For details, see Section 2.

of the parental drug, while the Pt and Cu derivatives slightly lower the efficacy of CTZ; this is in contrast with the wellknown high activity of Pt (superior to that of any other metal) as an anti tumor agent [9], suggesting that the mechanisms of action operating against the parasites are different from the cancer case. (ii) Complex 2 turned out to be by far the most active compound in our series. Perhaps more importantly, at the concentrations used, complex 2 was found to have a *trypanocidal* effect, while the parental drug displayed only *trypanostatic properties*², as determined by optical measurements and microscope observations. This quantitative and qualitative enhancement of the efficacy of CTZ stimulated us

² We define *trypanostatic* as the effect of diminishing or stopping the parasite proliferation rate; *trypanocidal* refers to diminishing the number of viable parasite cells.

to carry out further biological studies which are described below. (iii) The high activity of 2 also prompted us to carry out chemical modifications aimed at improving the activity and solubility properties through the incorporation of a bipy ligand and the formation of a charged complex 3; however, it is clearly seen that such modifications were actually detrimental to the antiparasitic behavior, since 3 is one of the least efficacious derivatives tested. In the absence of detailed mechanistic studies, we cannot offer a full explanation for this observation, but preliminary work described below indicates that hydrolysis of the Ru-Cl bonds in 2 (upon contact with the biological system) may be an important step toward the formation of the active species; therefore, substitution of the two chlorides by a strongly bound chelating bipy ligand probably hampers any hydrolytic process and thus this strategy does not seem adequate.

4.2. Further tests on the activity of complex 2 against epimastigotes and amastigotes of T. cruzi

In view of the very promising results described above for complex 2 we carried out further tests at different concentrations of the drugs. It was found that free CTZ produced a dose-dependent effect on the growth rate of *T. cruzi* epimastigotes with a median inhibitory concentration (IC₅₀) of 10^{-6} M, whereas for complex 2 the IC₅₀ was 10^{-7} M, indicating a 10-fold increase of the antiproliferative effect of CTZ by complexation to Ru. Furthermore, for the parental drug, complete growth arrest and cell lysis were observed only at concentrations as high as 10^{-4} and 3×10^{-5} M, respectively, while 2 was able to produce cellular lysis at 10^{-6} M, and growth arrest was already achieved at 3×10^{-6} M. Thus, the threshold to produce trypanocidal effects by CTZ is reduced also by a factor of 10 by complexation to ruthenium.

It is therapeutically more relevant to test potential new drugs on the infectious intracellular amastigote form of *T. cruzi*, which can be cultured in mammalian (Vero) cells as an in vitro model of an infection. The results of such tests for complex 2 in comparison with free CTZ can be seen in Fig. 4, where it is noted that eradication of the infection is achieved at 3×10^{-7} M for free CTZ, while a concentration as low as 3×10^{-8} M of 2 is enough to achieve the same effect. The corresponding EC₅₀ values are 10^{-8} and 5×10^{-9} M, respectively. This activity of complex 2 is comparable to the best results obtained by using combinations of an allylamine with ketoconazole [6c] and with the enantiomerically pure bistriazole D0870 [8].

Also, very importantly, as can be seen in Fig. 4(b) free CTZ has deleterious effects on the Vero cells at the concentrations required to eradicate the infection, while 2 does not affect the proliferation of these mammalian cells within the concentration range tested (up to 10^{-6} M); this is of importance in that it can be taken as an indication that complex 2 is non-toxic. More detailed studies on this aspect are clearly in order.

4.3. Preliminary studies on the mechanism of action of complex 2

4.3.1. Analysis of sterol content of treated and untreated parasites

As explained in Section 1, clotrimazole and other azole derivatives are known to inhibit parasite proliferation by blocking the cytochrome P-450 dependent C-14α-demethylation of lanosterol to ergosterol [6]. Thus, a first approximation to the study of the mechanism of action of 2 was to determine the sterol composition of parasites treated with the same concentration of 2 and CTZ (for details, see Section 2), in order to determine whether the potential of the antiproliferative effect in the former was due to a stronger inhibition of the synthesis of ergosterol. The results summarized in Table 10 show that the pattern of sterols isolated from parasites treated with complex 2 is comparable to that obtained from parasites treated with free CTZ, indicating that 2 is not a stronger inhibitor of the synthesis of ergosterol than clotrimazole itself, but instead the potentiation observed must be related to a different mode of action of the metal-based drug.

It is known [40] that azole-type SBIs exert their action on $cytP450_{DM}$ through binding of the unsubstituted N(3) atom to the heme group of the enzyme. In complex 2 the N(3)atom is actually bound to ruthenium; therefore, the fact that inhibition of sterol biosynthesis is taking place (as shown by the data in Table 10) indicates that the complex probably breaks down (very likely through hydrolytic processes) within the biological medium, releasing free CTZ which then goes to interact with the enzyme in the usual way. The 'RuCl₂' fragment liberated (possibly in a hydrolyzed form) could also interact with a variety of intracellular targets, thus producing additional effects that would result in the increased potency observed for 2 with respect to CTZ. A good candidate for such interaction would be the parasite's DNA, and we have begun to investigate this possibility as described in Section 4.3.2.

Table 10

Sterols isolated from epimastigotes of T. cruzi *

Treatment	Cholesterol (%)	Demethylated sterol ^b (%)	4.4' -Dimethylsterol * (%)
None	22.4	77.6	
CTZ	10.4	8.9	80.7
2	16.6	19.7	63.7

^a CTZ and **2** used as 10⁻⁶ M DMSO solutions. For details, see Section 2 and Refs. [6f,g].

⁵ This fraction is composed of lanosterol, 24-methylene-dihydrolanosterol, and 4,14-dimethyl-ergosta-8,24(28)-diene-3 β -ol (obtusifoliol).

^b This fraction is composed of ergosterol, 24-ethyl-5,7,22-cholest-triene-3β-ol, ergosta-5,7-diene-3β-ol, ergosta-7,24(28)-diene-3β-ol, and ergosta-5,7,24(28)-triene-3β-ol.

After irradiation

Before irradiation



Fig. 5. Interaction of 2 with DNA; schematic representation of the HPLC traces of (a) DNA; (b) CTZ; (c) DNA + CTZ; (d) 2; (e) DNA + 2 (*; this peak corresponds to the product of the DNA + 2 interaction). Numbers refer to retention times in min. For details see Section 2.

4.3.2. HPLC analysis of the interaction of CTZ and 2 with DNA

DNA is known to bind transition metals at various points of the molecule; this type of interaction is responsible, for instance, for the anticancer properties of cisplatin and related compounds [9]. It is therefore conceivable that the activity of complex 2 may also be related to some DNA binding capacity; in order to test this hypothesis, a series of qualitative preliminary experiments was conducted in which DNA was mixed with CTZ or with complex 2, and the possible interactions, before and after irradiation, were analyzed by HPLC, in comparison with pure samples of DNA, 2 and CTZ treated in the same manner.

The results of these studies are represented schematically in Fig. 5. The trace for free non-irradiated DNA (a) consists of two peaks of equal area at 2.87 and 3.2 min (retention times); upon irradiation, a main single peak appears at about 32 min at the expense of the original two peaks which now represent about 6% of the total. Free CTZ elutes as a single peak at 29.6 min which is unaltered upon irradiation (b). For the mixture DNA + CTZ the HPLC traces (c) are simply the sums of the individual traces (a + b) both before and after irradiation, indicating that no interaction takes place between CTZ and DNA under the conditions of the experiments. Complex 2 alone elutes as a single peak at 28.9 (d) which remains



Scheme 2. A possible mechanism of action of complex 2 against T. cruzi.

the same after irradiation of the solution. The trace (e) for the non-irradiated mixture of DNA+2 is the sum of the individual traces (c+d), but when this mixture is irradiated, a new peak (marked *) is observed at 23.6 min, besides the peaks corresponding to individually irradiated DNA and 2.

These results suggest a photochemically induced adduct formation between DNA and complex 2, in agreement with previous studies on the interaction of $RhCl_3(phen)_3$ [41] and $RuCl_2(phen)_2$ [42] with DNA which demonstrated that irradiation induces chloride exchange by water, followed by displacement of water by a DNA base. Further studies aimed at the isolation and characterization of such a Ru-DNA adduct are currently in progress.

Although we are not yet able to propose a mechanism for the anti T. cruzi action of complex 2, our combined results allow us to speculate a simple model represented in Scheme 2. A possible mechanism of action of this compound is as follows: the metal complex would act firstly as a transport agent through the parasite membrane and, once inside the cell, it would liberate the CTZ ligand allowing it to exert its known SBI action on cyt-P450_{DM}. The 'RuCl₂' fragment thus released would undergo a series of chloride ligand exchange reactions with water and eventually bind DNA. These combined effects may be responsible for the higher potency of the Ru complex, as well as for the qualitative change from a trypanostatic into a trypanocidal mode of action. Based on this hypothesis we are currently conducting further studies which are obviously needed in order to arrive at a more detailed proposal for a mechanism of action. These experiments, as well as in vivo tests against T. cruzi, will be the subject of future publications.

5. Supplementary material

Listings of atomic coordinates of the hydrogen atoms, anisotropic thermal parameters and structure factors are available as supplementary material from the authors.

Acknowledgements

We thank Sarah Pekerar for her assistance with the NMR experiments and Fundayacucho for Graduate Fellowships to K.L., M.N. and R.A. Generous support from the Commission of the European Communities (Contract No. CI 1-450-B (GDF)), the PNUD/World Bank/World Health Organization Program for Research and Training in Tropical Diseases (Grant 930161) and CONICIT (Grant S1-2696) is acknowledged.

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