Communications to the Editor

Toward a Novel Metal-Based Chemotherapy against Tropical Diseases. 1. Enhancement of the Efficacy of Clotrimazole against *Trypanosoma cruzi* by Complexation to Ruthenium in $RuCl_2$(clotrimazole)$_2$

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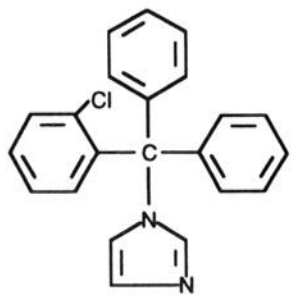
Received May 6, 1993

The development of efficacious agents against Chagas' disease is critical since this illness afflicts millions of people in Latin America. There is no effective treatment despite recent advances in the knowledge of the biochemistry of its causative agent, *Trypanosoma cruzi*.[1]

Azole derivatives are known to block the proliferation of parasites such as *Leishmania tropica*, *Leishmania mexicana*, and *T. cruzi*, by inhibiting the cytochrome P-450 dependent C(14) demethylation of lanosterol to ergosterol.[2,3] The use of metal complexes as chemotherapeutic agents is now also well-established, particularly in the cancer field.[4] Due to the similarities between the metabolism of tumor cells and pathogenic trypanosomes, analogous concepts may be applied to the treatment of this parasitic disease. Some antitumor and related metal complexes are moderately active against African trypanosomes,[5] but virtually no information is available concerning activity against American trypanosomiasis.

Here we present a novel approach toward the development of a chemotherapy against Chagas disease, consisting in the modification of clotrimazole (CTZ) (1-[(2-



CTZ

chlorophenyl)diphenylmethyl]-1*H*-imidazole, an azole-type antifungal agent that has a marginal anti *T. cruzi* activity) by the introduction of a transition metal.

Chemistry. The complex $RuCl_2(CTZ)_2$ (1) was prepared by reaction of $RuCl_2(NCCH_3)_4$ with 2 equiv of the ligand under mild conditions.[6] CTZ most probably binds to the metal through the unsubstituted N(3) atom, which is the best donor site of this molecule. Correspondingly,

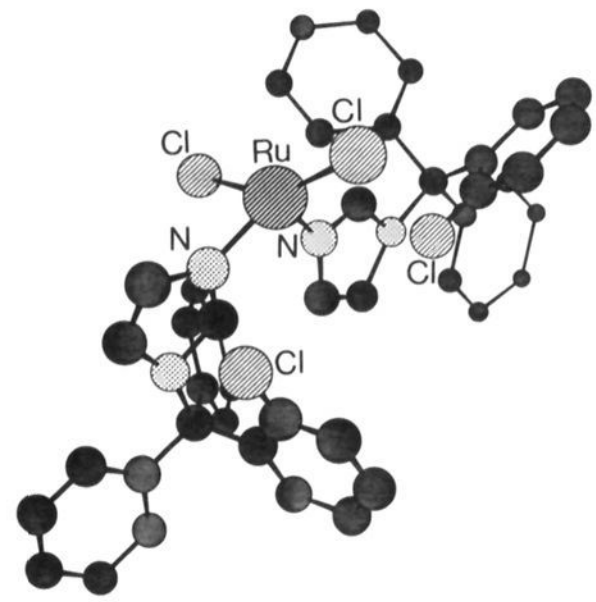


Figure 1. Optimum calculated[8] molecular structure of 1. Note the C_{2v} RuN_2Cl_2 core and the apposition of the chlorobenzene rings to the imidazole centers.

the largest ^1H and ^{13}C shifts with respect to the free ligand are observed for the protons and carbon that are located α to N(3).[7]

The proposed formulation corresponds to a 14-electron configuration, which is unusual for Ru(II). Twinning problems have precluded a crystal-structure determination, but we have performed theoretical calculations (including all the 87 atoms) on various possible geometries for this complex;[8] Figure 1 shows the optimum conformation which corresponds to an approximately C_{2v} arrangement of the $RuCl_2N_2$ core; this symmetry was previously shown to be the most stable situation for d^6 ML_4 fragments.[8b] Our calculations also indicate that the 18-e octahedral complex $RuCl_2(NCCH_3)_2$(clotrimazole)$_2$ would result in a higher energy than the optimum 14-e C_{2v} structure due to the steric hindrance caused by the clotrimazole ligands which is evident from partition energy analysis.[8c]

Independent support for the proposed structure was obtained from detailed ^1H NMR studies: (a) T_1 values for most aromatic protons of CTZ are approximately twice those obtained for compound 1, which suggests a molecular volume of the complex of roughly double that of CTZ. (b) A comparison of COSY and NOESY spectra for CTZ and 1 indicates a close proximity of the chlorophenyl and imidazole rings in the complex, not present in the parental compound, which is clearly predicted from the calculated structure (Figure 1).

An alternative formulation of complex 1 that must be considered is a dimeric structure containing Ru–Cl–Ru bridges. There is ample precedent for this type of dimer in ruthenium chemistry,[9] and our spectroscopic data do not allow us to rule out such possibility. The value of the molecular weight of 1 measured cryoscopically in $CHCl_3$ solution (808) is very close to the expected value for a monomeric formulation (861), but it is also known that Ru–Cl bridges may be broken with relative ease by solvents.

Since the biological tests described below were carried out using dmso solutions of 1, it must also be pointed out that this complex is stable in that solvent; the NMR spectra of 1 in deuterated dmso remains unchanged for several

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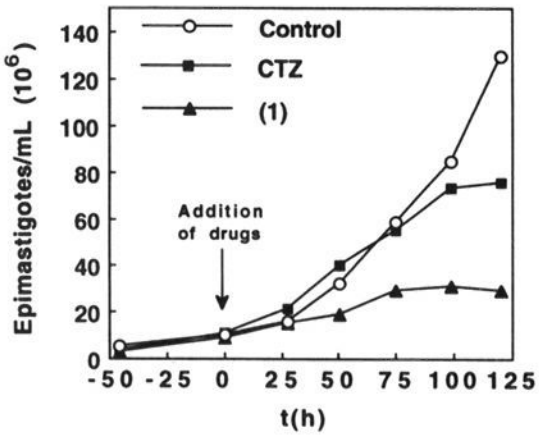

Figure 2. Effect of CTZ and complex 1 on the proliferation of epimastigottes of *T. cruzi*. An EP stock of the epimastigote form of *T. cruzi* cultivated as described in ref 2a was used. The initial cell density was 2×10^6 epimastigotes/mL; CTZ and 1 were added as dimethyl sulfoxide solutions (10^{-5} M) when the cultures reached a cellular density of 10^7 epimastigotes/mL. Parasite proliferation was followed daily by the use of an electronic particle counter and by direct counting with an hemacytometer.

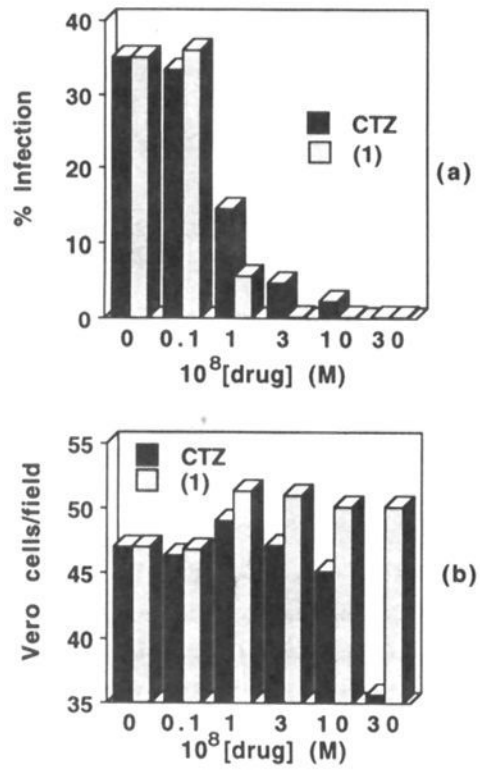

Figure 3. Concentration dependence of the effects of CTZ and 1 on the proliferation of (a) intracellular *T. cruzi* amastigotes and (b) Vero cells. Vero cells were infected with a 20:1 ratio of trypomastigotes per cell for 2 h and then washed with phosphate-buffered saline solution to remove the nonadherent parasites. Fresh medium, with and without the 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: percent of infected cells, number of amastigotes per infected cell, and the number of Vero cells per field of the microscope. For further details see ref 2b.

days at 37 °C, showing no evidence of displacement of the CTZ ligand by the solvent. Furthermore, $RuCl_2(dmso)_4$, which could conceivably be formed in this way, has been found to be inactive against *Trypanosoma rhodesiense*.[5a]

Biological Results and Discussion. Biological tests on the effect of complex 1 on the proliferation of the epimastigote form of *T. cruzi* are very encouraging. As shown in Figure 2, compound 1 causes a marked inhibition of the proliferation (*ca.* 90%) at 10^{-5} M; more importantly, it also produces cellular lysis after 96 h, while the parental compound (CTZ) has only a very modest effect on the growth rate at the same concentration. This means that complexation to Ru not only increases the activity of the parental drug but also modifies it from trypanostatic to *trypanocidal*. Experiments carried out at various concentrations of CTZ and of 1 in the range 10^{-7}–10^{-4} M provided EC_{50} values of *ca.* 3×10^{-6} M for CTZ and 10^{-7} M for 1.

It is therapeutically more relevant to test potential new drugs on the amastigote, which is the infectious intracellular form of *T. cruzi*. We find that also the amastigote form of the parasite, *grown on mammalian (Vero) cells*, is more susceptible to 1 than it is to free CTZ. As shown in Figure 3a, erradication of the infection is achieved with 3×10^{-7} M of CTZ while the same effect is achieved at a concentration as low as 3×10^{-8} M of 1. The corresponding EC_{50} values are 10^{-8} and 5×10^{-9} M, respectively. This activity of 1 is comparable to that of ketoconazole, one of the most efficient known drugs against *T. cruzi*.[10] Additionally, it is very important to note (Figure 3b) that 1 appears to be nontoxic to Vero cells in the range of concentrations tested, while CTZ cleary has deleterious effects on the proliferation of these mammalian cells at concentrations above 10^{-7} M.

In conclusion, we have discovered a new complex which displays a high trypanocidal activity against Chagas disease, combined with a low toxicity. Preliminary studies indicate that although 1 inhibits the biosynthesis of ergosterol, as does CTZ, this effect alone cannot account for the marked improvement observed with respect to the parental drug. Detailed studies on the mechanism of action of this complex, as well as further modifications of this and other related metal derivatives, are in progress and will be the subject of future papers.

Acknowledgment. We thank Sarah Pekerar for her assistance with the NMR experiments and Fundayacucho for Graduate Fellowships to K.L. and L.R. Generous support from the Commision of the European Communities (Contract No. CI 1-450-B (GDF)) and the PNUD/World Bank/World Health Organization Programme for Research and Training in Tropical Diseases (Grant 900359) is acknowledged.

Supplementary Material Available: Details of the synthesis and NMR data for compound 1, COSY and NOESY spectra for CTZ and 1, a table of optimum atomic coordinates for 1, and data on the effect of the concentration of CTZ and complex 1 on the proliferation of epimastigotes (6 pages). Ordering informatioin is given on any current masthead page.

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(6) (a) The synthesis of 1 is described in the supplementary material. Satisfactory analyses (C, H, N, Cl, Ru) were obtained.

(7) NMR data for CTZ and 1 ($CDCl_3$): $\delta_{H(2)}$ 7.45 (7.86); $\delta_{H(4)}$ 7.03 (6.84); $\delta_{C(4)}$ 128.3 (125.6). A complete table of NMR data is available as supplementary material.

(8) (a) Calculations were performed with the INDO-1 method using the GEOMOS computer program, for square planar *cis*- and *trans*-$RuCl_2(CTZ)_2$, the C_{2v} isomer in Figure 1, and *trans*-$RuCl_2(NCCH_3)_2(CTZ)_2$. Parameters were taken from Anderson, W. P.; Cundari, T. R.; Drago, R. S.; Zerner, M. C. Utility of the Semiempirical INDO/1 Method for the Calculation of the Geometries of Second-Row Transition-Metal Species. *Inorg. Chem.* **1990**, *29*, 3–5. A list of optimized atomic coordinates for the structure of 1 is available as supplementary material. (b) Elian, M.; Hoffmann, R. Bonding Capabilities of Transition Metal Carbonyl Fragments. *Inorg. Chem.* **1975**, *14*, 1058–1076.

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