Copper(II) and Cobalt(III) Pyridoxal Thiosemicarbazone Complexes with Nitroprusside as Counterion: Syntheses, Electronic Properties, and Antileukemic Activity

Marisa Belicchi-Ferrari,*,† Franco Bisceglie,†,‡ Claudio Casoli,|| Stéphanie Durot,§ Irène Morgenstern-Badarau,§ Giorgio Pelosi,† Elisabetta Pilotti,|| Silvana Pinelli,|| and Pieralberto Tarasconi†

Dipartimento di Chimica Generale ed Inorganica, Chimica Analitica, Chimica Fisica, Parco Area delle Scienze, 17/A, Università degli Studi di Parma, 43100 Parma, Italy, CIRCMSB, Unità di Ricerca di Parma, 43100 Parma, Italy, Laboratoire de Chimie Bio-organique et Bio-inorganique, FRE 2127, Bâtiment 420, Université de Paris XI, 91405 Orsay Cedex, France, and Dipartimento di Clinica Medica, Nefrologia e Scienza della Prevenzione, Università degli Studi di Parma, 43100 Parma, Italy

Received June 16, 2004

In this paper the syntheses of new pyridoxal thiosemicarbazone copper(II) and cobalt(III) complexes with nitroprusside as a counterion and tests on the antileukemic activity of three of these complexes toward human cell lines U937 and CEM are reported. Nitroprusside was chosen in order to test if its ability to release NO can increase the biological activity already shown by these complexes. The compounds were characterized by spectroscopic and magnetic methods and by single-crystal X-ray diffraction.

Introduction

Thiosemicarbazones are a class of compounds very promising in the treatment of many diseases, cancer in particular, and its development is still in progress.[1−3] Many thiosemicarbazones, such as marboran or triapine, are already used in medical practice. These compounds are thought to act as iron chelators, and their mechanism of action is explained as due to their ability to sequester iron from ribonucleotide reductase (RR). The inhibition of RR prevents the production of deoxyribonucleotides, and, as a consequence, these compounds interfere with DNA synthesis.[4] There are, however, a few aspects that are not explained by this model. First of all, metal complexes show an activity that, if compared with that of the corresponding ligands, is of the same order of magnitude and often larger. Second, if RR were the only target, an accumulation of cells in phase S would be observed because of the impossibility for the cell to complete DNA synthesis and cross the damage checkpoint. In contrast from the cell cycle analysis an accumulation in phase G2/M and a depletion of cells in phase S are often noticed. All this does not fit with the hypothesis of a simple ribonucleotide reductase inhibition by iron chelation. Other mechanisms can be envisaged, observing that many of these compounds induce apoptosis in leukemia cell lines. Therefore, as concerns the biological activity of these compounds and the way they interfere with the cell cycle, the mechanisms that lie behind the proliferation inhibition and the induction of apoptosis are still unclear and deserve more studies.

All this notwithstanding, the research and the study of thiosemicarbazone complexes as potential metal-based drugs is in development,[5] and, in particular, thiosemicarbazones bearing an aromatic heterocyclic moiety seem to present an implemented biological activity.[5−14] Among them, pyridoxal thiosemicarbazones have aroused particular interest. Continuing our research on the biological properties of pyridoxal thiosemicarbazone (H_2L) derivatives, we have synthesized new copper(II) and cobalt(III) complexes with nitroprusside as a counterion, and subsequently we have investigated the chemical and biological differences from the parent compounds having chlorides as counterions.[13,15] Nitroprusside has been chosen because it is already in use in medical practice to treat many different diseases thanks to the role of the nitric oxide released,[16−18] and in particular because of its regulatory and inductive role in apoptosis.[17,18] In the present work we report the synthesis, the chemical, spectroscopic, and magnetic characterization, X-ray structures, and antileukemic activity tests toward human cell lines U937 and CEM of two copper(II) complexes and one cobalt(III) complex with pyridoxal thiosemicarbazone and nitroprusside: $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n \cdot 2nH_2O$ **4**, $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n \cdot 6nH_2O$ **5**, and $[Co(HL)_2]_2[Fe(CN)_5(NO)] \cdot 8H_2O$ **6**.

Results and Discussion

Description of the Molecular Structures. The crystal structure of the monomer of complex $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n \cdot 2nH_2O$ **4** is reported in Figure 1.

The copper atom presents a square pyramidal coordination geometry with the three donor atoms (SNO) of the neutral ligand H_2L, a nitrogen atom of a C≡N group in the basal plane, while the axial position is occupied by an hydroxyl oxygen of a pyridoxal moiety in the $3/2 - x$, $-1/2 + y$, $1/2 - z$ position that joins the

* To whom correspondence should be addressed. Tel. +39-0521-905-420. Fax: +39-0521-905-557. E-mail: marisa.ferrari@unipr.it.
† Dipartimento di Chimica Generale ed Inorganica, Università degli Studi di Parma.
‡ Unità di Ricerca di Parma.
§ Université de Paris XI.
|| Dipartimento di Clinica Medica, Università degli Studi di Parma.

10.1021/jm049529n CCC: $30.25
Published on Web 02/12/2005

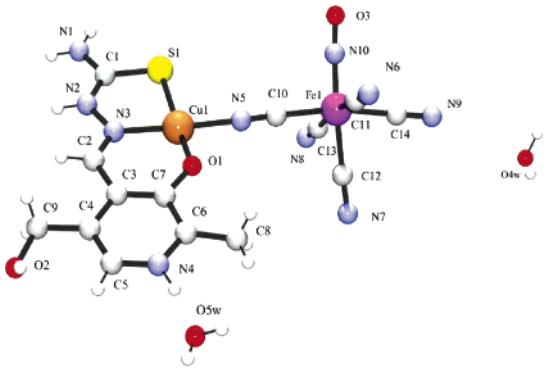

Figure 1. Ball and stick drawing of $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}\cdot 2H_2O$.

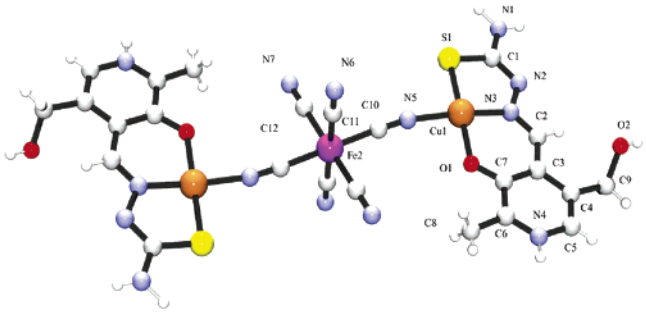

Figure 2. Ball and stick drawing of $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}$.

complexes forming polymeric chains where each complex is linked to the nitroprusside unit by a cyanide bridge.

The Cu−O1, Cu−N3, Cu−S1, and Cu−N5 coordination bond lengths in the basal plane and the axial Cu−O2 distance agree with the values found in copper complexes with the same ligand and analogous coordination geometry.[7,15] Average distances Fe−C and C≡N are 1.937 and 1.139 Å, respectively, while those of Fe−N and N−O are 1.647 and 1.133 Å, respectively, in agreement with those found in the literature.[19,20]

The molecular structure of complex $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n\cdot 6nH_2O$ **5** (Figure 2) is characterized by centrosymmetrical dimers of the copper complex.

The geometry of the copper atom is square pyramidal; each unit is linked to the other by the alcoholic oxygen of the pyridoxal in a $1 - x$, $1 - y$, z position that corresponds to the apex of the coordination pyramid. Dimers are joined by a nitroprusside anion bridge.

The iron atom lies on a symmetry center, and the cyanide group acts as a bridging ligand between two heterometallic units Cu(II)−Fe(III) giving rise to a nearly linear sequence Cu−N5≡C10−Fe with the Cu−N5−C10 angle of 169.2(3)° and the Cu−N5 bond (1.980(3) Å) in the equatorial plane. Because of the special position occupied by the iron atom, the NO group is difficult to localize. Among Fe−C distances, only Fe−C11 (1.835(4) Å) is fairly shorter than the others whose average value is 1.928 Å; for that reason it can be assumed that statistically the NO group occupancy percentage is higher in the position of the C11−N6 group.

Differently from the two polymeric copper complexes **4** and **5**, $[Co(HL)_2]_2[Fe(CN)_5(NO)]\cdot 8H_2O$ **6** crystal structure is formed by $[Co(HL)_2]^+$ octahedral cationic complexes, $[Fe(CN)_5NO]^{2-}$ anions, and crystallization water molecules. The crystal belongs to a centrosymmetric space group (P-1) and is formed by the racemic mixture of the two enantiomeric forms of the octahedral chiral complex. In Figure 3 is reported the Λ enantiomer with the nitroprusside anion. Also in this case the iron atom lies on a symmetry center in special position and does not allow the identification of NO among the other five CN groups.

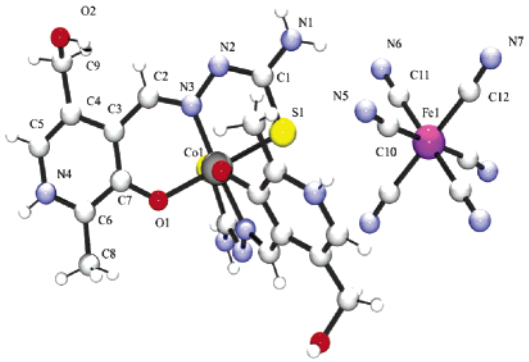

Figure 3. Ball and stick drawing of complex $[Co(HL)_2][Fe(CN)_5(NO)]^-$.

Both monodeprotonated ligands are in mer configuration with sulfur and oxygen atoms in cis and nitrogen atoms in trans position. The Co−S, Co−N, and Co−O coordination distances agree with the literature values found for Co(III) complexes.[14]

Electronic Properties. Polymeric complexes **4** and **5** were investigated also under a magnetic field by means of EPR and magnetic susceptibility measurements in order to deepen our understanding about the electronic properties of the complexes.

The EPR spectra of powdered $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n\cdot 2nH_2O$ **4** and that of $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n\cdot 6nH_2O$ **5** recorded at liquid helium temperature reveal for both an axial anisotropy centered at a magnetic field for which g =2. For **4** two observed transitions correspond to $g_{\parallel} = 2.21$ and $g_{\perp} = 2.05$ and for **5** $g_{\parallel} = 2.19$ and $g_{\perp} = 2.06$; the average value is consequently for both $g = 2.10$ in agreement with a S =1/2 state.

The variation of magnetic susceptibility in function of temperature shows for **4** a constant value of the product X_MT from room to very low temperature ($T <$ 10 K) and then a small decrease below 10 K while **5** behaves in the same way but it decreases when T goes below 50 K, revealing the presence of other interactions. The experimental constant value of the effective magnetic moment in this large temperature range is $\mu_{eff}^2 =$ 3.28 for **4** and $\mu_{eff}^2 = 6.5$ for **5**. The two [Cu(HL)] complexes in the dimeric unit $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}$ of **5** behave as two independent complexes at high temperature, while the interaction detectable below 50 K, as a decrease of the magnetic moment, suggests an antiferromagnetic interaction between the two copper centers.

Biological Test Results. Compounds **4** and **5** were evaluated in vitro for their cytotoxicities, using peripheral blood mononuclear cells (PBMC) as a control, against cell line U937 and CEM.

These two compounds showed no effect on PBMC also at rather high concentrations. The IC_{50} of the compounds presents values in the micromolar range, 10.8

μM (**4**) and 7.5 μM (**5**) on cell line CEM and 9 μM (**4**) and 6.5 μM (**5**) on cell line U937 without remarkable differences but presenting a certain degree of selectivity. An IC_{50} response and time-course effects in tumor cells were tested by flow cytometry. Cell cycle analysis showed that cells were arrested at the G_2/M phase following treatment with **4** and **5**. This action was dose- and time-dependent with a relative potency of the compounds, **4** being more effective than **5** on CEM and U937 cell line. Over 30% of CEM and 20% of U937 cells were arrested after 24 h incubation.

To examine the cell death response within the 24 h, an apoptotic time-course was performed on treated and untreated cell lines using Annexin-V assay. Significant detection of early stages of apoptotic cells was detected by a 6-h treatment of CEM cells with compound **4** (28.2%), and it was very evident after 12 h (57.1%). In contrast, treatment of U937 with the same compound induced a significant, but lesser, percentage of early apoptotic cells after 12−24 h (25.6%). In addition, a significant detection of late apoptotic cells was demonstrated by a 24-h treatment of CEM (42.7%), but not of U937 cells (12.8%). Concerning the action of compound **5** within the 24 h, a lower percentage of early apoptotic and late apoptotic cells were found in treated CEM and U937 cell line as compared to the **4**-treated cell lines. Taken together, these data suggest that compound **4** causes a more potent apoptotic response than compound **5** at the relative IC_{50}. This apoptotic response induced by compound **4**, conspicuous for CEM but not for U937 cells, could reflect a major compound susceptibility of the lymphoblastoid with respect to monoblastoid lineage.

In analogous cell lines, a direct relationship between apoptosis induction and telomerase inhibition by thiosemicarbazone compounds has been recently demonstrated (ref 15 and refs therein). Therefore, a study on telomerase activity was performed using compounds **4** and **5**. For both complexes, a direct action on telomerase can be excluded. In fact, in vitro experiments, performed preincubating CEM or U937 extracts with different concentration of **4** or **5**, showed no influence by these molecules on the enzymatic activity of telomerase (data not shown).

Telomerase activity was then studied using whole cell extracts, obtained from the treated cell lines used for the IC_{50} experiments. At 48 h, **4**- or **5**-treated U937 cells still possessed telomerase activity equivalent to that of untreated cells, demonstrating that growth inhibition and telomerase expression were not correlated. In contrast, **4**- or **5**-treated CEM cells showed a strong decrease of telomerase activity during the course of drug-induced cell death. In fact, telomerase activity was inversely correlated with the increase of PI-stained cells from 12 to 24 h, whereas it did not decrease in the early apoptotic stage characterized by Annexin-V-positive cells. Therefore, the telomerase activity reflected the number of cells reduced by treatment. Noteworthy is that in a previous experiment[15] concerning chloride derivatives we observed an opposite behavior: in fact, compound **2** induced apoptosis to a larger extent on cell line U937 than CEM and telomerase was not inhibited to a significant extent. At present, more work is in progress to understand the reasons that are at the basis of this apparently contradictory behavior.

Conclusions

Nitroprusside showed high anionic affinity toward the synthesized cationic copper complexes. In fact, notwithstanding the experimental conditions, it substituted not only the anionic but also the coordinated chlorine and did not allow isolation of entities with two different anions (Cl^- and nitroprusside) in the same unit.

From the biological viewpoint the two thiosemicarbazone copper complexes with 4 + 1 coordination geometry were found to be very good in inhibiting leukaemic cell proliferation of both CEM and U937 cell line and in inducing apoptosis. On the contrary, the $[Co(HL)_2]_2[Fe(CN)_5(NO)]\cdot 8H_2O$ **6** complex showed no activity, confirming that an accessible coordination position is fundamental for biological interactions. The study of the cell cycle, in particular, revealed an increase of cells in G_2/M phase. In our study, nitroprusside seemed to have no cooperative effect, probably because at the used concentrations, the NO amount released was not enough (taking into account also of the ubiquitous presence of radical scavengers such as superoxide dismutase) to induce apoptotic effects at least on U937 cells known in the literature to be NO sensitive.[17] To conclude, we can say that the absolute potency of these compounds against tumor cells is still low as compared to new generation antiproliferative compounds, but these compounds may constitute a basis for further attempts to increase antitumor activity.

Experimental Section

Synthesis of the Ligands. H_2L: pyridoxal (3-hydroxy-5-hydroxymethyl-2-methylpyridine-4-carbaldehyde) thiosemicarbazone was prepared as previously described.[21]

Synthesis of the Complexes. $[Cu(H_2L)(OH_2)Cl]Cl$ **1**, $[\{Cu(HL)(OH_2)\}_2]Cl_2\cdot 2H_2O$ **2,** and $[Co(HL)_2]Cl\cdot EtOH$ **3** were synthesized as reported in refs 7, 13, and 14, respectively.

$\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n\cdot 2nH_2O$ 4. The complex is obtained by exchange of the two chloride ions in complex $[Cu(H_2L)(OH_2)Cl]Cl$ **1** with a nitroprusside anion owning the same charge. Complex **1** (0.41 mmol) is dissolved by magnetic stirring and gentle heating in 30 mL of water. To this green solution is added an equimolar amount of $Na_2[Fe(CN)_5(NO)]\cdot 2H_2O$ dissolved in the minimum quantity of water. A dark green-brown powder is quickly formed and separated by filtration. By evaporation of the solvent, dark green crystals suitable for X-ray analysis are isolated. In an attempt to synthesize a complex from molecule **1** with only one chloride ion, the same reaction was carried out but with a molar ratio 2:1 of complex **1** with respect to nitroprusside, but the only product obtained was again complex **4**.

$\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n\cdot 6nH_2O$ 5. The complex is obtained by exchange of the two chloride ions in complex $[\{Cu(HL)(OH_2)\}_2]Cl_2\cdot 2H_2O$ **2** with the nitroprusside anion owning the same charge. Complex **2** (0.45 mmol) is dissolved by magnetic stirring and gentle heating in 30 mL of water. To this yellow-green solution is added an equimolar amount of $Na_2[Fe(CN)_5(NO)]\cdot 2H_2O$ dissolved in 15 mL of water. A dark green-brown powder is quickly formed and separated by filtration. By evaporation of the solvent, brown crystals suitable for X-ray analysis are separated.

$[Co(HL)_2]_2[Fe(CN)_5(NO)]\cdot 8H_2O$ 6. The complex is obtained by exchange of the chloride ion in complex **3** with the nitroprusside anion. Complex $[Co(HL)_2]Cl\cdot EtOH$ **3** (0.50 mmol) is dissolved by magnetic stirring and gentle heating in 30 mL of absolute EtOH. To this dark red solution is added an amount of $Na_2[Fe(CN)_5(NO)]\cdot 2H_2O$ dissolved in 15 mL of the

same solvent in a molar ratio 2:1 with respect to complex **3** in order to balance the charge of the anion. The mixture is heated at reflux temperature and stirred for 30 min. By slow evaporation of the solvent, red-brown crystals suitable for X-ray analysis are isolated.

Magnetic Measurements. The magnetic susceptibility of polycrystalline powder samples of complexes **4** and **5** was measured between 2 and 300 K at an applied magnetic field of 1 T using a cryogenic S600 Squid magnetometer. Data were corrected for the diamagnetism of the sample holder that was determined separately in the same temperature range and field, and the underlying diamagnetism of the samples was estimated from Pascal's constants.

X-Band EPR Spectra. EPR measurements were made with a Bruker ESP-380 spectrometer, on powder samples at liquid helium temperature.

Biological Data. Cell Culture. U937, a monoblastoid line, and CEM, an acute lymphoblastic leukemia, were obtained from American Type Culture Collection (ATCC, Rockville, MD) and cultured in RPMI 1640 medium (Gibco BRL, Life Technologies Italia, Milano) supplemented with 10% (v/v) fetal bovine serum (FBS) (Hyclone Europe, Cramlington, UK), 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine at 37 °C and in a humidified atmosphere containing 5% CO_2.

Viability and Proliferation Assays. Cell lines or PBMC were plated at 1×10^5/mL and 2×10^5/mL, respectively, in 96-well plates in the presence of increasing concentrations of compounds. IC_{50} are evaluated after 24 and 48 h of culture by ProCheck cell viability assay (Intergen Canada) and was performed according to the manufacturer protocol. Cells in the exponential phase of growth were seeded in duplicate in 30 mm tissue culture dishes. After seeding (24 h), exponentially proliferating cells were treated with the compounds and the cells were incubated for 48 h. The cell number was counted after 24−48 h of incubation (continuous exposure assay) using a hematocytometer by trypan blue staining.

Apoptotic Assay. For apoptosis determination the MBL MEBCYTO^R Apoptosis Kit Annexin-V Assay was used, this can better identify cells in an earlier stage of apoptosis than assays based on DNA fragmentation. Staining the cells with Annexin V−FITC and propidium iodide (PI) can be used in a bivariate analysis to distinguish between cells undergoing apoptosis (PI negative) and those that are necrotic or dead (PI positive). Cells (2×10^5) were incubated with FITC-labeled Annexin-V and propidium iodide (PI) at RT for 15 min in the dark and analyzed using a FACSCalibur (Beckton-Dickinson).

Telomerase Assay. The telomerase activity was studied by the telomeric repeat amplification protocol (TRAP),[22] the Intergen TRAP-eze telomerase detection kit (Intergen Company, Oxford, UK, or Purchase, New York). The TRAP assay procedure was performed according to the manufacturer protocol. The 12.5% nondenaturing polyacrylamide gel was stained with 1x SYBR green (Molecular Probes, Eugene, OR) and analyzed by Fluor-S MultiImager (Bio-Rad, Hercules, CA).

Cell Cycle Assay. Cells in the exponential phase of growth were treated with the copper complexes using an IC_{50} concentration, and, after 1, 6, 12, 24 h incubations, cells were washed in PBS w/o Ca^{2+} and Mg^{2+} and EDTA 0.5 mM and fixed with 3 mL of 96% ethanol. After fixation, $1-1.5 \times 10^6$ cells were washed once in PBS and stained in 2 mL of propidium iodide (20 μg/mL in PBS) and 25 μL of RNase-A (1 mg/mL in H_2O). Cells were left overnight at 4 °C, and thereafter the analysis was carried out using a FACSCalibur cytofluorimeter.

Acknowledgment. The authors thank MIUR (Ministero dell'Istruzione, Università e Ricerca) for financial support (MIUR, cofin:2001053898) and Dr. Bisceglie also CIRCMSB (Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici) for a scholarship.

Supporting Information Available: Physical measurements, microanalytical and spectroscopic data, crystallographic data, electronic properties, biological data, cell culture, biological tests results, and telomerase assay are available free of charge on the Internet at http://pubs.acs.org. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center with numbers CCDC 262341-43. Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. [fax, +44-(0)1223-336033; e-mail, deposit@ccdc.cam.ac.uk].

References

(1) Richardson, D. R. Iron chelators as therapeutic agents for the treatment of cancer. *Crit. Rev. Oncol./Hematol.* **2002**, *42*, 267−281.
(2) Lovejoy, D. B.; Richardson, D. R. Novel "hybrid" iron chelators derived from aroylhydrazones and thiosemicarbazones demonstrate selective antiproliferative activity against tumor cells. *Blood* **2002**, *100*, 666−676.
(3) Jütten, P.; Schumann, W.; Härtl, A.; Heinisch, L.; Gräfe, U.; Werner, W.; Ulbricht, H. A novel type of nonsteroidal estrone sulfatase inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1339−1342.
(4) Richardson, D. R.; Milnes, K. The Potential of Iron Chelators of the Pyridoxal Isonicotinoyl Hydrazone Class as Effective Antiproliferative Agents II: The Mechanism of Action of Ligands Derived From Salicylaldehyde Benzoyl Hydrazone and 2-Hydroxy-1-Naphthylaldehyde Benzoyl Hydrazone. *Blood* **1997**, *89*, 3025−3038.
(5) West, D. X.; Liberta, A.; Padhye, S. B.; Chikate, R. C.; Sonawane, P. B.; Kumbhar, A. S.; Yerande, R. G. Thiosemicarbazone complexes of copper(II): structural and biological studies. *Coord. Chem. Rev.* **1993**, *123*, 49−71.
(6) Byrnes, R. W.; Mohan, M.; Antholine, W. E.; Xu, R. X.; Petering, D. H. Oxidative stress induced by a copper-thiosemicarbazone complex. *Biochemistry* **1990**, *29*, 7046−7053.
(7) Belicchi-Ferrari, M.; Gasparri-Fava, G.; Tarasconi, P.; Albertini, R.; Pinelli, S.; Starcich, R. Synthesis, spectroscopic and structural characterization, and biological activity of aquachloro-(pyridoxal thiosemicarbazone) copper(II) chloride. *J. Inorg. Biochem.* **1994**, *53*, 13−25.
(8) Cory, J. G.; Cory, A. H.; Rappa, G.; Lorico, A.; Liu, M.-C.; Lin, T.-S.; Sartorelli, A. C. Inhibitors of ribonucleotide reductase: Comparative effects of amino- and hydroxy-substituted pyridine-2-carboxaldehydethiosemicarbazones. *Biochem. Pharmacol.* **1994**, *48*, 335−344.
(9) Kovala-Demertzi, D.; Nath Yadav, P.; Demertzis, M. A.; Coluccia, M. Synthesis, crystal structure, spectral properties and cytotoxic activity of platinum(II) complexes of 2-acetyl pyridine and pyridine-2-carbaldehyde N(4)-ethyl-thiosemicarbazones. *J. Inorg. Biochem.* **2000**, *78*, 347−354.
(10) Lakovidou, Z.; Papageorgiou, A.; Demertzis, M. A.; Mioglou, E.; Mourelatos, D.; Kotsis, A.; Yadav, P. N.; Kovala-Demertzi, D. Platinum(II) and palladium(II) complexes with 2-acetylpyridine thiosemicarbazone: cytogenetic and antineoplastic effects. *Anticancer Drugs* **2001**, *12*, 65−70.
(11) Belicchi-Ferrari, M.; Bisceglie, F.; Leporati, E.; Pelosi, G.; Tarasconi, P. Synthesis, Solution Chemistry, X-ray Structure and Biological Activity of Novel Pyridoxal Thiosemicarbazone Derivatives. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 781−788.
(12) Casas, J. S.; Rodríguez-Argüelles, M. C.; Russo, U.; Sànchez, A.; Sordo, J.; Vàzquez-Lòpez, A.; Pinelli, S.; Lunghi, P.; Bonati, A.; Albertini, R. Diorganotin(IV) complexes of pyridoxal thiosemicarbazone: Synthesis, spectroscopic properties and biological activity. *J. Inorg. Biochem.* **1998**, *69*, 283−292.
(13) Belicchi-Ferrari, M.; Gasparri-Fava, G.; Pelizzi, C.; Tarasconi, P.; Tosi, G. Thiosemicarbazones as coordinating agents. Part 2. Synthesis, spectroscopic characterization, and *X*-ray structure of aquachloro(pyridoxal thiosemicarbazone)manganese(II) chloride and aqua(pyridoxal thiosemicarbazonato)copper(II) chloride monohydrate. *J. Chem. Soc., Dalton Trans.* **1987**, 227−233.
(14) Belicchi-Ferrari, M.; Gasparri-Fava, G.; Pelosi, G.; Rodríguez-Argüelles, M. C.; Tarasconi, P. Cobalt(III) complexes with thiosemicarbazones as coordinating agents. Spontaneous resolution by crystallization and absolute configuration. *J. Chem. Soc., Dalton Trans.* **1995**, 3035−3040.
(15) Belicchi-Ferrari, M.; Bisceglie, F.; Pelosi, G.; Tarasconi, P.; Pinelli, S.; Bergamo, A.; Sava, G. Synthesis, characterization and biological activity of copper complexes with pyridoxal thiosemicarbazone derivatives. X-ray crystal structure of three dimeric complexes. *J. Inorg. Biochem.* **2004**, *98*, 301−312.
(16) Butler, A. R.; Megson, I. L. Non-Heme Iron Nitrosyls in Biology. *Chem. Rev.* **2002**, *102*, 1155−1165.

(17) Kim, P. K. M.; Zamora, R.; Petrosko, P.; Billiar, T. R. The regulatory role of nitric oxide in apoptosis. *Int. Immunopharmacol.* **2001**, *1*, 1421−1441.
(18) Terwel, D.; Nieland, L. J. M.; Schutte, B.; Reutelingsperger, C. P. M.; Ramaekers, F. C. S.; Steinbusch, H. W. M. *S*-nitroso-*N*-acetylpenicillamine and nitroprusside induce apoptosis in a neuronal cell line by the production of different reactive molecules. *Eur. J. Pharmacol.* **2000**, *400*, 19−33.
(19) Chen, Z. N.; Wang, J. L.; Qiu, J.; Miao, F. M.; Tang, W. X. Crystal Structure and Magnetic Properties of a 2D Sheetlike.mu.-Cyanido- and.mu.-Oxamidato-Bridged Fe(III)-Cu(II) Bimetallic Complex. *Inorg. Chem.* **1995**, *34*, 2255−2262.
(20) Shyu, H. L.; Wei, H. H.; Wang, Yu Preparation, characterization and crystal structure of $[Ni(bpy)_3][Fe(CN)_5(NO)]3H_2O$ and one-dimensional cyano-bridged $[Ni(en)_2Fe(CN)_5(NO)]H_2O$. *Inorg. Chim. Acta* **1997**, *258*, 81−86.
(21) Belicchi-Ferrari, M.; Gasparri-Fava, G.; Leporati, E.; Pelizzi, C.; Tarasconi, P.; Tosi, G. Thiosemicarbazones as coordinating agents. Solution chemistry and *X*-ray structure of pyridoxal thiosemicarbazone trihydrate and spectroscopic properties of its metal complexes. *J. Chem. Soc., Dalton Trans.* **1986**, 2455−2461.
(22) Kim, N. W.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. Specific association of human telomerase with immortal cells and cancer. *Science* **1994**, *266*, 2011−2015.

JM049529N