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

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Appendix 1

Physical Measurements. Elemental analyses (C, H, N, S) were performed with a Carlo Erba model EA 1108 automatic analyzer. IR spectra (4000 – 400 cm^{-1}) for KBr discs were recorded on a Nicolet 5PC FT. Melting points were determined with a Gallenkamp instrument.

Microanalytical and spectroscopic data.

Compound 4. Anal. Calcd. for $C_{14}H_{16}CuFeN_{10}O_5S$: C, 30.32; H, 2.52; N, 25.24; S, 5.77%. Found: C, 30.29; H, 2.64; N, 24.92; S, 4.98. IR: (KBr discs, cm^{-1}) 3460 (m, br) ν(OH), 3386 (s) 3371 (m) 3150 (w) ν(NH), 2191 (m) 2157 (m) 2146 (m) ν(C≡N), 1933 (s) ν(NO), 1633 (m) 1611 (m) 1504 (mw) ν(C=N) + ν(C=C), 927 (w) ν(C=S). M. p.: >300 °C.

Compound 5. Anal. Calcd. for $C_{23}H_{34}Cu_2FeN_{14}O_{11}S_2$: C, 29.70; H, 3.66; N, 21.08; S, 6.90%. Found: C, 30.10; H, 3.70; N, 21.20; S, 6.90. IR: (KBr discs, cm^{-1}) 3440 (s, br) ν(OH), 3356 (m) ν(NH), 2919 (s) ν(CH), 2183 (mw) 2146 (mw) ν(C≡N), 1937 (s) ν(NO), 1652 (m) 1606 (m) 1499 (m) ν(C=N) + ν(C=C), 825 (w) ν(C=S). M. p.: >300 °C.

Compound 6 Anal. Calcd. for $C_{41}H_{60}Co_2FeN_{22}O_{17}S_4$: C, 35.19; H, 4.00; N, 22.03; S, 9.15%. Found: C, 35.22; H, 4.01; N, 22.18; S, 9.21. IR: (KBr discs, cm^{-1}) 3424 (m, br) ν(OH), 3291 (m) 3092 (m) ν(NH), 2134 (mw) ν(C≡N), 1912 (s) ν(NO), 1631 (m, sh) ν(C=N) + ν(C=C), 925 (w) ν(C=S). M. p.: >300 °C.

Crystallography. Relevant data concerning data collection and details of structure refinement are summarized in Table 1. Intensity data were collected on a SMART 1000 Bruker AXS diffractometer with Mo-Kα radiation for compound **4** and on a Philips single-crystal computer controlled by the θ–2θ technique with Mo-Kα radiation for **5** and **6**. The structures were solved using direct methods (SIR-97[1]) and refinements were carried out by full matrix least-squares cycles SHELXL97[2] for all compounds. Anisotropic thermal motion was assumed for all non-hydrogen atoms. Hydrogen atoms were located on a difference map and refined for compounds **4** and **5** (the water hydrogen atoms in complex **5** were not located). In compound **6** the hydrogen atoms were calculated with standard geometry and not refined. Atomic scattering factors were taken from ref. 3. An ENCORE91 computer was employed with the program PARST[4] for the geometrical description of the structures and ORTEP[5] and PLUTO[6] for the structure drawings.

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(2) Sheldrick, G. *SHELXL 97 A Program for Structure Refinement*, University of Goettingen, Germany, **1997**.

(3) International Tables for X-Ray Crystallography, Kynoch Press, 4, Birmingham, UK, **1974**.

(4) Nardelli, M. *PARST95. An update to PARST: a system of Fortran routines for calculating molecular structure parameters from the results of crystal structure analyses, J. Appl. Cryst.* **1995**, *28*, 659.

(5) Johnson, C. K.; Burnett, M.N. *ORTEPIII.* Report ORNL-6895, Oak Ridge National Laboratory, Tennessee, USA, **1996**.

(6) Spek, A.L. *PLATON. A Multipurpose Crystallographic Tool*, Utrecht University, Utrecht, The Netherlands, **1999**.

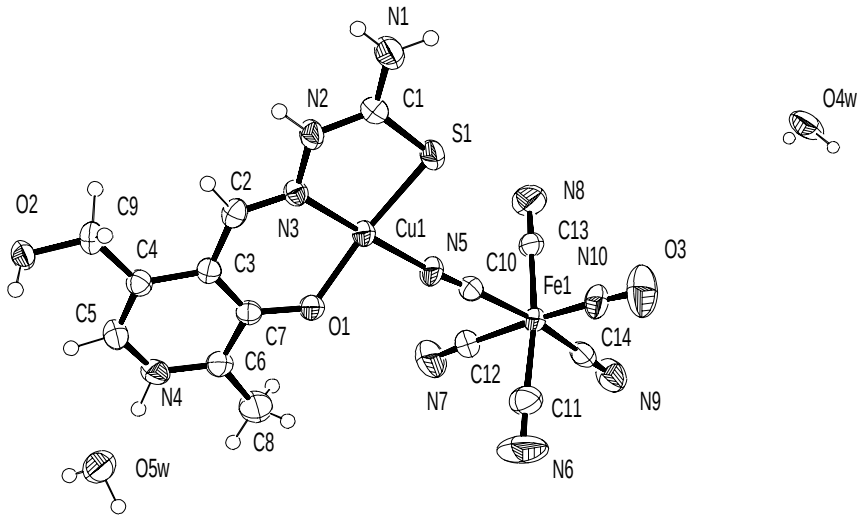


Figure 1. Ortep view of the monomer $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}\cdot 2H_2O$. Ellipsoids are at 50% probability level.

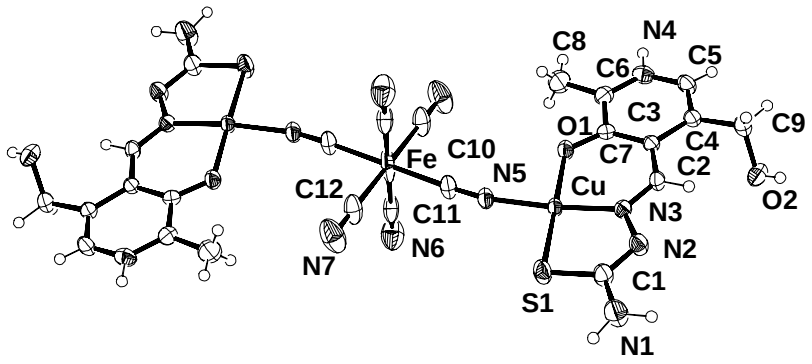


Figure 2. Ortep view of $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}$.

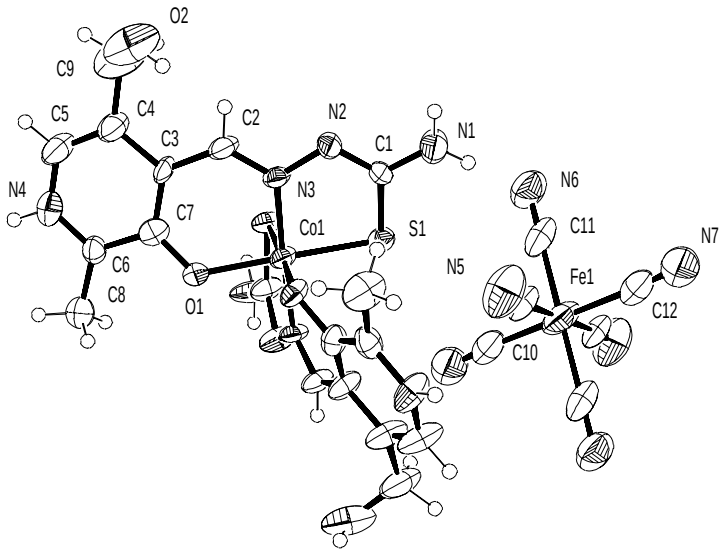


Figure 3. Ortep view of complex $[Co(HL)_2][Fe(CN)_5(NO)]^-$.

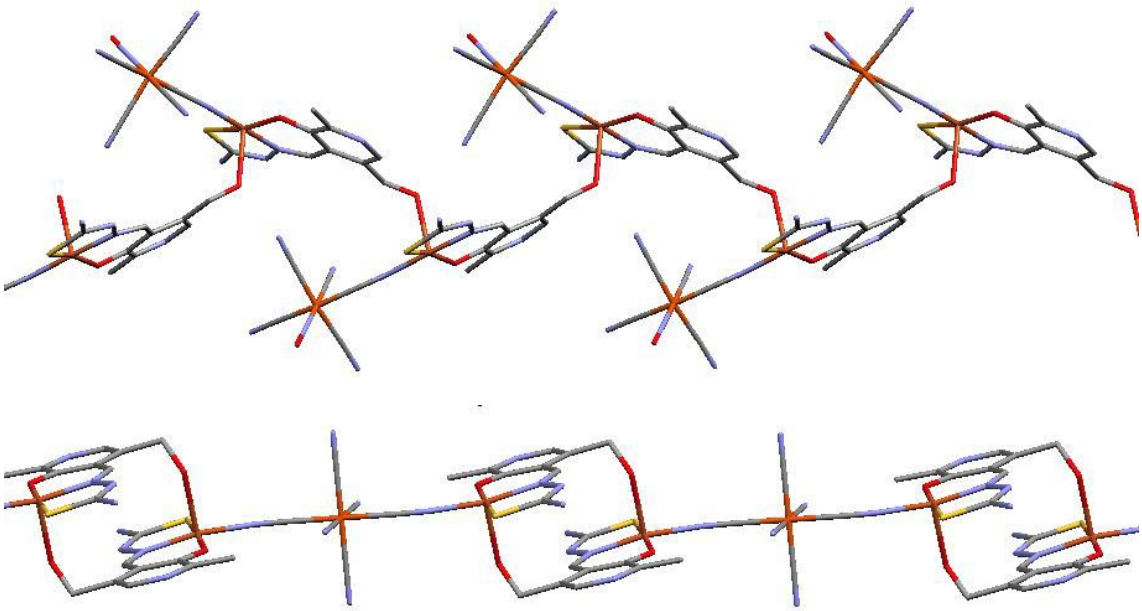


Figure 4. Capped-stick view of the polymer $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n$ (above) and of the chain of dimers $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n$ (below)

Table 1. Main crystallographic data for the compounds studied.

	(**4**)	(**5**)	(**6**)
Formula	$C_{14}\,H_{16}\,Cu\,Fe\,N_{10}\,O_5\,S$	$C_{23}\,H_{34}\,Cu_2\,Fe\,N_{14}\,O_{11}\,S_2$	$C_{41}\,H_{60}\,Co_2\,Fe\,N_{22}\,O_{17}\,S_4$
M. W.	555.80	929.67	1435.02
Crystal System	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1/n$ (No. 14)	$P2_1/n$ (No. 14)	P-1 (No. 2)
a, b, c [Å]	13.157(3) 13.641(3) 14.205(4)	13.946(4) 7.163(1) 18.389(5)	15.278(3) 13.533(3) 7.977(1)
α, β, γ [°]	90 115.98(1) 90	90 93.37(2) 90	84.40(1) 76.78(1) 77.22(1)
V [$Å^3$]	2292(1)	1833.8(8)	1563.9(5)
Z	4	2	1
D(calc) [g/cm^3]	1.611	1.684	1.524
μ(MoKα) [/mm]	1.698	1.727	0.968
F(000)	1124	948	740
Crystal Size [mm]	0.40 x 0.20 x 0.70	0.30 x 0.20 x 0.60	0.32 x 0.40 x 0.60
Temperature (K)	298.15	298.15	298.15
Radiation [Å]	MoKα 0.71069	MoKα 0.71069	MoKα 0.71069
θ Min-Max [°]	3.0, 30.0	3.0, 30.0	3.0, 30.0
Dataset	-14 14 ; -15 15 ; -15 15	-19 19; -10 10; 0 25	-17 18; -15 16; 0 9
Tot., Uniq. Data	16587, 16143	10654, 5329	5490, 5490
Nref, Npar	12894, 354	5329, 289	5490, 394
R, wR2, S	0.0479, 0.1600, 0.99	0.0498, 0.1474, 0.89	0.0611, 0.1970, 0.758
Min. and Max. Resd. Dens. [$e/Å^3$]	-0.46, 0.77	-1.82, 1.64	-0.53, 0.93

Table 2. Selected bond distances (Å) and angles (°) for $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n \cdot 2nH_2O$ **4**

Fe1-N10	1.647(3)	S1-C1	1.711(4)	N9-C14	1.143(5)
Fe1-C14	1.939(4)	O1-C7	1.305(3)	N7-C12	1.131(6)
Fe1-C12	1.936(4)	O2-C9	1.419(4)	N8-C13	1.148(5)
Fe1-C10	1.950(3)	O3-N10	1.133(4)	N6-C11	1.141(5)
Fe1-C11	1.934(4)	N1-C1	1.306(6)	C2-C3	1.438(5)
Fe1-C13	1.926(3)	N2-N3	1.365(5)	C3-C4	1.429(4)
Cu1-S1	2.263(1)	N2-C1	1.349(4)	C3-C7	1.406(5)

Cu1-O1	1.907(2)	N3-C2	1.305(4)	C4-C5	1.351(6)
Cu1-O2^{I}	2.361(2)	N4-C5	1.334(5)	C4-C9	1.522(5)
Cu1-N3	1.974(3)	N4-C6	1.339(4)	C6-C7	1.421(6)
Cu1-N5	1.957(3)	N5-C10	1.135(4)	C6-C8	1.480(8)

N10-Fe1-C14	93.05(15)	O1-Cu1-O2^{I}	90.42(8)	C2-C3-C7	122.5(3)
N10-Fe1-C12	176.26(17)	O1-Cu1-N5	90.62(10)	C4-C3-C7	119.4(2)
N10-Fe1-C10	96.91(14)	O2^{I}-Cu1-N3	86.70(9)	C3-C4-C5	118.5(3)
N10-Fe1-C11	93.99(16)	O2^{I}-Cu1-N5	95.31(9)	C3-C4-C9	121.0(2)
N10-Fe1-C13	95.02(15)	N3-Cu1-N5	177.39(10)	C5-C4-C9	120.5(3)
C14-Fe1-C12	83.41(16)	Cu1-S1-C1	96.81(12)	N4-C5-C4	121.0(3)
C14-Fe1-C10	170.00(13)	Cu1-O1-C7	127.03(18)	N4-C6-C7	117.9(3)
C14-Fe1-C11	89.06(15)	N3-N2-C1	119.5(3)	N4-C6-C8	119.2(3)
C14-Fe1-C13	90.99(14)	Cu1-N3-N2	117.32(19)	C7-C6-C8	122.9(4)
C12-Fe1-C10	86.62(14)	Cu1-N3-C2	126.9(2)	O1-C7-C3	125.2(2)
C12-Fe1-C11	84.77(16)	N2-N3-C2	115.7(3)	O1-C7-C6	116.1(3)
C12-Fe1-C13	86.27(15)	C5-N4-C6	124.5(3)	C3-C7-C6	118.7(3)
C10-Fe1-C11	89.39(14)	Cu1-N5-C10	175.4(3)	Fe1-C14-N9	178.0(3)
C10-Fe1-C13	89.01(13)	Fe1-N10-O3	176.8(3)	Fe1-C12-N7	177.4(4)
C11-Fe1-C13	170.98(14)	S1-C1-N1	121.5(3)	Fe1-C10-N5	177.8(3)
S1-Cu1-O1	165.78(7)	S1-C1-N2	120.0(2)	Fe1-C11-N6	177.8(3)
S1-Cu1-O2^{I}	103.22(7)	N1-C1-N2	118.5(3)	Fe1-C13-N8	178.0(3)
S1-Cu1-N3	85.83(8)	N3-C2-C3	124.0(3)	O2-C9-C4	112.7(2)
S1-Cu1-N5	92.08(9)	C2-C3-C4	118.1(3)		

I =3/2-x,-1/2+y,1/2-z

Table 3. Selected bond distances (Å) and angles (°) for $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n \cdot 6nH_2O$ **5**

Cu-S1	2.249(1)	O1-C7	1.286(4)	N6-C11	1.133(6)
Cu-O1	1.925(2)	O2-C9	1.432(5)	N7-C12	1.121(5)
Cu-O2	2.369(3)	N1-C1	1.354(5)	C2-C3	1.448(5)
Cu-N3	1.965(3)	N2-N3	1.388(4)	C3-C4	1.418(4)
Cu-N5	1.980(3)	N2-C1	1.314(4)	C3-C7	1.417(4)
Fe-C10	1.928(3)	N3-C2	1.292(4)	C4-C5	1.364(5)
Fe-C11	1.835(4)	N4-C5	1.355(5)	C6-C7	1.434(5)
Fe-C12	1.927(4)	N4-C6	1.323(4)	C6-C8	1.481(6)
S1-C1	1.728(3)	N5-C10	1.139(4)		

S1-Cu-O1	174.61(7)	Cu-O1-C7	127.0(2)	C4-C3-C7	119.3(3)
S1-Cu-O2	99.82(7)	Cu-O2-C9	129.2(2)	C3-C4-C5	119.4(3)
S1-Cu-N3	85.97(8)	N3-N2-C1	113.7(3)	N4-C5-C4	120.1(3)
S1-Cu-N5	93.22(9)	Cu-N3-N2	120.1(2)	N4-C6-C7	118.7(3)
O1-Cu-O2	85.46(10)	Cu-N3-C2	125.7(2)	N4-C6-C8	120.1(3)
O1-Cu-N3	92.43(10)	N2-N3-C2	114.2(3)	C7-C6-C8	121.2(3)
O1-Cu-N5	87.46(11)	C5-N4-C6	124.4(3)	O1-C7-C3	125.8(3)
O2-Cu-N3	96.12(10)	Cu-N5-C10	169.2(3)	O1-C7-C6	116.0(3)
O2-Cu-N5	93.90(11)	S1-C1-N1	117.1(3)	C3-C7-C6	118.1(3)
N3-Cu-N5	169.94(11)	S1-C1-N2	125.6(3)	Fe-C10-N5	176.6(3)
C10-Fe-C11	89.75(15)	N1-C1-N2	117.3(3)	Fe-C11-N6	179.7(4)
C10-Fe-C12	91.40(14)	N3-C2-C3	125.8(3)	Fe-C12-N7	179.9(4)
C11-Fe-C12	90.29(17)	C2-C3-C4	118.9(3)		
Cu-S1-C1	94.62(12)	C2-C3-C7	121.8(3)		

Table 4. Selected bond distances (Å) and angles (°) for $[Co(HL)_2]_2[Fe(CN)_5(NO)] \cdot 8H_2O$ **6**

Co1-S1	2.204(3)	N1-C1	1.315(12)	C2-C3	1.422(13)

Co1-S1A	2.220(3)	N1A-C1A	1.345(14)	C2A-C3A	1.428(15)
Co1-O1	1.959(6)	N2-N3	1.391(10)	C3-C4	1.442(15)
Co1-O1A	1.982(7)	N2-C1	1.325(12)	C3-C7	1.410(12)
Co1-N3	1.911(7)	N2A-N3A	1.379(12)	C3A-C4A	1.418(17)
Co1-N3A	1.911(7)	N2A-C1A	1.338(14)	C3A-C7A	1.415(13)
Fe1-C10	1.848(11)	N3-C2	1.314(13)	C4-C5	1.353(16)
Fe1-C11	1.945(14)	N3A-C2A	1.300(14)	C4-C9	1.546(16)
Fe1-C12	1.940(14)	N4-C5	1.349(12)	C4A-C5A	1.367(18)
S1-C1	1.727(8)	N4-C6	1.336(14)	C4A-C9A	1.544(14)
S1A-C1A	1.706(9)	N4A-C5A	1.331(13)	C6-C7	1.425(14)
O1-C7	1.301(13)	N4A-C6A	1.362(15)	C6-C8	1.488(12)
O1A-C7A	1.319(13)	N5-C10	1.094(15)	C6A-C7A	1.418(15)
O2-C9	1.38(2)	N6-C11	1.155(18)	C6A-C8A	1.485(13)
O2A-C9A	1.414(15)	N7-C12	1.137(18)		

S1-Co1-S1A	92.91(11)	Co1-N3-N2	120.3(5)	C5-C4-C9	119.1(11)
S1-Co1-O1	177.1(2)	Co1-N3-C2	123.2(6)	C3A-C4A-C5A	120.7(11)
S1-Co1-O1A	90.0(2)	N2-N3-C2	116.5(8)	C3A-C4A-C9A	121.2(9)
S1-Co1-N3	86.4(2)	Co1-N3A-N2A	120.6(5)	C5A-C4A-C9A	117.9(10)
S1-Co1-N3A	90.4(2)	Co1-N3A-C2A	124.8(6)	N4-C5-C4	122.3(11)
S1A-Co1-O1	90.0(2)	N2A-N3A-C2A	114.6(8)	N4A-C5A-C4A	119.1(10)
S1A-Co1-O1	177.0(2)	C5-N4-C6	122.9(9)	N4-C6-C7	118.9(9)
S1A-Co1-N3	92.2(2)	C5A-N4A-C6A	125.8(9)	N4-C6-C8	118.4(9)
S1A-Co1-N3A	86.6(2)	S1-C1-N1	119.7(7)	C7-C6-C8	122.8(9)
O1-Co1-O1A	87.1(3)	S1-C1-N2	123.4(7)	N4A-C6A-C7A	116.2(9)
O1-Co1-N3	93.3(3)	N1-C1-N2	116.9(9)	N4A-C6A-C8A	119.5(9)
O1-Co1-N3A	89.9(3)	S1A-C1A-N1A	118.3(7)	C7A-C6A-C8A	124.2(9)
O1A-Co1-N3	88.7(3)	S1A-C1A-N2A	125.3(7)	O1-C7-C3	124.2(8)
O1A-Co1-N3A	92.8(3)	N1A-C1A-N2A	116.3(9)	O1-C7-C6	116.9(9)
N3-Co1-N3A	176.6(3)	N3-C2-C3	127.2(9)	C3-C7-C6	118.9(9)
C10-Fe1-C11	88.7(5)	N3A-C2A-C3A	125.2(9)	O1A-C7A-C3A	124.0(9)
C10-Fe1-C12	87.3(5)	C2-C3-C4	118.6(9)	O1A-C7A-C6A	115.4(9)
C11-Fe1-C12	89.3(5)	C2-C3-C7	122.3(9)	C3A-C7A-C6A	120.6(9)
Co1-S1-C1	95.1(3)	C4-C3-C7	119.0(8)	O2-C9-C4	104.7(10)
Co1-S1A-C1A	94.1(4)	C2A-C3A-C4A	119.7(10)	O2A-C9A-C4A	110.2(9)
Co1-O1-C7	123.4(6)	C2A-C3A-C7A	122.6(9)	Fe1-C10-N5	173.7(11)
Co1-O1A-C7A	119.5(6)	C4A-C3A-C7A	117.6(9)	Fe1-C11-N6	177.9(11)
N3-N2-C1	113.1(8)	C3-C4-C5	117.9(10)	Fe1-C12-N7	174.6(13)
N3A-N2A-C1A	111.8(8)	C3-C4-C9	122.4(9)		

Appendix 2

Electronic Properties.

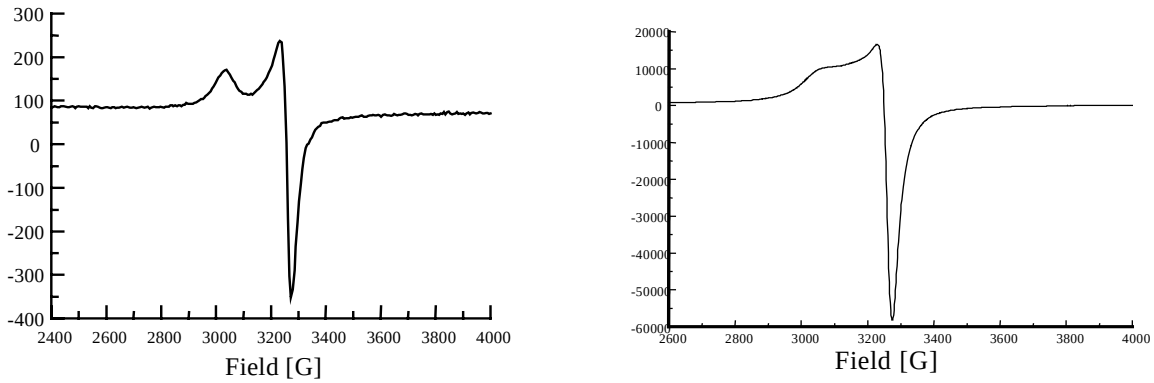


Figure 5. EPR spectrum of powdered $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n \cdot 2nH_2O$ **4** (left) [attenuation 20 dB, modulation 10 G, gain 30 dB, t_c = 21 ms, sweep time = 84 s] and of $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n \cdot 6nH_2O$ **5** (right) [attenuation 17 dB, modulation 10 G, gain 27 dB, t_c = 82 ms, sweep time = 336 s]

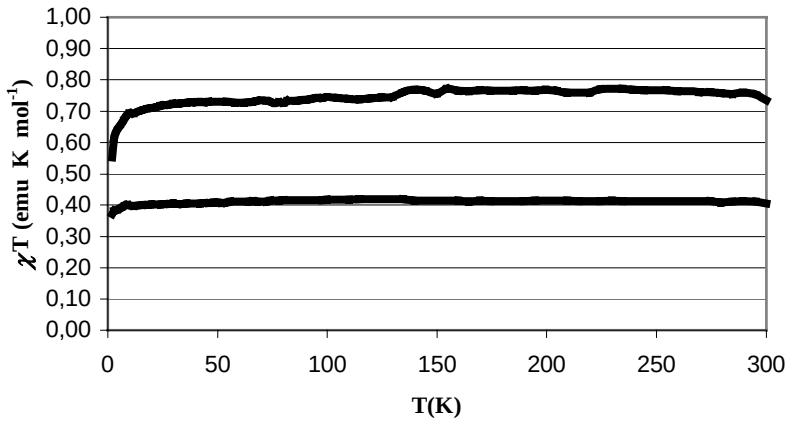


Figure 6. X_MT vs T magnetic data for powdered $\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n \cdot 2nH_2O$ **4** (line below) and $\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n \cdot 6nH_2O$ **5** (line above).

Appendix 3

Biological Data
Cell Culture.

PBMC used for the control were purified from 10 healthy subjects by density gradient centrifugation on a Ficoll/sodium diatrizoate solution (Nycomed Pharma As, Oslo, Norway). PBMCs, were cultured in RPMI 1640 medium, supplemented with 10% FBS, at a concentration of 2 x 10^5 cells per well, in the absence or in the presence of rIL-2 (20U/ml) and phytohemaglutinin (PHA) at 1µg/ml for 72 hours before treatment with compounds.

Biological tests results

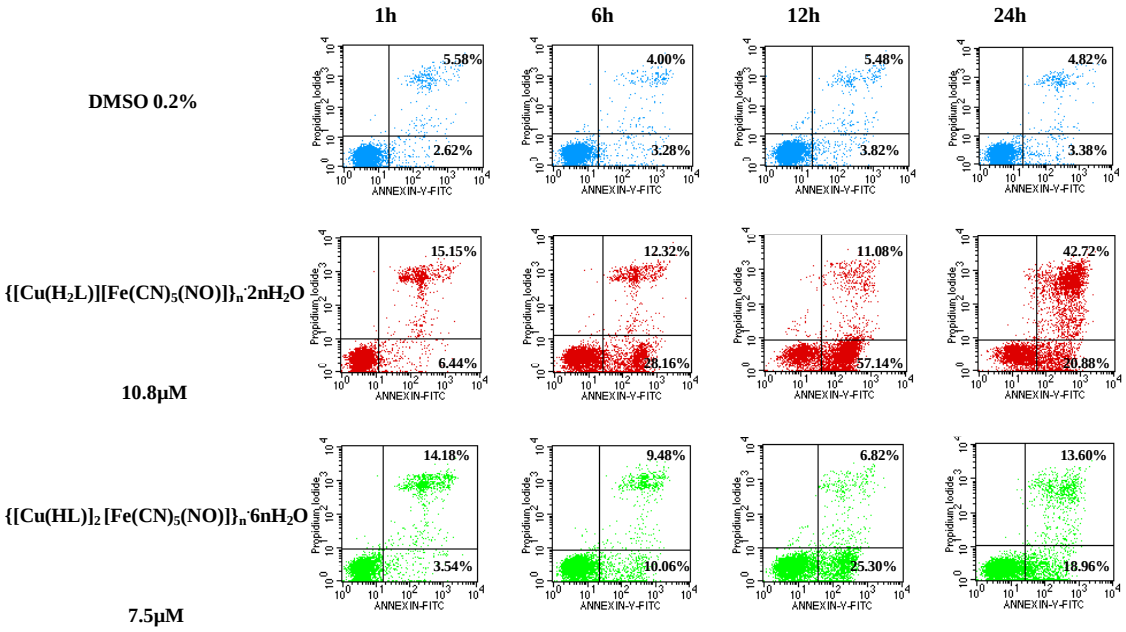


Figure 7. Quantification of apoptosis for cell line CEM. Numbers on the dot plot refer to the percentage of cells undergoing early stages of apoptosis (lower right quadrant) and later stages of apoptosis or dead (upper right quadrant).

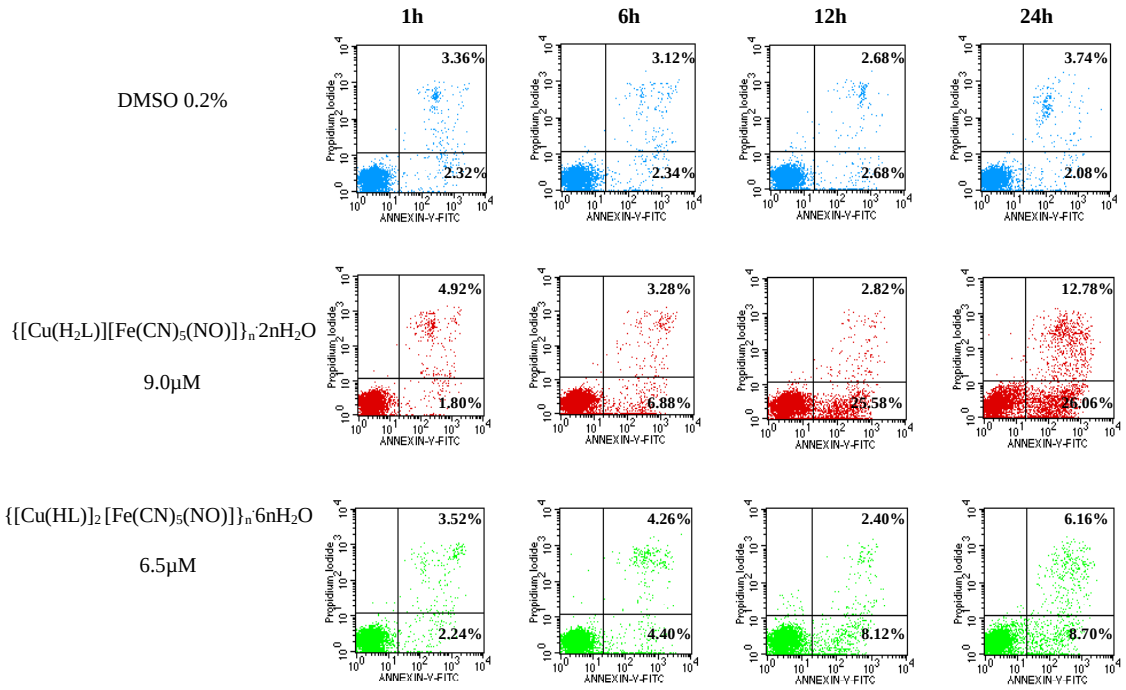
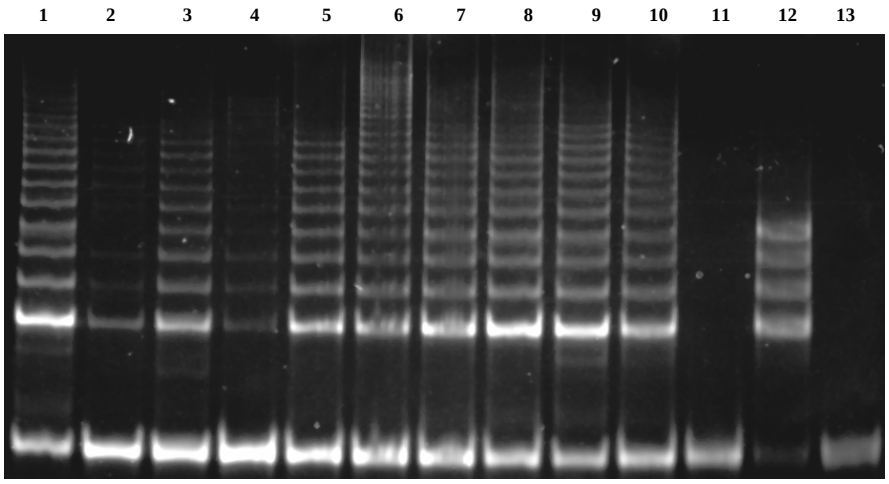


Figure 8. Quantification of apoptosis for cell line U937. Numbers on the dot plot refer to the percentage of cells undergoing early stages of apoptosis (lower right quadrant) and later stages of apoptosis or dead (upper right quadrant).

Telomerase Assay. 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). The quantitative value of telomerase activity was expressed as a Total Product Generated (TPG) unit using the following formula:

TPG = [(TSRTS/ ICS)/ (TNC/ ICT)] x100 (using 0.1 amol of TSR8)

where TSRCT=Telomerase sample-RNase treated sample, ICS=Internal control of sample, TNC=TSR8-negative control and ICT=Internal control of TSR8.



1= untreated control

2= **4** 48h

3= **4** 24h

4= **5** 48h

5= **5** 24h

6= untreated control

7= **4** 48h

8= **4** 24h

9= **5** 24h

10= **5** 48h

11= + RNase-A

12= TSR8

13= negative control

$\{[Cu(H_2L)][Fe(CN)_5(NO)]\}_n \cdot 2nH_2O$ **4**

$\{[Cu(HL)]_2[Fe(CN)_5(NO)]\}_n \cdot 6nH_2O$ **5**

Figure 9. TRAP ASSAY for evaluation of telomerase activity using whole cell extracts of U937 cells.