Supporting Information

Ni(II), Cu(II), and Zn(II) diethyldithiocarbamate complexes show various activity against proteasome in a breast cancer cell line

Table of Contents

Table 1. Basic Information & Reaction Yields.

Compound	**Formula**	**Mr**	**Color**	**Yield (%)**	**Dimer**
$Cu(EtDTC)_2$	$CuC_{10}H_{20}N_2S_4$	360.06	brown	60	yes
$Zn(EtDTC)_2$	$ZnC_{10}H_{20}N_2S_4$	361.89	white	45	yes
$Ni(EtDTC)_2$	$NiC_{10}H_{20}N_2S_4$	355.24	green	70	not

Crystal Structure Determination

Complexes were crystallized from mixtures of diethylether and acetone.

The intensity data were collected on a KUMA KM-4 CCD kappa-axis diffractometer using a graphite monochromatized Mo-Kα radiation (λ = 0.71069 Å). The structure was solved by direct methods. Non-hydrogen atoms were refined anisotropically while hydrogen atoms were inserted in calculated positions and isotropically refined assuming a "ride-on" model. The crystal data for compound **1** and other pertinent information are summarized in Table 1. Table 2 contains atomic parameters of non-hydrogen atoms and a list of interatomic distances and angles is in Table 3. The programs used were: SHELX-97 [1] program package for the structure determination and structure refinement and tables, and the drawings were made by XP program of Bruker SHELXTL V5.1 [2] program package.

CCDC 654111 - 654116 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html

[or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk]

[1] G.M.Sheldrick: SHELX-97 program package, University of Goettingen 1997
[2] G.M. Sheldrick: SHELXTL V 5.10, Bruker AXS Inc., Madison, Wl, 1997

Table 2. Crystal Data & Structure Refinement.

Empirical formula	$C_{10}H_{20}N_2S_4Ni$	$C_{10}H_{20}N_2S_4Zn$	$C_{20}H_{40}N_4S_8Cu_2$
Formula weight	355.24	361.89	720.12
Temperature	120 K		
Wavelength	0.71069 Å		
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	P2(1)/c	P2(1)/n	P2(1)/n
Unit cell dimensions	a = 6.0571(12)Å b = 11.445(2)Å c = 11.549(2)Å α = 90° β = 95.89(3)° γ = 90°	a = 9.763(2)Å b = 10.684(2)Å c = 15.658(3))Å α = 90° β = 103.97(3)° γ = 90°	a = 9.6740(19)Å b = 10.554(2)Å c = 15.498(3)Å α = 90° β = 101.67(3)° γ = 90°
Volume	796.4(3) A^3	1584.9(5) A^3	1549.6(5) A^3
Z	2	4	2
Calculated density	1.481 $Mg.m^{-3}$	1.517 $Mg.m^{-3}$	1.543 $Mg.m^{-3}$
Absorption coefficient	1.724 mm^{-1}	2.058 mm^{-1}	1.929 mm^{-1}
F(000)	372	752	748
Crystal size	0.25 x 0.10 x 0.07 mm	0.25 x 0.20 x 0.15 mm	0.25 x 0.20 x 0.10 mm
θ range for data collection	3.55 to 27.25 °	3.29 to 27.24 °	3.31 to 27.16 °
Limiting indices	-7<=h<=7,-14<=k<=12,-14<=l<=14	-12<=h<=9,-13<=k<=13,-17<=l<=19	-12<=h<=11,-12<=k<=13,-19<=l<=19
Reflections collected / unique	6983 / 1658 [R_{int} = 0.0733]	11500 / 3272 [R_{int} = 0.0868]	10737 / 3219 [Rint = 0.0542]
Completeness to 2θ=25,00	99.6 %	99.7 %	99.7 %
Absorption correction	Psi-scan	Psi-scan	Psi-scan
Max. and min. transmission	1.000 and 0.757	1.000 and 0.839	1.000 and 0.535
Refinement method	Full-matrix least-squares on F^2		
Data / restrains / parameters	1658 / 0 / 81	3272 / 0 / 158	3219 / 0 / 158
Goodness-of-fit on F^2	1.051	1.059	1.063
Final R indices [I>2σ(I)]	R1 = 0.0372, wR2 = 0.1013	R1 = 0.0369, wR2 = 0.0971	R1 = 0.0298, wR2 = 0.0810
R indices (all data)	R1 = 0.0378, wR2 = 0.1023	R1 = 0.0376, wR2 = 0.0981	R1 = 0.0305, wR2 = 0.0817
Largest diff. Peak and hole	1.064 and -0.592 e.$Å^{-3}$	1.075 and -0.905 e.$Å^{-3}$	0.693 and -0.756 e.$Å^{-3}$

Figure 1. Molecular structures of Monomer Complexes.

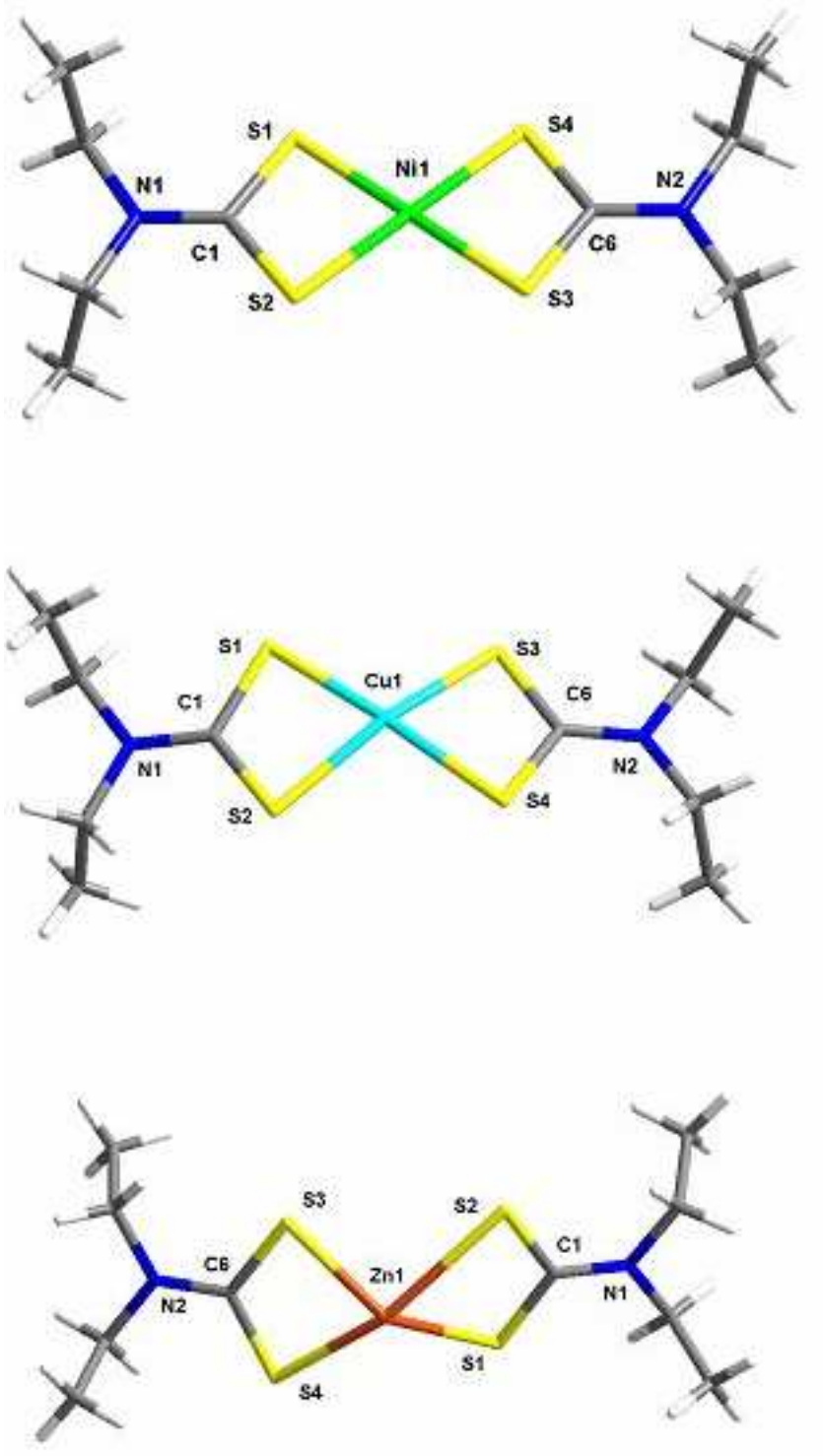


Figure 2. Molecular Structures of Dimers.

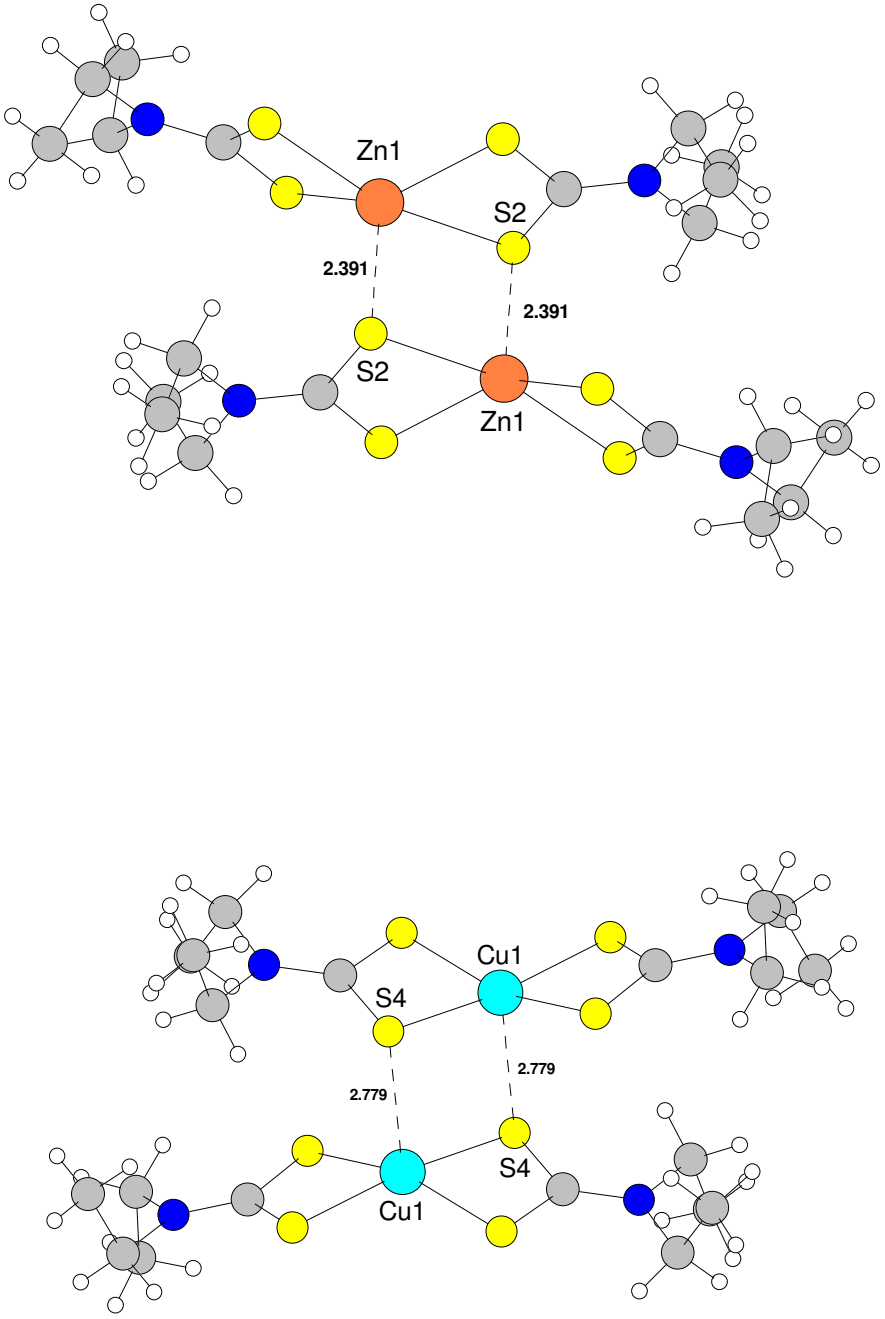
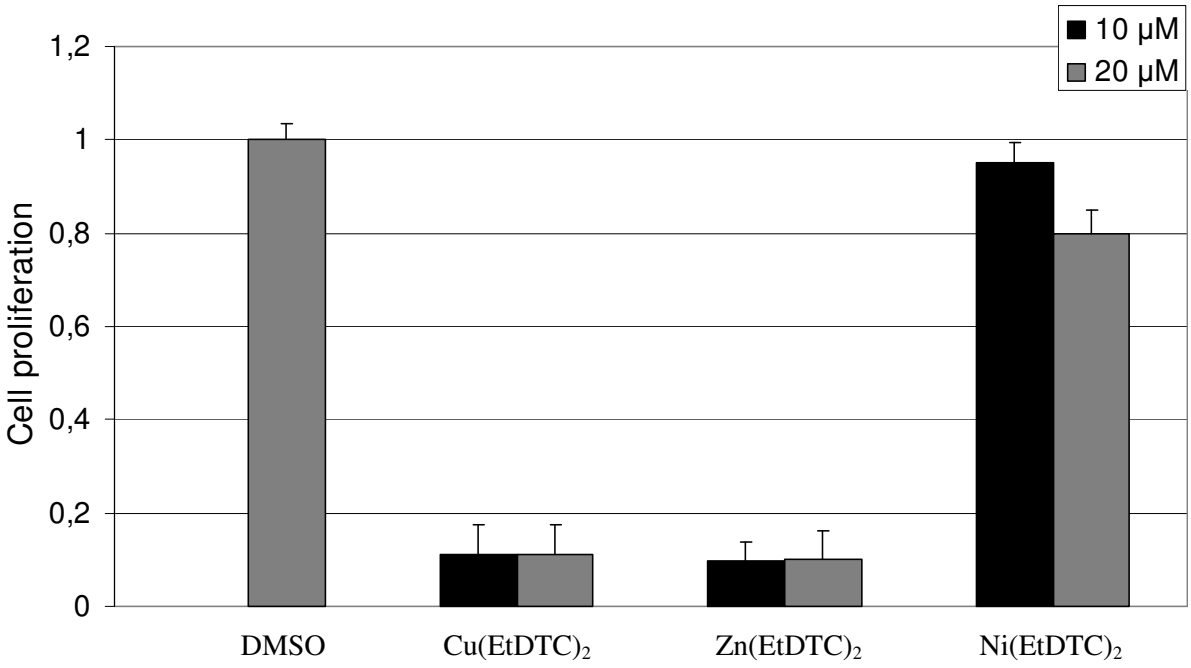
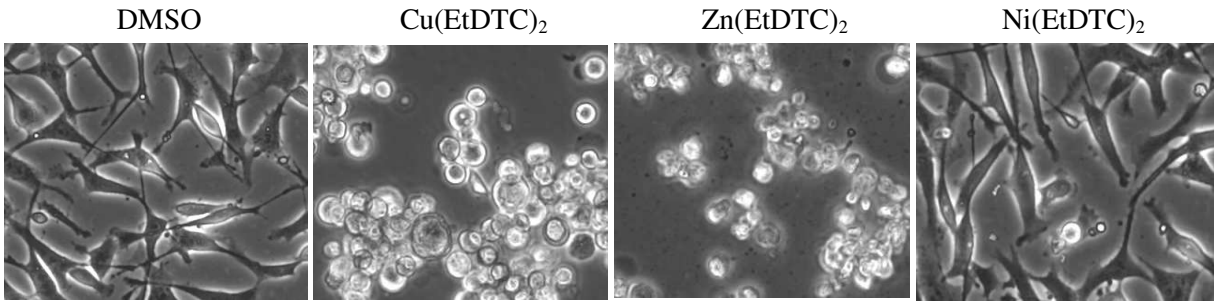


Figure 3. Inhibition of Breast Cancer MDA-MB-231 Cell Proliferation.



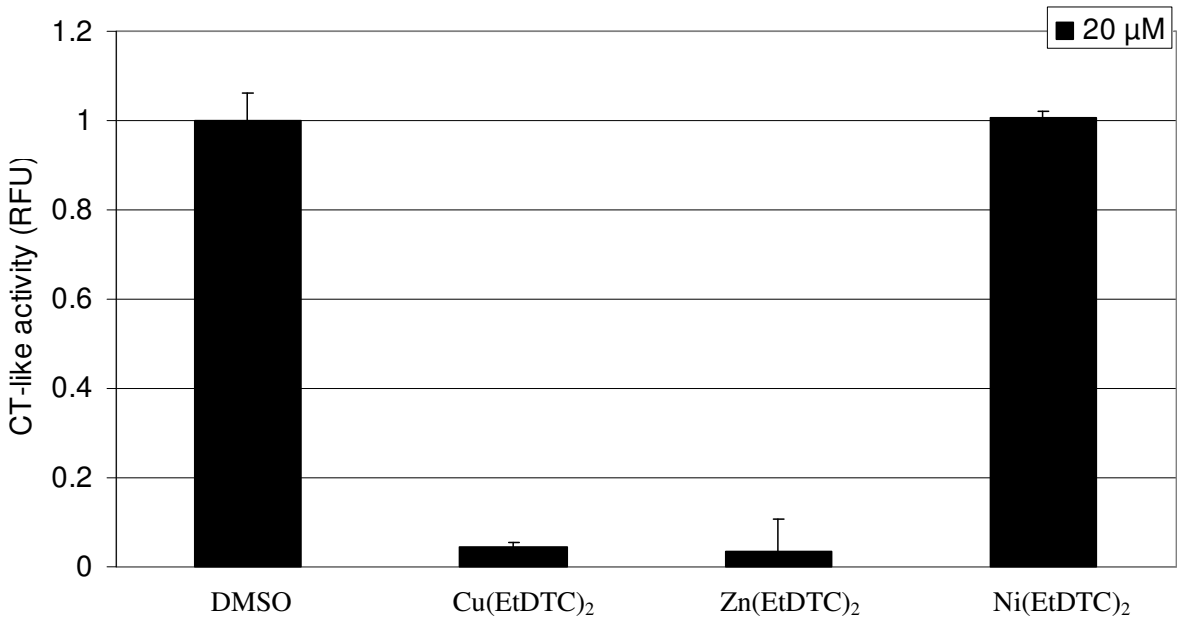
MDA-MB-231 cells were plated in 96-well plate and treated with $Cu(EtDTC)_2$, $Zn(EtDTC)_2$, or $Ni(EtDTC)_2$ for 24 hours, as indicated. DMSO was used as a solvent control. After the treatment, medium was removed and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) (1 mg/ml PBS) was added to each well. The cells were then incubated at 37°C for 4 h to allow for complete cleavage of the tetrazolium salt by metabolically active cells. Next, MTT was removed and 100 µl of DMSO was added, followed by colorimetric analysis using a multilabel plate reader at 560 nm (Victor^3; PerkinElmer (Wellesley, MA, USA)). $Cu(EtDTC)_2$ and $Zn(EtDTC)_2$, were equally potent by inhibiting about 90% of cell proliferation at both concentrations used, while $Ni(EtDTC)_2$ inhibited less than 10% of cell proliferation at 10 µM and about 20% at 20 µM.

Figure 4. Apoptotic Morphological Changes in MDA-MB-231Cell Line.



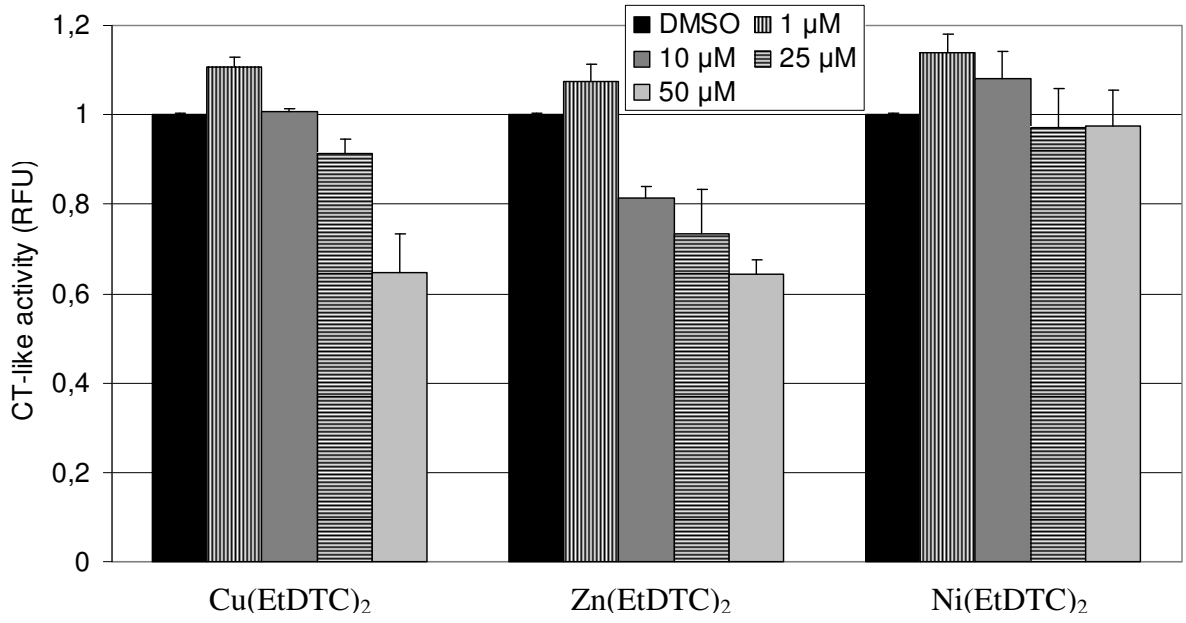
MDA-MB-231 cells were treated with 20 μM concentration of each compound for 24 hours. A Zeiss (Thornwood, NY) Axiovert 25 microscope with phase contrast was used for cellular morphology imaging. Apoptotic morphological changes were observed in the cells treated with $Cu(EtDTC)_2$ and $Zn(EtDTC)_2$.

Figure 5. Proteasomal Activity in Intact MDA-MB-231 Cells Treated with Diethyldithiocarbamate Complexes.



MDA-MB-231 cells were treated with each compound at 20 µM for 24 hours, harvested and used for whole cell extract preparation. Ten (10) µg of cell extract was then incubated with fluorogenic peptide substrate Suc-LLVY-AMC (specific for CT-like activity), in 100 µl of assay buffer (25 mM Tris–HCl, pH 7.5). After 2 h incubation at 37˚C, inhibition of proteasomal CT-like activity was measured by the release of hydrolyzed AMC groups. $Cu(EtDTC)_2$ and $Zn(EtDTC)_2$ inhibited ~90% of the proteasomal CT-like activity, while no inhibition was found after $Ni(EtDTC)_2$ treatment.

Figure 6. Inhibition of Purified 20S Proteasome by Diethyldithiocarbamate Complexes.



Purified rabbit 20S proteasome (17.5 ng) was incubated with fluorogenic peptide substrate Suc-LLVY-AMC (specific for CT-like activity), in 100 µl of assay buffer (25 mM Tris–HCl, pH 7.5) in the presence of different compounds at various concentrations or equivalent volume of solvent DMSO as a control. After 2 h incubation at 37˚C, inhibition of proteasomal CT-like activity was measured by the release of hydrolyzed AMC groups. All three compounds showed only minor effect against the purified rabbit 20S proteasome, with $IC_{50} > 50\mu M$.