Similar but different: Antiproliferative activity and cancer-selectivity of two copper complexes

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ABSTRACT

Background: Copper is an essential nutrient necessary for all domains of life, although too little or too much copper can be toxic. Certain complexes of copper are being investigated for anticancer and/or antimicrobial properties. Previous studies conducted by our group identified novel copper complexes with potent antiproliferative activity against malignant cells and selectivity for cancer cells compared to non-malignant cells. **Objective:** The purpose of this project is to compare the antiproliferative activity of two copper complexes, named KB-L4-16 and KB-L4-17, in A549 lung adenocarcinoma cells and in IMR90 non-malignant lung fibroblasts. KB-L4-16 and KB-L4-17 are constitutional isomers (same molecular formula but different structures) that differ in the attachments of their side groups. We wanted to test whether these small structural differences alter the biological activity or selectivity of the complexes. **Methods:** A colorimetric assay (MTT assay) and the clonogenic assay were used to assess proliferation and survival of cells that were treated with various concentrations of the copper complexes. **Results:** Our results indicate that both copper complexes are more cytotoxic to A549 lung cancer cells compared to IMR90 non-cancer cells. However, KB-L4-16 was both more potent against cancer cells and more selective for cancer vs. non-cancer compared to KB-L4-17. In MTT assays, KB-L4-16 had a GI_{50} value (concentration required for 50% inhibition of cell growth) of 35 nM in A549 cells compared to the GI_{50} value of 180 nM for KB-L4-17. In IMR90 cells, GI_{50} values were 900 nM and 1,900 nM for KB-L4-16 and KB-L4-17, respectively, representing cancer-selectivity ratios (GI_{50} IMR90/GI_{50} A549) of 25.7 and 10.6. Clonogenic assays confirmed the greater potency of KB-L4-16 compared to KB-L4-17. **Conclusions:** These results indicate that small changes in the structures of copper complexes can have surprisingly large effects on their antiproliferative activity and cancer-selectivity. Although the mechanism of action is still unclear, it is possible that structure influences copper complex shape, which may affect cellular uptake or interactions with proteins. Further research is necessary to elucidate the mechanisms of KB-L4-16 and KB-L4-17 and to evaluate their potential as cancer therapeutics.

BACKGROUND

An essential nutrient and neccesary for the growth and development of all domains of life [1].

Imbalances in homeostasis have been linked to several pathologies, including cancers, neurodegeneration (ex. Menkes syndrome), growth abnormalities, etc [1].

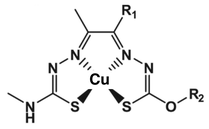
Exists in three oxidative states with differing affinities for different coordinating groups: Cu^{+} , Cu^{2+} , Cu^{3+} [2].

Transported into cells by copper transporters and delivered to specific target proteins by copper chaperones [1].

Cofactor for metalloenzymes like superoxide dismutase, cytochrome C oxidase, lysyl oxidase, etc [1].

COPPER

NOVEL COPPER COMPLEXES

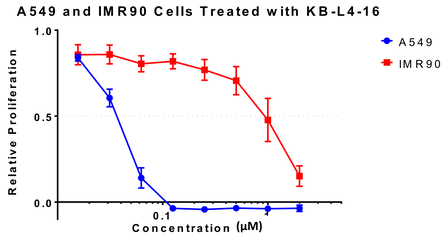


Initally made and used for the purposes of clean energy [3] but found to have selective activity against cancer cells [4].

Anticancer mechanisms are not fully understood. Other copper complexes reported to modulate intracellular ROS, inhibit protesome activity, minimize angiogenesis, and induce apoptosis.

GOAL FOR THIS PROJECT: Compare antiproliferative activity of two related copper complexes, **KB-L4-16 & KB-L4-17** (see structures in "Conclusions" panel), in human cell lines.

RESULTS



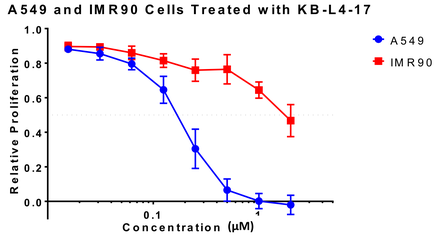


Table 1. Growth Inhibition Values of Proliferation Assays

Copper complex	GI_{50} (μM) in A549 cells	GI_{50} (μM) in IMR90 cells	Selectivity Ratio
KB-L4-16	0.035	0.9	25.7
KB-L4-17	0.18	1.9	10.6

Figure 2: MTT cell proliferation assays evaluating cytotoxicity of KB-L4-16 (top left) and KB-L4-17 (top right) in A549 lung cancer cells compared to IMR90 non-malignant lung fibroblasts. These results indicate that KB-L4-16 is more potent than KB-L4-17 with a >5-fold smaller GI_{50} against A549 cells (Table 1). KB-L4-16 is also more cancer-selective than KB-L4-17 with a >25-fold difference in GI_{50} between A549 cancer cells and IMR90 non-cancer cells (Table 1), offering a greater therapeutic window.

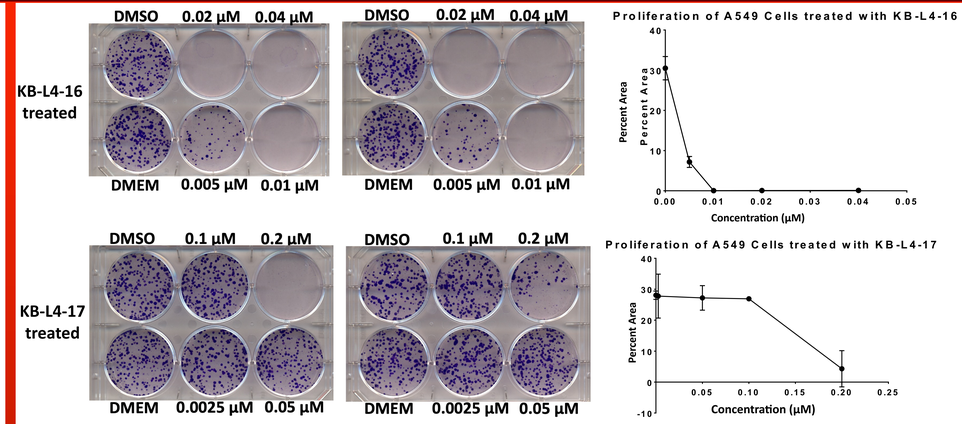


Figure 3: Clonogenic assays of A549 cells treated at serially diluted concentrations of KB-L4-16 (top left panels) and KB-L4-17 (bottom left panels). Concentrations for testing were selected based on previously determined GI_{50} values in MTT assays (see Figure 2). The graphs above show quantitation of results. Consistent with the data in Figure 2, KB-L4-16 was more potent than KB-L4-17 at inhibiting colony formation.

METHODS

Cell Lines: Lung adenocarcinoma cells (A549) and non-malignant fibroblast cells (IMR-90) were purchased from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM) and Eagle's minimal essential medium (EMEM), respectively, containing 10% Fetal Bovine Serum and 62.5 μg/mL penicillin and 100 μg/mL streptomycin. Additionally, EMEM was supplemented with 1 mM sodium pyruvate, and non-essential amino acids. Cells were grown in an incubator at 37˚C in 5% CO_2.

MTT Proliferation Assay: The effects of the metal complexes KB-L4-16 and KB-L4-17 were assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay [4,5]. A549 and IMR-90 cells were seeded in quadruplicate wells within a 96-well plate at a density of 1000 live cells/well and 5000 live cells/well, respectively. The cells were allowed to adhere overnight and then were treated with serial dilutions of the metal complexes. Plates were incubated for 72 hrs, and then MTT was added for 4 hrs followed by lysis solution overnight. Plates were read using a spectrophotometer (Biotek instruments), and data was analyzed using GraphPad Prism 6 application.

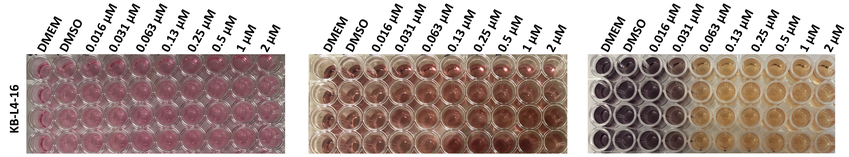


Figure 1: Illustration of an MTT cell proliferation assay. Photographs of cells plated and treated with various concentrations of the copper complexes (left), following addition and incubation with MTT (middle), and subsequent incubation with lysis buffer (right).

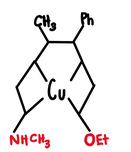
Clonogenic Assay: A549 cells were plated at a density of 1000 live cells/well in 6-well plates and incubated overnight. Cells were treated with metal complexes and vehicle controls. After 10 days, cells were fixed and stained with 4% paraformaldehyde and 0.04% Accustain Crystal Violet Solution. Colonies formed were analyzed via the ColonyArea plugin for ImageJ software [6].

Data Analysis: For MTT proliferation assays, the culture medium background (media alone) was subtracted from each well, and OD_{570} were normalized to the untreated cells (no copper complexes). The averages were plotted (see Figure 2) and data represent the average +/- SEM from at least three independent experiments. Clonogenic assays were performed in duplicate. Plates were scanned (Epson Expression 1680), aligned, and areas were selected to minimize background interference using the ColonyArea application on ImageJ to obtain the percent areas. Average percent areas +/- SD were plotted (see Figure 3).

CONCLUSIONS

- **KB-L4-16 and KB-L4-17 are structurally and electronically similar and were expected to have similar biological activities.**
- **Both KB-L4-16 and KB-L4-17 copper complexes inhibit the proliferation of lung adenocarinoma A549 cells in a selective manner (i.e., they have much less effect on non-malignant fibroblast IMR90 cells).**
- **Surprisingly, KB-L4-16 was found to be markedly more potent and more cancer-selective compared to KB-L4-17.**
- **Our results suggest that KB-L4-16 could be a useful agent against cancer due to its greater activity and selectivity.**
- **Results suggest a complex structure-activity relationship, in which the the differences in these constitutional isomers may have profound implications on the activity of these copper complexes (A-C).**

KB-L4-16



KB-L4-17

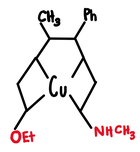


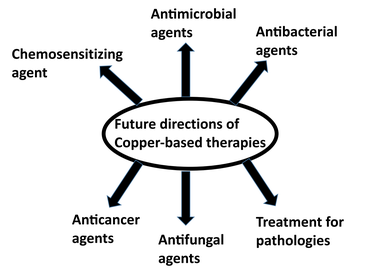
Table 2. Reduction potential

Copper complexes	Reduction potentials (Volts)
KB-L4-16	-0.850
KB-L4-17	-0.859

Ⓐ Differing structures can result in changes in their shape, influencing their cellular uptake.

Ⓑ Differing shapes can also affect their interactions with proteins

Ⓒ Differing shapes may affect their functions in other known, cited mechanisms of actions such as acting as antiangiogenic agents, ROS scavengers, inducers of apoptosis, and inhibitors of protesomes.



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