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Avaliação *in vitro* do Potencial Antitumoral de Novo Composto Sintético

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Daniel da Silva Arcanjo

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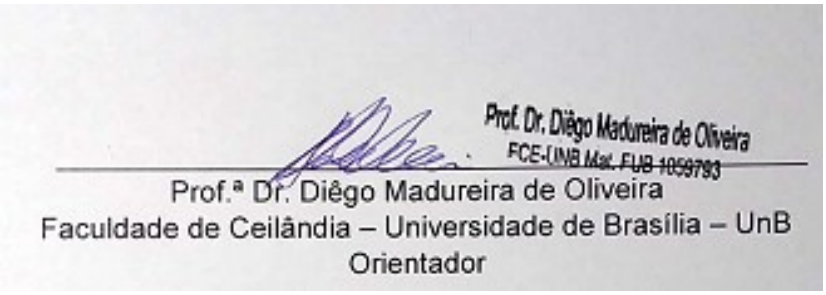

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Figuras

Siglas

MDA MB-231(Linhagem celular de câncer de mama)

$[Zn\ (L1)\ (\mu\text{-}CH3COO)]_2$ (Refere se a formula do complexo usado no trabalho)

HL^1 (Refere-se ao ligante sintetizado para a complexação)

Zn (II) (Elemento Zinco na Valencia 2+)

DNA (Sigla de material Genético ácido desoxirribonucleico)

Au, Ag, Cu, Ru, Rh, Pt, Pd (Elementos metálicos Ouro, Cobre, Rutênio, Platina e Paládio)

MDR (Gene de resistência relacionado a resistência de fármacos)

DMSO (Dimetilsulfóxido, usado neste trabalho como diluente da Droga)

IC_{50}% (Concentração necessária para reduzir 50% das células)

PBS (Tampão fosfato)

BAX (complexo de enzima do maquinário de replicação do DNA)

Resumo

Ditiocarbazatos e seus complexo metálicos tem se mostrado uma interessante classe farmacológica e demonstram grande poder de redução e se coordenam a diferentes centros metálicos, além de sua capacidade de coordenar a bases de Schiff. Este trabalho tem como objetivo avaliar o perfil de sensibilidade da linhagem celular de câncer de mama MDA-MB-231 *in vitro* do composto metálico à base de um ditiocarbazato (HL1) $[Zn(L^1)(\mu\text{-}CH3COO)]_2$. A linhagem de câncer de mama foi submetida ao tratamento do composto e logo após a mensuração da atividade metabólica mitocondrial foi conduzida pelo teste clássico de MTT mostrando um IC_{50} de 2,0 μM. O experimento de marcação com iodeto de propídio mostrou fragmentação de DNA superior ao observado no grupo controle, sugerindo que o composto se liga em algum nível ao material genético. O tratamento também alterou a adesão celular nas primeiras 48 horas, indicando morte celular. Dadas as descobertas deste trabalho, esta classe de moléculas apresenta-se como uma fonte promissora e versátil de protótipos de novas drogas antitumorais.

Palavra Chave: Compostos metálicos, complexos de ditiocarbazato, câncer de mama MDA MB-231.

Abstract

Dithiocarbazates and their metal complexes have been shown to be an interesting pharmacological class and demonstrate great reduction power and are coordinated to different metallic centers, in addition to their ability to coordinate the Schiff bases. This work aims to evaluate the sensitivity profile of the in vitro breast cancer cell line MDA-MB-231 of the metal compound based on a dithiocarbazate (HL1) [Zn(L^1)(μ-CH_3COO)] 2. The breast cancer line was submitted to treatment of the compound and soon after the mitochondrial metabolic activity measurement was conducted by the classic MTT test showing an IC 50 of 2.0 μM. The propidium iodide labeling experiment showed DNA fragmentation higher than that observed in the control group, suggesting that the compound binds to some level with the genetic material. Treatment also altered cell adhesion in the first 48 hours, indicating cell death. Given the findings of this work, this class of molecules presents itself as a promising and versatile source of prototypes of new antitumor drugs.

Key Word: cancer: Metallic compound, dithiocarbazates complexes, cancer cell MDA MB-231.

Introdução

Considerando a resposta ineficiente de alguns tipos de câncer a quimioterápicos tradicionais, a busca por novas substâncias e formulações de novos antitumorais é constante [1]. No entanto, novos compostos são descobertos e enfrentam problemas como viabilidade farmacológica e clínica, de síntese e toxicidade [2]. As estratégias para o desenvolvimento de compostos sintéticos em terapia antitumoral vêm mudando ao longo dos anos. São inúmeros os métodos de síntese e técnicas, mostrando efeitos promissores inicialmente, mas em sua grande maioria isso não se confirma em testes clínicos [3]. Tendo em vista que a maioria dos compostos antitumorais apresentam falhas devido à resistência tumoral, mortes relacionadas a esta patologia em sua grande maioria estão correlacionadas às falhas em tratamentos [4]. Entretanto, existe um grande interesse acadêmico e industrial na busca de novos tratamentos e novos compostos antitumorais, visando maior segurança e eficácia [5]. Expondo essa problemática outras substâncias que sejam menos tóxicas e eficientes tornam se necessárias, a identificação de novos compostos químicos pode servir de pistas para um novo caminho de antitumorais [6]. Levando em consideração a alta atividade redox de alguns íons metálicos, compostos inorgânicos a base de ditiocarbazatos com centro metálico demonstram interessante atividade antitumoral *in vitro* [7]. O presente trabalho buscou executar testes de triagem de atividade antitumoral em complexo de ditiocarbazato com centros metálicos de Zn(II) investigando subsequentemente o mecanismo de indução de morte celular.

O composto a ser testado foi sintetizado e teve a estrutura determinada na Universidade de Brasília pelo grupo de pesquisa LASIC do Instituto de Química.

Revisão de Literatura

Câncer

Células tumorais apresentam uma série de características que as diferenciam de células somáticas normais: dentre as quais podemos destacar crescimento, independência de sinalização, capacidade de reverter o mecanismo de senescência celular, crescimento independente de ancoragem e pôr fim a alto potencial invasivo e metastático. O câncer de mama é o tipo de câncer mais comum que afeta mulheres em todo o mundo [8]. Até 2030 o número de casos de câncer de mama deve aumentar em 50% sendo 3,2 milhões de novos casos [9]. Apesar dos avanços em tratamentos de câncer, este se torna entre as mulheres o mais comumente diagnosticado e a principal morte por câncer. Além disso o câncer de mama apresenta subtipos diferentes de acordo com a expressão de receptores, podendo responder de distintas maneiras à terapia farmacológica [10]. Esta perda de controle está relacionada à consequência de mutações em um ou mais genes, que regulam o crescimento celular e a morte celular programada [11]. Ainda que inúmeras modalidades de tratamentos sejam colocadas no mercado, a quimioterapia convencional é uma das formas mais eficazes de se tratar neoplasias. Esta modalidade é comumente usada na clínica para o tratamento de malignidades inoperáveis e adjuvante para tratamentos pós cirúrgicos. Embora a quimioterapia seja uma forma convencional de tratamento, o desenvolvimento de resistência a múltiplas drogas (MDR) aos agentes quimioterápicos é uma limitação fundamental para o sucesso da terapia, entretanto a maioria dos quimioterápicos apresentam efeitos adversos, muito destes danosos a saúde sendo um fator limitante ao sucesso da terapia [12]. Com base neste cenário, ainda que a

quimioterapia convencional seja amplamente utilizada, compostos inorgânicos conjugados a metais vêm ganhando espaço neste contexto, devido às inúmeras capacidades desses compostos, dentre as quais podemos destacar a capacidade de reverter resistência tumoral, sendo uma alternativa a quimioterápicos convencionais [13].Portanto, exibida essas limitações, extensas investigações sobre terapias alternativas contra o câncer baseada em compostos conjugados a metais se tornam essenciais para tratamentos mais eficazes.

Tratamentos do câncer

Complexos metálicos usados na terapia do câncer

Os íons metálicos desempenham papéis importantes em processos biológicos. Com a aplicação da química inorgânica e medicinal novas formulações derivadas de complexos metálicos ajudam na melhor compreensão e estratégia para novos tratamentos [14]. O aparecimento de compostos inorgânicas começou na década de 60, com o intuito de trazer uma nova abordagem para terapia anticâncer. Os primeiros e mais utilizados até hoje são os compostos derivados da platina (Pt), trazendo uma nova perspectiva de tratamento [15]. Dentre outros metais mais utilizados e que apresentam efeitos satisfatórios está o rutênio (Ru), que mostra uma toxicidade abaixo dos compostos de platina [16].

O uso de metais e compostos metálicos na medicina vem de milênios mostrando uma evidência empírica para terapêuticas de diversas patologias. Metais de transição desempenham um papel muito significativo na biologia e sistemas enzimáticos, isso devido ao alto potencial redox, estabilidade e capacidade de desempenhar vários números de coordenação, os tornando um excelente alvo para estudo, além de sua geometria variada e suas propriedades magnéticas [17].

Complexos metálicos tem sido empregado na terapia oncológica de inúmeras formas, pela formação de adutos de DNA em sua quebra de fita ou alquilação, mas dentre as mais comuns está a ligação do metal aos heteroátomos de bases nucleosídicas, fazendo uma ligação mais estável impedindo desta forma o processo de replicação de DNA [18]. Uma outra grande vantagem de usarmos

metais na terapêutica oncológica, é a sua capacidade de fazer bases de Schiff, uma estrutura que doa elétrons, com efeitos eletrônicos e estéreis baixos, reduzindo espécies que estão ativas em um sistema biológico [19]. Compostos metálicos de transição se mostram com um importante efeito na bioinorgânica, pois, além de serem estáveis e apresentarem alta afinidade por sítios ativos, possibilitam alvos de tratamento para diversos sistemas biológicos [20].

A capacidade de formar quelatos torna-se também um alvo estratégico, pois a sua alta capacidade de coordenação aumenta a lipofilicidade da molécula, melhorando o caráter e a capacidade do tipo de ligação tornando um interessante alvo, mais seletivo para estruturas de DNA.

Sem sombra de dúvidas, a principal aplicação de complexos metálicos está em sua capacidade de coordenação e de se ligarem ao DNA de diferentes formas causando a morte celular. Ainda hoje, como citado acima, a cisplatina (Pt) e o rutênio (Ru) ainda continuam sendo os principais metais usados. No entanto sua alta toxicidade e baixa viabilidade de redução além da resistência tumoral, faz deste um campo promissor para o estudo, visando o desenvolvimento de novos compostos.

Atividade farmacológica de complexos sintéticos na terapia anticâncer

Entender mecanismos de interação de complexos metálicos com diferentes sitios de DNA, tentando comprender sua natureza de ligação, vem a ser um dos desafios de síntese e caracterização de compostos [25]. Grande parte das moléculas descritas na literatura tem seu mecanismo elucidado sendo eles ligação covalente em sitios especificos, intercalação ou alquilação em pares de base do DNA .

Grande parte dos íons metálicos conseguem se coordenar a ditiocarbazatos, formando complexos metálicos. Basicamente o desenho de sua estrutura é um composto contendo um carbono com dupla ligação no nitrogênio, podendo apresentar um grupo aril ou alquil (>C=N-R < Arila ou Alquila). Esse mecanismo se torna interessante pois estas moleculas comportam se como bases de lewis, estabilizando moléculas reativas em sistemas biológicos [21]. Deixando

claro que metais que se coordenam a ditiocarbazatos apresentam grande potencial antitumoral [22].

Complexos com os metais (Au, Ag, Cu, Ru, Rh, Pt, Pd) , são alvos de inúmeros trabalhos [24], mas complexos com metais de Cu e Au vêm apresentando resultados mais significativos em testes *in vitro.* Um exemplo de complexo de ditiocarbazto com centro metalico de cobre cobre combinado com ligantes bidentados apresenta atividade inibitoória com complexo BAX enzimatico Bcl2 , onde possivelmente pode estar relacionado com ativação de processos pró apoptóticos, atuando de uma forma diferente de ligantes normais impedindo o processo de imortalização celular [23] . O mesmo ocorre em compostos auríferos, mostrando um efeito mais seletivo para se ligar ao DNA. Compostos auríferos apresetam um orbital d^8 eletrônico o que facilita uma geometria quadrado planar a se ligar em bases nitrogenadas, além de seus baixos niveis de energia, propiciando uma formação de adutos de DNA [26].

Metais como Co(II), Ni(II) e Zn(II) também desempenham um papel fundamental na formação de complexos, estes metais conseguem realizar a formação de complexos com ligantes polidentados com capacidade quelante formando estruturas de alto interesse farmacológico, além de sua boa capacidade eletrolíticas e de suas diversificadas geometrias podendo realizar várias coordenações. Estes metais se apresentam com um alto comportamento redox [27]. Outro metal bastante utilizado e que apresenta um grande interesse farmacológico é o Cd(II), devido sua alta capacidade de coordenação e sua atividade anti oxidante[28].

Apesar de promissores os complexos metálicos e seus derivados tem ainda seu mecanismo de ação pouco elucidados [29]. Os mecanismos de ligações e a natureza destes indicam que boa parte destes complexos formam adutos de DNA. Estudos mais recentes indicam que o segredo pode estar na estabilização de diferentes proteínas que regulam a síntese de DNA [30]. O grande potencial farmacológico indica uma via de mão dupla, ao passo que estes compostos conseguem realizar um grande número de coordenações pela sua geometria, além de formarem bases de Schiff essencial para o potencial redox. Mostrando

que sua caracterização, síntese e toxicidade se tornam um problema pela grande dificuldade [31].

Justificativa

Apesar dos avanços na compreensão biológica do tratamento do câncer, tais avanços ainda não se reverteram em melhoria na terapia de muitos pacientes. Considerando os dados prévios acerca de componentes de mesma natureza dos que serão testadas, são estes bons protótipos para testes iniciais como os propostos.

Objetivos:

Objetivo geral

Determinar o potencial uso terapêutico em oncologia de um complexo de Zinco(II) sintético através de abordagem *in vitro*.

Objetivos específicos

a) Determinar efeito do composto na viabilidade celular e calcular IC_{50}%.

b) Avaliar se o composto promove dano de DNA.

c) Determinar os efeitos do composto na morfologia celular.

d) Testar a toxicidade de intermediários da rota sintética do composto.

Artigo científico

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Indication (Experimental study)

Evaluation of *in vitro* antitumor effects of a novel synthetic Zn(II) complex

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Key Word: cancer, metallic compound, ditocarbazates complexes.

Abstract

Metallic complexes have shown to be an important pharmacological class, synthetic inorganic compounds derived from dithiocarbazates show interesting possibilities as the possibility of biding to different metal centers, due the high power of coordination besides their ability to perform Schiff mechanism. This work

aims to evaluate the MDA-MB-231 breast cancer cell line sensitivity profile *in vitro* to the metal dithiocarbazate based compound on $[Zn(L^1)(\mu\text{-}CH_3COO)]_2$.The cells were subjected to the treatment with the compound, and then mitochondrial metabolic activity was measured by the MTT classic test leading to an IC_{50} of 2.0 μM. Cytometry tests with propidium iodide staining indicated DNA fragmentation in treated cells compared to the control, suggesting that the Zinc(II) complex is able to bind to the genetic material of this cells. The cell adhesion was also affected by the treatment in the first 48 hours, indicating cell death. Given the findings of this work, this class of molecules may be considered a promising and versatile source of prototypes of new antitumor drugs.

Introduction

Considering the inefficient response to certain types of cancer and the development of new anti-tumor agents, the search for new synthetic compounds and treatments become essential for alternative therapies. strategies for antitumor development have been changing over the years [1]. A range of new compounds are synthesized every day, but only a small portion of these show promising effects, being a small part of these selected for clinical trials [2].

Observing most of the antitumor compounds, much of its mechanism of action is focused on cell proliferation causing low selectivity to treatment, This type of chemotherapy is commonly used in the treatment of inoperable malignancies and adjuvant for post-surgical treatments, although conventional chemotherapy is widely used, new compounds have highlighted due to their high ability to reverse the resistance mechanism. Inorganic compounds conjugated to metals have been

gaining ground in this scenario, due to their property to reverse tumor resistance, being an alternative to conventional chemotherapeutics [3].

Recent studies by Claudia et al show complexes with specific metal atoms that play a role in ability to stabilize nitrogenous bases and atoms. the basic structure of dithiocarbaztes provides a several possibilities of coordination, due this mechanism, the coordination with different metals become a pharmaceutical target [4]. The dithiocarbazates have an interesting possibility of coordinating to different metallic centers, being able to be found in the two tautomeric forms of thiol and thione, depending on the conditions under which they are submitted and the variation of pH. Although they present potential electrons donor atoms , they coordinate to different metal ions in mono, bi or polydentate forms [5].

Dithiocarbazates are considered Schiff bases, such complexes when combined can control to some extent the activity and process of tumor cell senescence [7]. Another point should be taken into consideration is the geometry of these complexes, for interaction with certain receptors can promote the pharmacodynamic action and versatility in its synthesis, allowing variations in their molecular structure that may enhance this action [8]. Although the cited data, the majority of work with metal complexes with biological activity focus on the synthesis and characterization of these often limited to statements of superficial pharmacological potential of these molecules. Compounds to be tested were synthesized and had the structure determined by the University of Brasilia LASIC research group of the Institute of Chemistry.

Material and methods:

Cell culture

The cytotoxic activity of the compounds was tested against human breast cancer MDA-MB-231 cell line (the main breast cancer cells used around the world to perform single cell line studies). Whereas it was isolated from a very aggressive metastatic triple negative tumor, it is a very useful *in vitro* model for screening of new antitumor drugs. The cells were cultured on a 10 cm diameter plastic plates at controlled conditions (humid atmosphere of 5% CO_2 at 37°C) in DMEM culture media supplemented with 10% (v/v) fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 µg/mL), which was replaced each two-three days.

Mtt Assay

Cell viability was measured by the MTT reduction method. This test is based on the ability of viable cells to metabolize yellow colored 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) through their mitochondrial dehydrogenases into the purple stained formazan product. The cells were plated at a density of 10,000 cells/well (96-well plates were used) and then subjected to the treatments of interest (antineoplastic drugs, ligands and complexes tested at concentrations ranging from 0 to 3µM). After 72 hour exposure to the compounds, culture medium was exchanged for the MTT solution and the plates were incubation for two hours. After that, the cells were lysed for spectrophotometric quantification at a wavelength of 595 nm. Dimethyl sulfoxide (DMSO) was used as diluent for all compounds and it was present in all the groups, including control. Results were expressed as percentage of control viability.

Flow Cytometry

According to the manufacturing instruction the detection of necrotic cells or apoptosis late stage the use of propidium iodide (PI), a fluorescent DNA intercalant to which the whole plasma membrane is impermeable at low concentrations for this assay, will be used 12-well plates in one density of 100,000 cells / well. These plates were incubated under standard conditions and subjected to the treatment of interest. After exposure to compound ($[Zn(L1)(\mu\text{-}CH_3COO)]_2$), the cells were trypsinized, centrifuged and washed with PBS, then the supernatant was discarded and the pellet resuspended in appropriate buffer solution to which FITC-labeled propidium iodide (fluorophore) according to the manufacturer's instructions. After 15 minutes of incubation, the reading was performed on the conventional flow cytometer with SSC / FSC reading and F2 and F3 filters. After treatment of interest, the percentage of cells in culture at each stage of the cell cycle, was estimated from reading by flow cytometry, the intensity of cell fluorescence after permeabilization with ice methanol and exposed to propidium iodide.

Morphometric analysis

For the evaluation of the morphological aspect the adhesion area was estimated by measuring the largest axis and its perpendicular axis to calculate an index. Cell culture morphology monitoring was performed using a phase contrast microscopy and obtaining micrographs with a 40x magnification, using a digital camera attached to the microscope and connected to a computer carrying the specific editing program. 21 cells of each photograph were measured in different concentrations.

Data analysis

All data receive appropriate statistical treatment, and the measure of central tendency adopted in the case of quantitative variables, chosen according to the distribution of values (admitted asymmetry limits to consider the next normal distribution will be of -1 and +1). Statistical tests adopted, parametric and non-parametric, will be displayed in the presentation of results, and p values considered statistically significant will be those below 0.05. For the IC_{50} calculation, the data will be submitted to linear regression hand with software equations bank GraphPad Prims 7.0.

Qualitative microscopic analysis

All the photos taken in the experiment, were used the NIKON Eclipse inverted phase contrast microscope (TS100) was used to perform a qualitative analysis of cellularity and morphology.

Synthesis and composition

2-acetylpridine-S-allyl-dithiocarbazate(HL^1)

For the synthesis of 3 mmols of the complexing agent 2-acetylpyridine-S-allyl-dithiocarbazate (HL1) in an ice bath with stirring, solubilizing 198 mg of potassium hydroxide in 30 ml of ethanol. Then, 0.15 mL of hydrazine monohydrate. After 40 minutes, 0.18 mL of carbon disulfide was added,by dripping the solution. After 60 minutes, was added 0.26 mL of allyl bromide. And then 60 minutes later, 0.34 mL of 2-acetylpyridine were added to the condensation reaction. The condensation step was carried out in an oil bath at 150°C. There was obtained a yellow colored

solid which was filtered from the stock solution. Yield: 81%. Melting Point: 113°C. Elemental Analysis (%): C 52.56; H 5.21; N 16.72 (Theoretical values), C 51.46; H 4.75; N, 16.28. (Experimental values).

bis (μ-acetate) bis (2-acetylpyridine-S-allyl-dithiocarbazate) - zinc (II)

The complex $[Zn(L^1)(\mu\text{-}CH3\ COO)]_2$ was synthesized from the reaction of (0.1mmol) of the complexing agent (HL^1) solubilized in 5 mL of chloroform with (0.1 mmol) $Zn(CH_3COO)_2$ in 5 ml of methanol, remaining in 2 hours under constant stirring, the reaction product was filtered from solution after slow evaporation of solvents. Yield: 56%. Melting Point: 198C. Elemental Analysis (%): C 41.66; H 4.03; N 11.21 (theory), C 41.15; H 3.77; N 11,13 (experimental values)

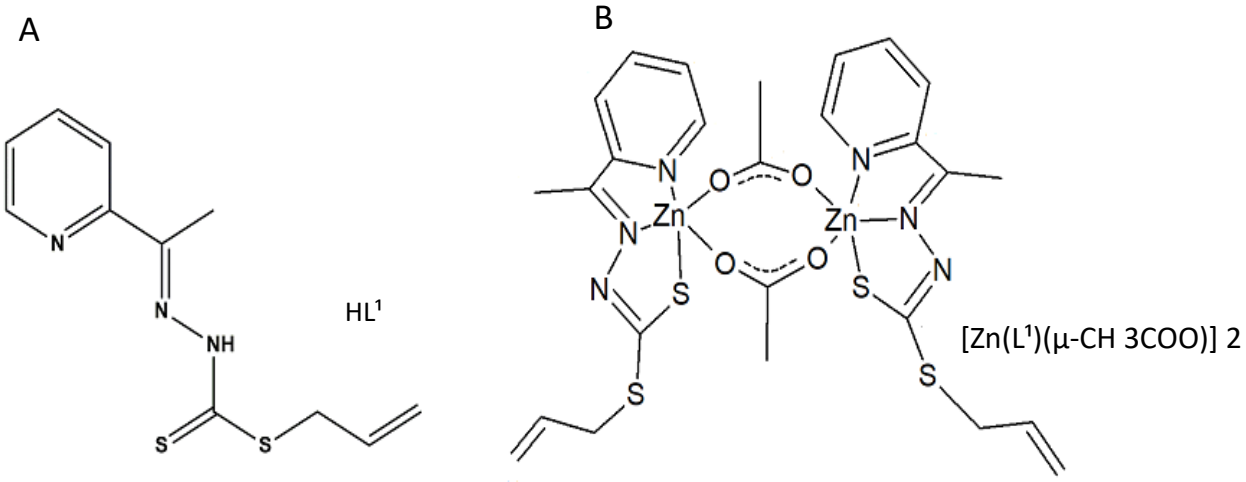

Figure 01. Synthesized chemical structures, In (A) compound 2-acetylpyridine-S-allyl-dithiocarbazate HL^1 and (B) bis (μ-acetate) bis (2-acetylpyridine-S-allyl-dithiocarbazate) - Zinc(II).

Results:

Evaluation of the cytotoxic effects in cell line

The cytotoxicity of the Zn(II) complex was performed on MDA MB-231 breast cancer cells, increasing the concentrations of the metal compound. After 72 h of

incubation, cell viability was assessed by the classic MTT assay. The results showed that the effect on the compound $[Zn(L^1)(\mu\text{-}CH_3COO)]_2$ had a high cytotoxic impact at low concentrations, showing that in a short time the compound reduced cell viability by more than half. The action of the ligand (HL^1) in (Figure 1A) the cells was measured, presenting low activity in relation to the complex $[Zn(L^1)(\mu\text{-}CH_3COO)]_2$ (Figure 1B). The same experiment was conducted but this time testing the salt from the inorganic synthesis, observing if it presents some activity . It may be noted that in relation to the complex, the compound does not exhibit some damaging cell activity.

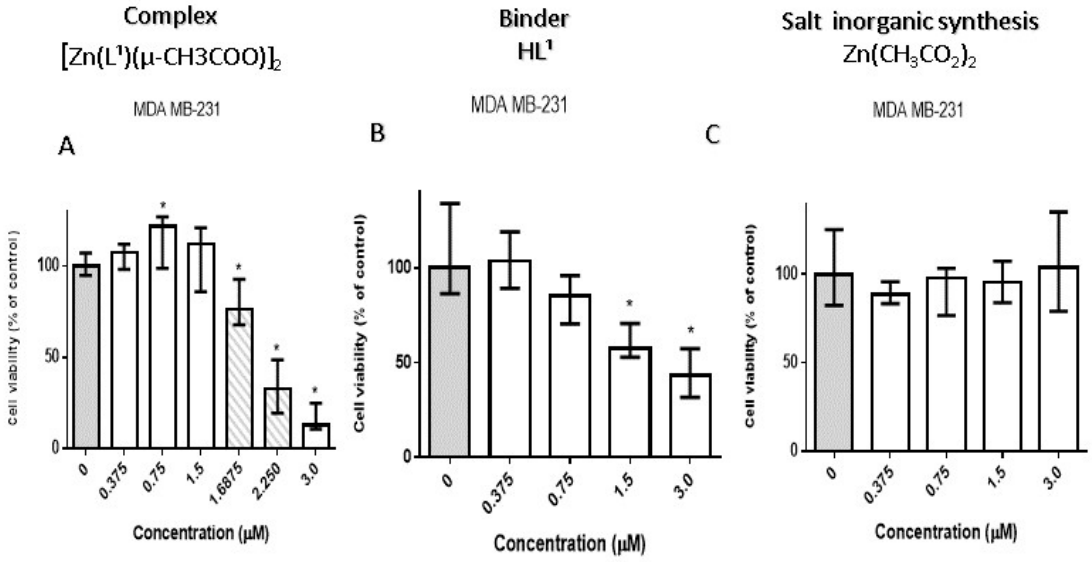

Figure 02. Analysis of short-term cytotoxic effect in MDA-MB 231 treated for 72h in increasing concentrations measured by the MTT classic assay, compound, *P < 0.05 compared to respective controls (nonparametric Kruskal-Wallis test followed by Dunns Comparison test). Data are shown by median and range.

Qualitative morphological analysis

Changes in the morphologies of the treated MDA MB-231 cell line were observed using an inverted-phase contrast microscope (NIKON Eclipse TS100). After 48h of treatment can identify some differences in relation to the control, presenting different morphologies of those observed in the control (more rounded forms and little confluence, showing few adherent cells), such alterations as loss of cytoplasmic area and decrease in volume are related to the cell death process, confirming the cytotoxic action of the complex (Figure 03) .

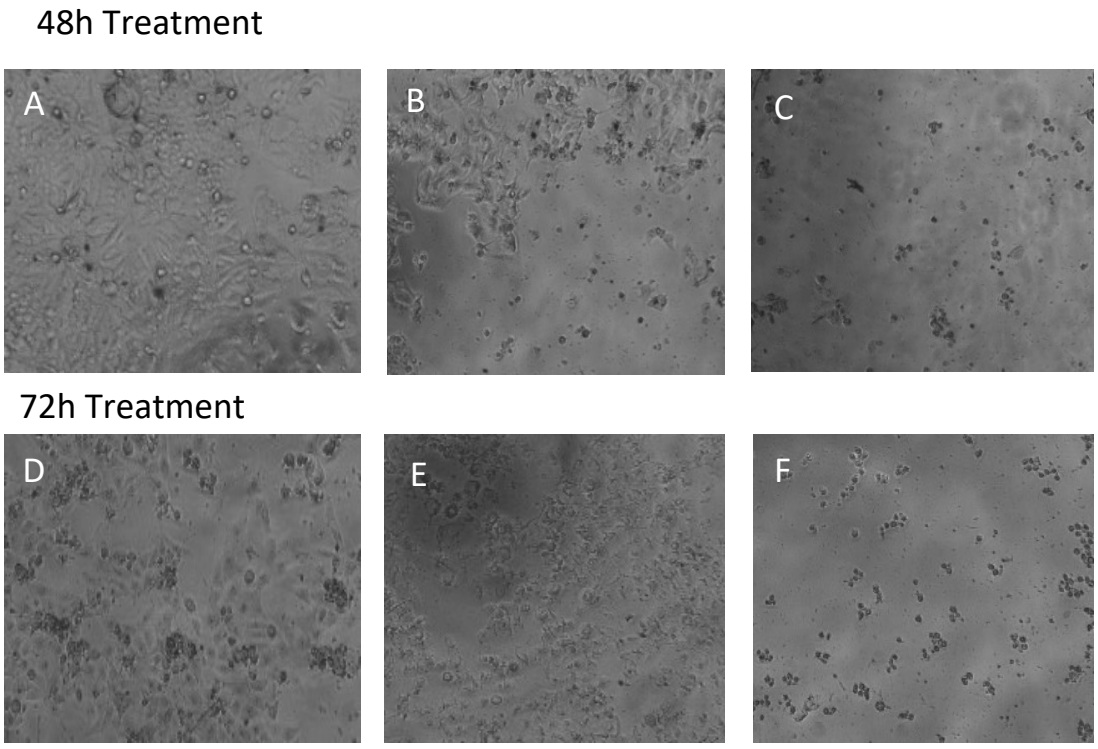

Figure 03. Photos obtained by phase contrast microscopy with a 40x magnification objective, Figures A and D, representing the control of MDAMB-231 supplemented with fetal bovine serum, and cultured whit DEMEM, Figures B and E treated with the compound $[Zn(HL^1)(\mu\text{-}CH_3COO)]_2$ at a concentration of 2,250 µM, Figures C and F presenting the highest concentration 3.0 µM.

Another interesting finding of this work is based on morphometric studies, measuring the estimated area of cell adhesion in treatment times of 48h and 72h. It can be noticed that there is an attenuating difference between the control and treated groups corroborating the finding of IC_{50}, however if we compare the times of treatments and their concentrations we can notice that this difference becomes subtle between the times of 48 and 72h, showing that there is little difference between treatment time, indicating that there may be a dose response effect of the compound on the cell line , such findings can be seen in (Figure 04).

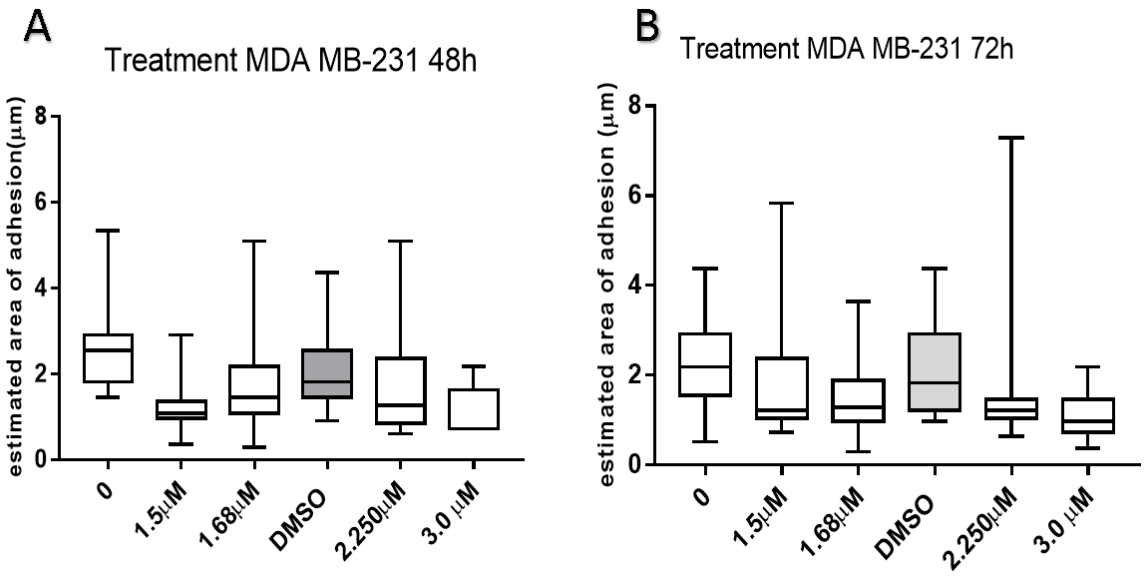

Figure 04. The estimated area of adhesion of the cell line MDA MB-231 in 48 and 72h of treatment. In the X axis the concentrations of the compound and in the Y axis morphometric pattern with the area of adhesion. The measure of central tendency used was the media.

DNA fragmentation

For the cytometry assays 24 h of exposure to 3 μM of the compound was used. The total number of cells was obtained by cell counting from the neubawer chamber, using the principle of exclusion of trypan blue, differentiating viable cells from nonviable cells. In Figure 05 graph (A) we can see that the number of cells presented in the control was greater than in the treated one, indicating that the compound [Zn (L1)(μ-CH_3COO)]$_2$ had an effect on the modulated concentration reducing part of the cells in relation to the control. In figure 05 graph (B) , we can see DNA fragmentation, such finding indicated a high percentage of fragmented DNA in the control and treated group, this data indicates that the high concentration of 3.0 μM of the compound affected to a large extent the treated groups, exerting a high cytotoxic effect in only 24h demonstrating that this compound exerts high cytotoxic activity in low concentrations and also in a short period of time. However, the flow cytometry experiments had some limitations, such as contamination of the cells by bacteria, and the time of exposure to the compound, bringing interferences to the experiment.

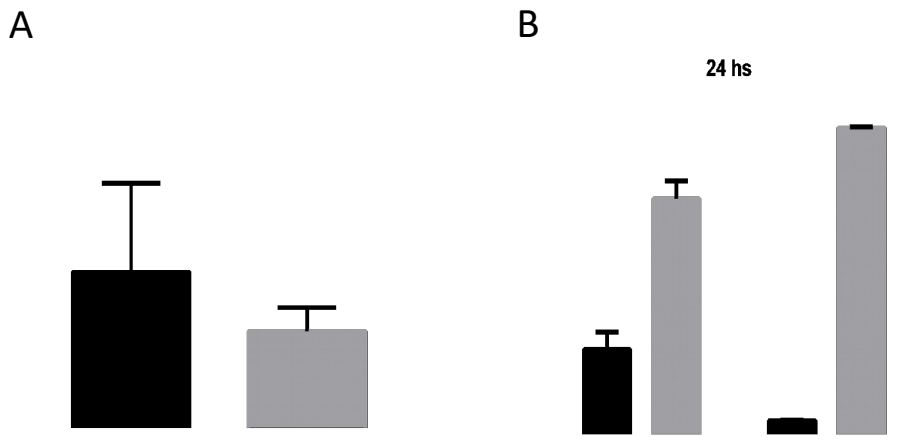

Figure 05. Graph (A), total number of control cells and treated at the 24-hour time of the compound [Zn(L1) (μ-CH_3COO)]$_2$ at a concentration of 3.0 μM. Graph (B), DNA fragmentation in percentage, relating control and treatment.

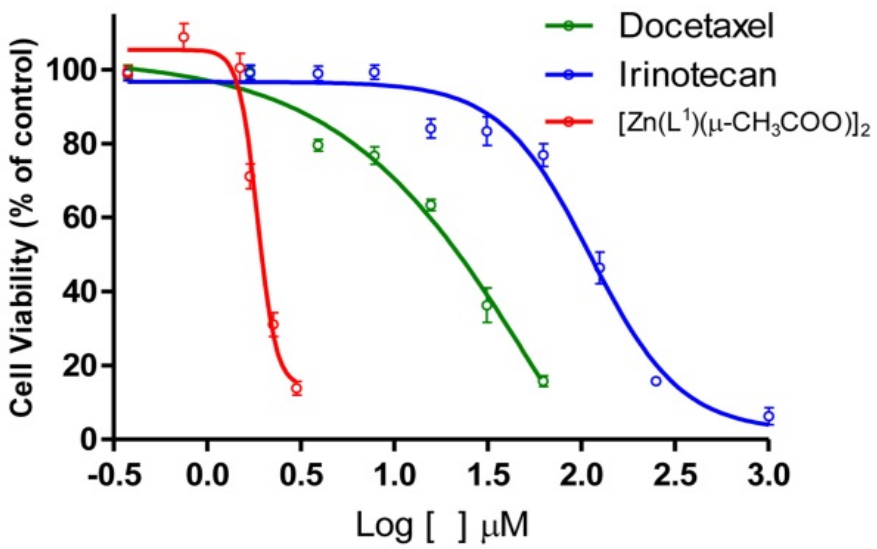

Figure 06. Comparison of the IC_{50} of the compound $[Zn(L1)(\mu\text{-}CH_3COO)]_2$. between chemotherapeutic agents, Irinotecan and Docetaxel values estimated by non-linear regressions.

The Figure 06 shows values estimated by non-linear regressions graphs. Comparing the zinc complex with classic antitumor drugs used against breast cancer. The IC_{50} of the zinc complex was 2.0μM (R^2 = 0.94), showing that it is much more toxic to these tumor cells than Docetaxel and Irinotecan, which presented IC_{50} of 22.0μM (R^2 = 0.94) and 110.0μM (R^2 = 0.94), respectively.

Discussion

Current studies aim to develop more effective drugs, with a primary focus on compounds that induce selective toxicity in cancer cells. Tests performed on the synthetic compound have shown important role in tumor regression [33]. The metal complexes exhibit a non-selective function, given their high ability to bind to some

nonspecific DNA sites. This compound showed satisfactory results in relation to screening of candidate compounds antitumor. The Cell line MDA MB-231 were used in this study changes in their morphological and metabolic mechanisms can be noticed after the first 48 hours of treatment with the zinc-based compound $[Zn(L^1)\ (\mu\text{-}CH_3COO)]_2$.

These data point to the compound $[Zn(L^1)(\mu\text{-}CH_3COO)]_2$ as a good prototype for the development of novel antineoplastic agents. Metal complexes are mostly known for altering the cell cycle and cell division [34]. The MDA-MB-231 cell line is known as a chemotherapeutic resistant cell line. The toxic profile of some chemotherapics is only evident at high concentrations. However, when we compare the toxic effect of some chemotherapeutic agents frequently used in the clinic for the treatment of breast cancer, we obtain promising results when we compare the complex $[Zn(L^1)\ (\mu\text{-}CH_3COO)]_2$.

Notably, when we compared the zinc complex to the antineoplastics used in the antitumor treatments, we noticed an immense difference in toxicity. Such activity has already been described in the work of Adhikari et al., demonstrating the zinc complex of the added tryptophan 1,4,7,10-tetraazacyclododecane activity, comparing classical antineoplastics such as cisplatin, exhibiting a difference in toxicity, the complex being less toxic than cisplatin [35]. However, when comparing similar structures in this work we can observe a more prominent effect of the dithiocarbazate complex when compared to that of tryptophan, it being clear that different structural changes and different center of coordination make the compounds have different cytotoxic profiles

The basic structure of the dithiocarbazates provides several possibilities of coordination, when combined with an organic synthesis process this activity can be amplified, due to this mechanism the coordination with different metals making an extremely versatile molecule [36].

Another point to be taken into account in this work is the morphological effect of the adhesion tests and the effect of DNA fragmentation showed by flow cytometry, in both tests we see that the complex $[Zn(L^1)(\mu\text{-}CH_3COO)]_2$. Exerts high cytotoxic effect. In the adhesion test we observed that at 48h the compound reaches a high cytotoxic effect. When we compared the concentrations of 2.0μM to the respective controls we can notice an immense difference of cellular adhesion, being a clear effect of cell death. When comparing the times of 48 and 72h note a small difference in adhesion, which suggests a dose response effect, and that its activity is in the first 48h.

The data point the compound $[Zn(L^1)(\mu\text{-}CH_3COO)]_2$ suggest that the complex is able to bind to the DNA causing cell death, although the controls experience fragmentation because of experimental interference, when we compare the treated groups we see that there is a difference of fragmentation rate between the control and the treatment groups. Notably the treated group showed a greater fragmentation given the time and concentration adopted, it being clear that at 3.0 μM the dose adopted is very toxic to the cells, however such a finding shows that the compound binds to the DNA in some way by triggering the signal cascade causing cell death.

Cytotoxicity should not be the only parameter analyzed considering the structure of the zinc dimer and the geometry of the compound, we take into account its capacity for coordination and interaction with the receptors. In addition to versatility in coordination and geometry, which at some level potentiates the effect of the compound, regulating in some aspect's cellular tumor processes.

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Conclusion

The data presented in this work indicate a strong action of the cytotoxic effect of the experimental compound $[Zn(L^1)(\mu\text{-}CH_3COO)]_2$ with respect to the ligands and the salt of the inorganic synthesis, with emphasis on the IC_{50} of the complex presenting a value of 2.0 μM. Another gain from this work is in the DNA fragmentation experiment, indicating that the complex can bind to the genetic material in some way, causing the death of the cell. The cellular adhesion data and the micrographs obtained by phase contrast microscopy reinforce the finding of cytotoxicity and cytometry, making it clear that cell behavior changes in the first 48h presenting unusual morphologies. These findings reinforce that when we associate the ligand (HL^1) with the metal center of Zn (II) in an inorganic synthesis, the biological activity is drastically reduced., but mainly, they point to this class of molecules as a promising and versatile source of prototypes of new antitumor drugs.

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