STUDY OF NITROKETENE DITHIOACETAL-METAL COMPLEX AS A POTENT CANCER CONTROL THERAPEUTIC AGENT

J.Kavitha[1],
1. Department of Chemistry, V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India
e-mail Id: kavithaj@vvvcollege.org

L.Sakthikumar[2]
2. Department of Chemistry, S.B.K College, Aruppukottai, Tamilnadu, India
e-mail Id: lsakthisbk@gmail.com

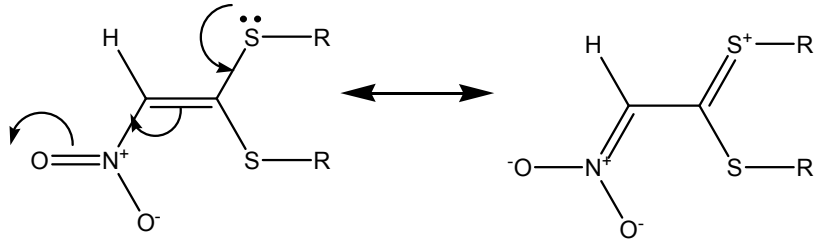
ABSTRACT:

In synthetic organic chemistry, the nitroketene dithioacetal motif (NKDA) based organo sulfur compounds are well known intermediates as a two carbon push–pull system. The nitro group acts as a powerful electron-withdrawing group and the two alkylated sulfur atoms readily donate their lone-pair of electrons to make the entire NKDA motif frame work as highly polarized system. Thorough literature search in the field of nitroketene dithioacetal chemistry showed that there is no report that NKDA has been used as inorganic ligand. It made a new route of synthesizing transition metal complexes with Nitroketene dithioacetal as one ligand which is an organic moiety. In this paper, we seek to give an overview of previous reviews on the cytotoxic effect of metal-based complexes while focusing more on newly designed NKDA-metal complexes and their cytotoxic effect on the cancer cell lines, as well as on new approach to NKDA-metal complexes drug design and molecular target in cancer therapy. We are optimistic that the concept of selective targeting remains the hope of the future in developing therapeutics that would selectively target cancer cells and leave healthy cells unharmed. Cell lines are useful models for doing research since they provide large amounts of consistent cells for prolonged used. Because most cellular characteristics are maintained in cell lines, they provide reliable in experimental results. Experimental results can be compared among other research reports, in which, the same cell lines were used. L929 cell line is usually used as tool in many standard testing. It is a cell line which popular in many experiment aspects such as material biocompatibility testing, drug cytotoxicity testing and cell biology studies. This research is aimed to prove the differentiation potency of L292 mouse fibroblast cell line compare with human dermal fibroblast by conducting in frequently used differentiation inducing media which their ingredients different from the media used in the above reports.

Key words: NKDA-Metal complexes, anticancer activity, L929 Mouse fibroblast, cancer cell line, cytotoxic effect.

1. INTRODUCTION:

In synthetic organic chemistry, the nitroketene dithioacetal motif (NKDA) based organo sulfur compounds are well known intermediates as a two carbon push-pull system[1,2]. The nitro group acts as a powerful electron-withdrawing group and the two alkylated sulfur atoms readily donate their lone-pair of electrons to make the entire NKDA motif frame work as highly polarized system. The push and pull polarization of E-and Z- sulfur lone pair of electrons with respect to the nitro group of the NKDA motif is possibly conjugated is represented as below



Cell culture is the process by which cells are grown under controlled conditions, generally outside their natural environment. After the cells of interest have been isolated from living tissue, they can subsequently be maintained under carefully controlled conditions. These conditions vary for each cell type, but generally consist of a suitable vessel with a substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (CO2, O2), and regulates the physio-chemical environment (pH buffer, osmotic pressure, temperature). Most cells require a surface or an artificial substrate (adherent or monolayer culture) whereas others can be grown free floating in culture medium (suspension culture). The lifespan of most cells is genetically determined, but some cell culturing cells have been "transformed" into immortal cells which will reproduce indefinitely if the optimal conditions are provided. In practice, the term "cell culture" now refers to the culturing of cells derived from multicellular eukaryotes, especially animal cells, in contrast with other types of culture that also grow cells, such as plant tissue culture, fungal culture, and microbiological culture (of microbes). The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture. Viral culture is also related, with cells as hosts for the viruses. The laboratory technique of maintaining live **cell lines** (a population of cells descended from a single cell and containing the same genetic makeup) separated from their original tissue source became more robust in the middle 20th century. Cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. Growing viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines. The injectable polio vaccine developed by Jonas Salk was one of the first products mass-produced using cell culture techniques. This vaccine was made possible by the cell culture research of John Franklin Enders, Thomas Huckle Weller, and Frederick Chapman Robbins, who were awarded a Nobel Prize for their discovery of a method of growing the virus in monkey kidney cell cultures. The development of more potent metal-based drugs have been investigated over the last three decades and it has been discovered that inorganic compounds have enormous impact in medicine[3]. Some metal complexes have been found to have antimicrobial and antiviral properties and could be effective against diseases[4]. This had led to numerous

investigations on metal-drug interactions and more studies on metal complexes with arm of discovering more effective chemotherapeutic agents to fight diseases. Also, it is known that some drugs at via chelation or by inhibitory metalloenzymes but for most drugs that act as potential ligands a lot of studies are being carried out to know how metal binding influences their activities[5]. Development of inorganic drugs is broadly divided into two categories, diagnostics and therapeutics. As diagnostics researches are been undertaken in radiopharmaceutical (γ,β+) magnetic resonance imaging (MRI) and X-ray contrast agents. As therapeutics, inorganic compounds are used chelating agents, metal-mediated antibiotics, antibacterial, antiviral antiparasitic, treatment of rheumatoid anthritis and radiosensitizers[6,7]. L929 cell line is usually used as tool in many standard testing. It is a cell line which popular in many experiment aspects such as material biocompatibility testing, drug cytotoxicity testing and cell biology studies. This research is aimed to prove the differentiation potency of L292 mouse fibroblast cell line compare with human dermal fibroblast by conducting in frequently used differentiation inducing media which their ingredients different from the media used in the above reports. In this review, we seek to give an overview of previous reviews on the cytotoxic effect of metal-based complexes while focusing more on newly designed NKDA-metal complexes and their cytotoxic effect on the cancer cell lines, as well as on new approach to metal-based drug design and molecular target in cancer therapy. We are optimistic that the concept of selective targeting remains the hope of the future in developing therapeutics that would selectively target cancer cells and leave healthy cells unharmed.

2. EXPERMENTAL

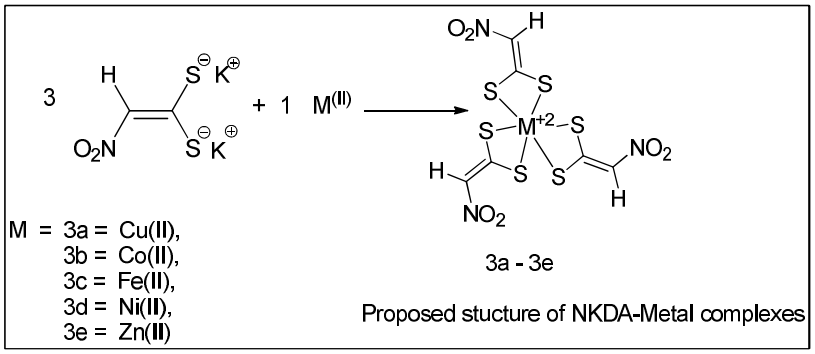
General procedure for the synthesis of NKDA - Metal Complexes (3a – 3e)

STEP 1: Synthesis of Dipotasium salt of 2-nitro-1,1-ethylene dithiolate[8-10]:

To a well stirred ice cold solution of nitromethane 1(10 ml, 0.1869mole) and carbondisulphide 2(11.3 ml, 0.1870 mole), a solution of potassium hydroxide(21g, 0.3743 mole) in 60ml of HPLC grade methanol was added drop by drop for 40 mins using a pressure equalizing funnel. The stirring was continued for 3hrs at 0°c-5ºc using freezing ice salt mixture. The reddish brown free flowing powder formed in the reaction mixture was quickly filtered using Buckner funnel under section and washed with dry methanol (25ml) followed by dry ether(25 ml)to yield 23g (58%) of reddish brown colour powder 3. The salt 3 was quickly transferred into a brown colour sample bottle and kept in dark to avoid photo decomposition.

STEP 2:Synthesis of Mixed Ligand NKDA Metal Complexes:

10 mmole (3.372g) of Nitroketene dithioacetal(NKDA) was dissolved in 20 ml of distilled water. 10 ml of aqueous :solution of the metal salt M (3 mmole, where M = $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $ZnCl_2.4H_2O$, $FeCl_3$ and $CuCl_2$) were added to the NKDA solution. The colour change and pH were recorded. The mixture was refluxed for overnight and left to stand. The precipitates formed were filtered, washed with distilled water and methanol. The precipitate formed were stored in well-labeled containers and dried over $CaCl_2$ in a dessicator.



Scheme 1: Schematic representation of synthesis of NKDA-Metal complexes

3. RESULTS AND DISCUSSION:

Materials and Methods:

- L929 cells (ATCC CCL 1)
  - Minimum Essential Medium Eagle (MEM)
  - Bovine Calf Serum (BCS), iron supplemented or New born Calf Serum (NCS)
- L-Glutamine, 200 mm
  - Non-essential Amino Acids (NEAA), 100X
- Dulbecco's Phosphate Buffered Saline, 1X (without Ca2+ or Mg2+)
- Trypsin Solution, 1X
- Antibiotic/ Antimycotic Solution,100X
- Crystal Violet or Methyl Violet, 0.1-0.4% in aqueous alcohol solution
- Reference lots

Analar grade chemicals and reagents were used in all the synthetic steps.

Metal Salts: The metal salts used were all purchased in the pure form from Sigma Aldrich Chemicals. They include copper(II) chloride hexahydrate, iron(III) chloride hexahydrate, Cobat(II) chloride hexahydrate, zinc(II) sulphate pentahydrate, iron(II) sulphate, zinc(II) chloride and nickel(II) chloride hexahydrate.

Solvents: The solvents used include: Distilled water, Ethanol, Methanol, Acetone, Chloroform, Petroleum ether, Benzene and Dimethylsulfoxide (DMSO).

Melting points were noted using IN-LAB equipment. UV-Vis spectra were recorded using Shimadzu spectrometer in DMSO solvent. TheIR spectra were recorded in Shimadzu instrument using KBr pellet. ^{1}H &^{13}C NMR data were recorded in Bruker-300 MHz NMR using DMSO as solvent. The FAB-mass was recorded using FAB-MS in a JEOL JMS-HX110 high field high resolution mass spectrometer. C, H, N elemental analysis was Carried out using Thermo Finnigan-EA11112 instrument.Energy Dispersive X-Ray Analysis(EDX) was recorded using Shimadzu EDX-7000/8000 Energy Dispersive X-ray Fluorescence Spectrometer.

UV-Visible spectral studies

The Co (II) complex exhibited bands at 282 and 585 nm which are attributed to the electronic charge transfer transition of $^{4}T_{1g}(F) \longrightarrow {}^{4}A_{2g}(F)$ and $^{4}T_{1g}(F) \longrightarrow {}^{4}T_{1g}(P)$ respectively. The

electronic spectra of Ni (II) complex shows bands corresponding to electronic transition of $^3A_{2g}(F) \longrightarrow ^3T_{1g}(P)$ and $^3A_{2g}(F) \longrightarrow ^3T_{2g}(F)$ at 284 and 683 nm respectively. These assignments correspond to Ni (II) octahedral complex. The spectrum of Cu (II) complex shows bands at 540 and 745 nm in the visible region which are attributed to the electronic transition of $^2b_{2g}(D) \longrightarrow ^2b_{1g}(D)$ and $^2e_{2g}(D) \longrightarrow ^2b_{1g}(D)$ at 540 and 745 nm respectively. Finally, the electronic configuration of Zn (II) complexes were (d10) which confirms the absence of any (d-d) transitions.

Infrared Spectra of the NKDA-Metal Complexes

The infrared spectra of Nitroketene dithioacetal – Metal complexes are shown in Table 3.3. The IR spectrum of NKDA showed a strong peak at 1410 cm^{-1} which were assigned to N-CH=C stretching vibrations. These peaks have been shifted in the metal complexes. The strong broad band at 650 cm^{-1} due to C-H (sp^2 CH bend), observed in the free NKDA is also present in all the complexes. This band remains unchanged upon complexation although slightly shifted in most of the complexes.Some new medium bands around 410-440 cm^{-1} in most of the complexes, were tentatively assigned to (M-S) vibrations. Infrared spectral bands of ligand (NKDA) observed at 1170 cm^{-1} (ν N-O) and at 1410 cm^{-1} (ν N-CH=C) remained almost unchanged in position on complexation indicating absence of bonding through nitrogen.However, (ν C-S) of the ligand undergoes red shift of 600- 700 cm^{-1} on complexation indicating bonding through sulphur in all complexes. The systematic shift in C-S bonds of ligands clearly indicates formation of metal sulphur bond. The olefinic protons in ^{1}HNMR were appeared in the range of ~6.70 ppm for the complexes 1c and 1e. Similarly in ^{13}CNMR the olefinic carbon atoms were appeared in the range of ~146.1ppm for NKDA- Fe and NKDA-Zn complexes1c & 1e. Another olefinic carbons were appeared in the range between ~113ppm for the complexes 1c and 1e. C, H, N elemental analysis data of the complexes 1a – 1e showed good agreement with the calculated values for the confirmation of molecular weight of the products.In the FAB mass spectrum of complex 1a, the molecular ion peak was observed at *m/z 465*, which is in agreement with the molecular weight (469.0405) of the proposed structure. Similarly, the FAB mass spectrum of NKDA-Co complex, the molecular ion peak was observed at *m/z* 463, which is in good agreement with the molecular weight (464.4277) of the proposed structure. EDX analysis data confirmed the presence of elements and the corresponding metals in the complexes 1a-1e. PXRD measurements were carried out to examine the phase and structure of the synthesized complexes. The PXRD pattern of the complexes shows a broad line parallel to the amorphous nature appears at a 2θ range between 15-30°. The surface morphology of the complexes was observed by scanning electron microscopy.

Anticancer Cell line Report - Culturing Procedure

1. Place culture vessels (samples and reference lots, as appropriate) in a laminar flow hood along with the culture medium components which have been pre-warmed to 37°C.
2. Prior to harvesting, cells must be at least 75% confluent with good morphology. Aspirate medium and wash cells twice with 1X PBS before trypsinization.
3. Add Trypsin to disaggregate the cells. Incubate culture vessels at 25°C or 37°C and monitor cell detachment under the microscope. Detachment time will vary.
4. After cells detach, add medium to stop trypsinization and to disperse the cells.
5. Transfer cells to a sterile conical tube and place on ice.

6. Determine cell quantity, e.g. Trypan Blue dye exclusion assay.
7. Determine the number of cell required for each product by multiplying the plating density by the surface area. Plating density for L929 cell line is 1.5 x 104/cm2.
8. Dilute cells into growth medium and seed cell culture product.
9. Incubate cells in a 37°C incubator with 5% CO2 for four days. A confluent monolayer of L929 cells should be formed.
10. Decant medium. Add reagent alcohol, 95%, for 5 to 10 minutes for fixation, then decant. Add crystal violet or methyl violet stain, 0.1-0.4%, to cover the surface for 5 to 10 minutes, then decant and wash with water.
11. Evaluate the monolayer when dry.

Prepare growth medium for L929 cell line as follows

MEM 1X 500.0 mL

BCS 57.0 mL

L-glutamine 5.7 mL

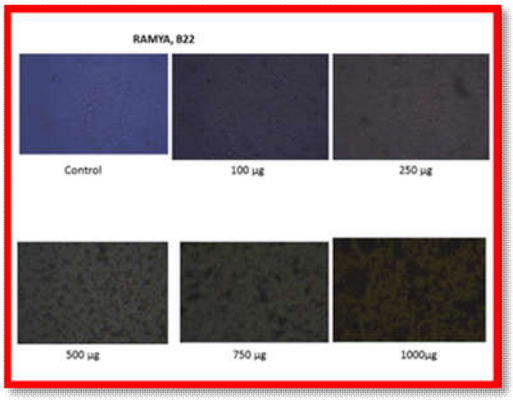
NEAA 5.7 mL

Antibiotic/Antimycotic 5.7 mL

Total 574.1 mL

Cell Morphology

Cell morphology was observed under microscope. Growthcurve and doubling time were determined. Number of viable cells was assayed by MTT method. MTT solution (5 mg/ml MTT in DMEM without phenol red) was incubated with cells at 37°C for 30 min. MTT solution was removed and violet formazan dye entrapped in viable cells was dissolved by dimethyl sulfoxide. The absorbance measured at 570 nm using a microplate reader. Amount of cells was determined using a standard curve established from known number of cells. Doubling time was determined form equation obtained from the growth curve. Cell morphology and number determinationCell morphology was observed under microscope. Growth curve and doubling time were determined. Number of viable cells was assayed by MTT method. MTT solution (5 mg/ml MTT in DMEM) without phenol.



Morphology of L929 Mouse Fibroblast

Cell culture

Sources of cell line : NCCS

Justification : **L929** is an established and well-characterized cell line that has demonstrated reproducible results.

Culture medium : MEM medium supplemented with fetal bovine serum

Test details

Assay method and Rationale : MTT-Direct

Test sample preparation : Water

Evaluation criteria

S.NO	Grade (%)	Reactivity
1.	0	None
2.	1-20	Slight
3.	21-50	Mild
4.	51-70	Moderate
5.	>71	Severe

Observation

Concentration (µg)	**Cytotoxicity (%)**	**Viability (%)**	**Cytotoxic reactivity**
100	42.5	57.5	Mild
250	43.0	57.0	Mild
500	46.8	53.2	Mild
750	73.o	27.0	Severe
1000	45.0	55.0	Mild

4. CONCLUSION:

A great deal of research has been conducted in the therapeutic application of metal based complexes, We synthesis more new library of transition metal complexes with Nitroketene dithioacetal compound shows good result in binding the metal with the biological compound. Then for each metal we had studied the spectral characterisation in UV, IR, Fab Mass Spectrometer, C, H, N, Elemental analysis, EDX analysis. This method is then undertaken to anticancer activity in L929 cell line in mouse. As per ISO 10993:5 the samples showed **Mild To Severe Cytotoxic** reactivity to **L929** cells after 24 h contact.L929 mouse fibroblasts and human dermal fibroblasts werequite different both in cell size and doubling time. By the condition used in this research, L929 mouse fibroblasts could be induced to undergo while taking in less concentration there will be good anticancer activity and will taken for future work. This report and the accumulated results from other reports reveal the plasticity of L929 mouse fibroblasts that can be successfully induced to undergo differentiation by diverse culture conditions. Their revealed potency allows L929 mouse fibroblast cell line to become a useful cell line for various experiment aspects. Human dermal fibroblasts unsuccessful induced to undergo adipogenic differentiation this may be due to inducing medium in this research differ from those researches which special inducing media were used.

Acknowledgement: The authors sincerely thanks to the management AND FIST Instrumentation Centre of V.V. Vanniaperumal College for Women, Virudhunagar for providing necessary facilities and constant encouragement.

REFERENCES:

1. Interdiscipl.Sci.Rev.978,3,134-147, Rosenberg, B.latinumcomplexes for the treatment of cancer.
2. Gompper. R and Schaefer. H Chem. Ber, 1967, 100,91.
3. Dalton Trans, pp; 4929-4937 Hambley TW (2007) Developing new metal based therapeutics challenges and opportunities.
4. Reddy, M. R. & Erion, M. D. J. Enzyme. Inhib, 1998. 14, 1-14.
5. Taylor. R. D. Jewsbury, P. J. & Essex, J. W, J. Comput, Aided Mol.Des.2002.16. 151-166.
6. Biochem Biophys. Res.Commun. 1981. 103,521-15, Hatchikian, E.C. A cobalt porhyrin containing protein reducible by hydrogenase isolated from Desulfovibro desulfuricans(Norway).

7. J Cell Mol Med.2003. 7, 333-335, Parvu, L.

8. Der Pharma Chemica, 2014, 6(2):294-298, Synthesis, characterization and antibacterial activity of alkyl, benzyl and chloro substituted benzyl derivatives of nitroketene dithioacetals **L. Sakthikumar, J. Kavitha and R. Mahalakshmy.**

9. Der Pharma Chemica, 2017, 9(12):70-75, ISSN 0975-413X CODEN (USA): PCHHAX, Electro-oxidation and Determination of Sulfur-containing Amino Acid on Screen-printed Carbon Electrode Modified with NZ **Kavitha J, Mahalakshmi R**

10. Indo American Journal of Pharmaceutical Sciences, IAJPS,2018,05(01),645-658, ISSn 2349-7750, Synthesis,spectral and biological studies of mixed ligand transition metal complexes of nitroketene dithioacetal with ephedrine , **J. Kavitha, L. Sakthikumar, R. Mahalakshmy**