UNIVERSIDAD AUTONOMA DE NUEVO LEÓN

FACULTAD DE CIENCIAS QUIMICAS

# DETERMINATION OF THE CYTOTOXIC CAPACITY OF ORGANOTIN COMPOUNDS DERIVATIVES FROM SALICYLIDENE BENZOYLHIDRAZONE

By

JESSICA CECILIA BERRONES REYES

As partial requirement for the Degree of
MASTER OF SCIENCE with
Orientation in Pharmacy

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DETERMINATION OF THE CYTOTOXIC CAPACITY OF

ORGANOTIN COMPOUNDS DERIVATIVES FROM

SALICYLIDENE BENZOYLHIDRAZONE

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# RESUMEN

QFB Jessica Cecilia Berrones Reyes Julio 2015

Universidad Autónoma de Nuevo León

Facultad de Ciencias Químicas

Título del estudio: DETERMINACION DE LA CAPACIDAD CITOTOXICA DE COMPUESTOS DE ESTAÑO DERIVADOS DE SALICILDENBENZOILHIDRAZONAS

Número de páginas: 92

Candidato para el grado de Maestría en Ciencias con Orientación en Farmacia.

Área de estudio: Farmacia.

Propósito y Método de Estudio: La aplicación exitosa de complejos metálicos en el tratamiento de numerosas enfermedades, como el cáncer, es un área en plena expansión en química biomédica e investigaciones. Estudios de compuestos organoestánicos, muestran la versatilidad y las atractivas características de estas moléculas. A su vez, se ha reportado el desarrollo de nuevos compuestos a partir de bezoilhidrazinas, las cuales poseen el fragmento azometino (-N=CH-), pilar clave para el diseño de nuevos fármacos con actividad biológica. Con base en lo anterior, estamos interesados en realizar la síntesis de cuatro compuestos de estaño derivados de benzohilidrazonas, y determinar su capacidad citotóxica.

Contribuciones y Conclusiones: En este trabajo de investigación se reportaron cuatro nuevas estructuras de compuestos de estaño derivados de benzoilhidrazinas, los cuales fueron caracterizados mediante diversas técnicas espectroscópicas y espectrométricas, así como difracción de rayos X. De igual forma la capacidad citotóxica de cada compuesto fue estudiada y comparada con fármacos metalados como cisplatino y carboplatino. Y en algunos casos fue posible el uso de su capacidad luminiscente para estudiar su comportamiento intracelular.

FIRMA DEL ASESOR:

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Dr. Victor M. Jiménez Pérez

SUMMARY

QFB Jessica Cecilia Berrones Reyes July 2015

Universidad Autónoma de Nuevo León

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Study Title: DETERMINATION OF THE CYTOTOXIC CAPACITY OF ORGANOTIN COMPOUNDS DERIVATIVES FROM SALICYLIDENE BENZOYLHIDRAZONE

Page number: 92

Candidate for the degree of Master of Sciences with Orientation in Pharmacy.

Study area: Pharmacy.

Purpose and study method: The successful application of metal complexes in the treatment of many diseases, including cancer, is a rapidly expanding area in biomedical chemistry and research. Organotin compounds studies show the versatility and the attractive features of these molecules. In the same manner, has been reported the development of new compounds derivative from bezoylhidrazones, which possess the azomethine fragment (-N = CH-), key pillar for designing new drugs with biological activity. Based on the above, we are interested in to synthesize four tin compounds derivatives from benzoylhidrazones, and the evaluation of their cytotoxic capacity.

Conclusions and contribution: In this research work, we reported four new tin compounds derivatives from bezoylhidrazones, which were characterized by spectroscopic and spectrometric techniques and X-ray diffraction In the same manner, cytotoxic ability of each compound was studied and reported and compared with metaled base drugs like cisplatin and carboplatin. Also, in some cases it was possible to use their luminescent ability to study their intracellular behavior.

ASSESOR SIGNATURE:

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Dr. Victor M. Jiménez Pérez

This thesis was realized at Laboratory of materials III into the postgraduate area of Facultad de Ciencias Químicas from Universidad Autónoma de Nuevo León under the management of Ph.D. Victor Manuel Jiménez Pérez.

This project was carried out whit the financial support from CONACYT (558050), and it was complemented with two short stays with the collaboration of Ph.D. Arturo Chávez and Ph.D. Rasika Dias.

## DIVULGATION PROJECT AND STAYS PERFORMED

The study realized in this thesis generated a publication:

- Víctor M. Jiménez-Pérez, María C. García-López, Blanca M. Muñoz-Flores, Rodrigo Chan-Navarro, Jessica Berrones, H. V. Rasika Dias, A. Chávez-Reyes, Jesús A. Serrano, Rosa Santillán, and M. E. Ochoa. New Application of Fluorescent Organotin Compounds Derived from Schiff Bases: Synthesis, X-ray Structures, Photophysical Properties, Cytotoxicity and Fluorescent Bioimaging. ***J. Mater. Chem. B***, **2015**, 3, 5731-5745.

Two shorts stays were performed:

- September 1$^{st}$ 2014 to February 20$^{th}$ 2015. Stay in Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional en Monterrey (Cinvestav), under the management of Ph.D. Arturo Chávez.

- March 15$^{th}$ to May 31$^{st}$ 2015. Stay in the department of Chemistry and Biochemistry of the University of Texas at Arlington, under the management of Ph.D. Rasika Dias.

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But most of all I thank my family, who throughout my life have ensured my welfare and education being my support at all times so that I could achieve my dreams, for motivating me and shake my hand when I felt that the road was finished.

I dedicate this thesis

TO MY PARENTS. They put their complete trust in me in every challenge that I face, without hesitation or a moment in my intelligence and ability. It is for them that I am what I am now, with you forever , my heart and my thanks.

MY SIBLINGS. Thanks to those important people in my life who were always ready to give me all your help.

MY GRANDPARENTS. For his infinite love, affection, understanding and support, for your prayers and wise counsel

TO GOD. Because he has been with me every step I take, giving me strength to continue.

With all my love for all people who believed in me

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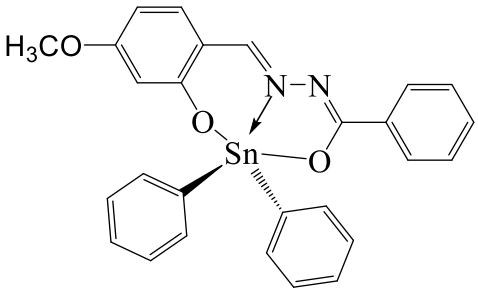
## LIST OF ABBREVIATIONS

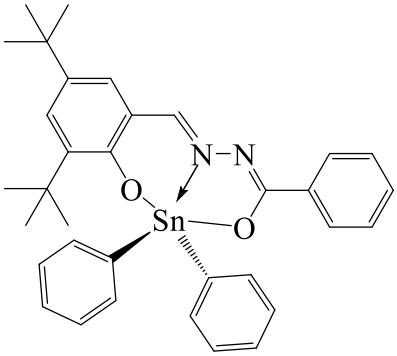
| | | | |
|---|---|---|---|
| **ATR** | attenuated total reflection | **µg** | microgram |
| **Å** | Armstrongs | **MP** | melting point |
| **$CO_2$** | carbon dioxide | **µm** | micrometer |
| **$^{13}C$** | carbon NMR | **ml** | mililiter |
| **$^oC$** | Celsius degrees | **mmol** | milimol |
| **δ** | chemical shift | **nm** | nanometer |
| **COSY** | correlation spectroscopy | **NMR** | Nuclear magnetic resonance |
| **J** | coupling constant | **OLED** | organic light-emitting diode |
| **DNA** | deoxyribonucleic acid | **ppm** | Parts per million |
| **$CDCl_3$** | deuterated chloroform | **PBS** | phosphate-buffered saline |
| **DMSO** | dimethyl sulfoxide | **PVC** | polyvinyl chloride |
| **DMEM** | Dulbecco's Modified Eagle's medium | **pH** | potential of Hydrogen |
| **HETCOR** | 2-D heterocorrelation | **$^1H$** | proton NMR |
| **FBS** | fetal bovine serum | **RNA** | ribonucleic acid |
| **HGFI** | gingival biopsy cells | **$cm^2$** | square centimeters |
| **g** | grams | **SD** | standard deviation |

| | | | |
|---|---|---|---|
| **Hz** | hertz | **$(CH_3)_4Si$** | tetramethylsilane |
| **hr** | Hours | **$(CH_3)_4Sn$** | tetramethyltin |
| **IC50** | inhibitory concentration of 50% of cell growth | **TOF** | time of flight |
| **IR** | infrared spectroscopy | **$^{119}$ Sn** | tin NMR |
| **K** | Kelvin degrees | **UV** | ultraviolet |
| **MHz** | Megahertz | **λ** | wavelength |
| **A375** | melanoma cells | **WHO** | World Health Organization |
| **B15F10** | melanoma murine cells | | |

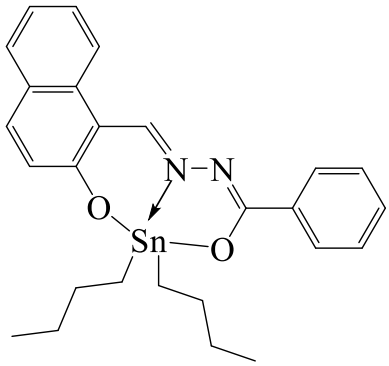
## LIST OF ORGANIC COMPOUNDS



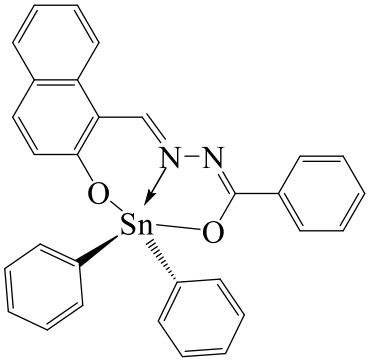

Compound **1**.




Compound **2**.




Compound **3**.




Compound **4**

# 1. INTRODUCTION

"Cancer" is a generic term that encompasses a large group of diseases that develop as a result of abnormalities in the genetic material of cells and can affect any part of the body; is also spoken of "malignant tumors" or "malignancies". A feature of cancer is the rapid multiplication of abnormal cells that extend beyond their usual limits and can invade adjacent parts of the body or spread to other organs, a process known as metastasis (**Figure 1**).

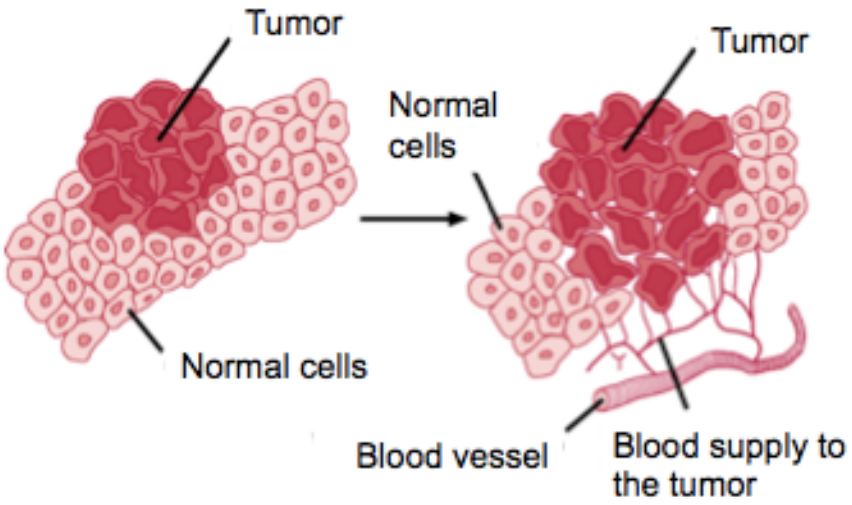
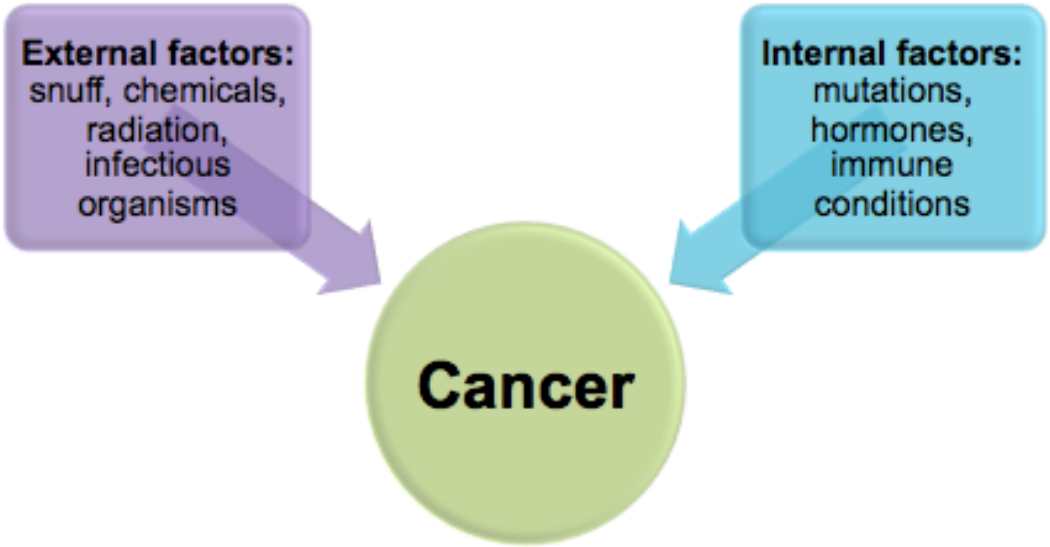



**Figure 1**. Rapid multiplication of cancer cells.

In an observation on a global scale, cancer is one of the major causes of deaths, is responsible for one in every eight deaths throughout the world.[i] According to the WHO (World Health Organization) this disease is attributed

about 7.6 million deaths (approximately 13% of the total) that occurred worldwide in 2008. [ii]

It encompasses more than 100 different diseases with different risk factors and epidemiology that originate from the majority of cell types and organs of the human body. It is known that cancer can be caused by external factors (snuff, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from the metabolism) (**Scheme 1**). These causal factors may act together or in sequence to initiate or promote carcinogenesis. The development of most types of cancer requires multiple steps that take place over many years.[iii]




**Scheme 1.** Factors that may favor the development of cancer.

Genetic abnormalities found in cancer cells may consist of a point mutation, translocation, amplification, deletion, and gain or loss of an entire chromosome. There are genes that are more likely to suffer mutations that trigger cancer. These genes, when in its normal state, are called proto-oncogenes, and when mutated are called oncogenes. What these genes usually encode are growth factor receptors, so that genetic mutation renders produces receptors to be permanently activated or encoding growth factors themselves, and the mutation can cause growth factors to be produced in excessive and uncontrolled manner.[iv]

The tissue from which cancer cells originate classifies Cancer. The type of tumor and the extent of disease largely influence the diagnosis. Often in early stages, symptoms may be interpreted as originating from other pathologies. Although the first manifestations of the disease come from symptoms, blood tests or X-rays, definitive diagnosis requires histological examination under a microscope. The histological classification after biopsy of the lesion is used to determine the most appropriate treatment. The treatment options depend mainly on the type of cancer that is suffering from well as the status, taking into account other individual factors such as age, general condition, patient preferences, etc.[v]

For cancer treatment careful selection of one or more therapeutic modalities, surgery, radiotherapy or chemotherapy is required. The goal of each

one of them is to cure the disease, or significantly prolong the survival and possibly improve the quality of life for patients.

The applications of appropriate treatments that combine surgery, radiotherapy and chemotherapy have allowed improving greatly on the quality of life for cancer patients and to lower mortality.

Although surgery continues to be the primary treatment in our days has managed to be less radical and this is largely due to the use of radiotherapy, which can eliminate the tumor mass with great precision and once unthinkable power. However, this form of therapy is local and only has an effect if malignant cells are located within the irradiated field, is ineffective in combating metastasis. Here comes into play its role chemotherapy, which is the third weapon with which they have to fight cancer.

It is known that chemotherapy is the treatment with drugs that can destroy these cells or stop them from dividing (**Figure 2**). However, one of the major situations that arise against them is the fact that it can also damage healthy cells, causing the so-called side effects.

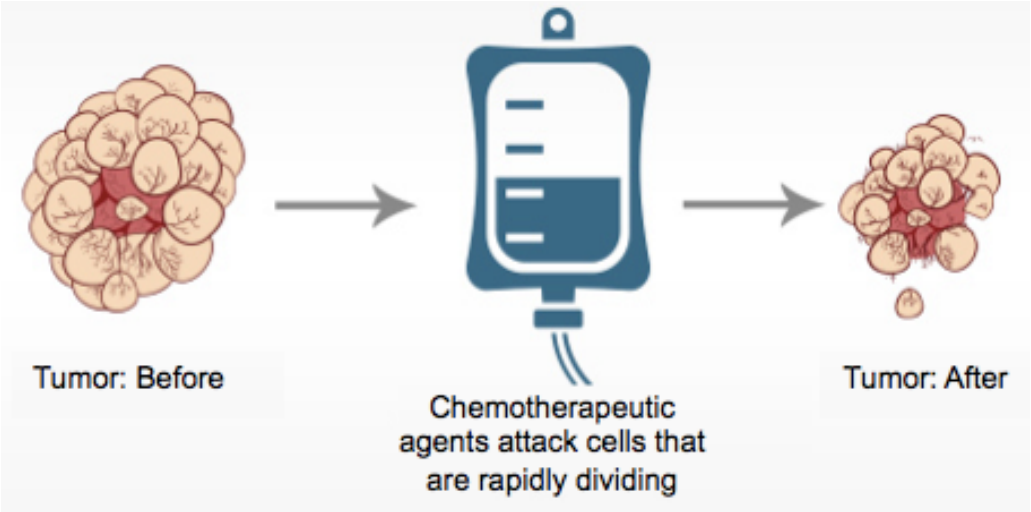



**Figure 2**.General action of drugs in cancer treatment.

Chemotherapy is used to treat many types of cancer. Currently more than 100 chemotherapy drugs are used, either on their own or in combination with other medications or treatments. As the investigations continue, it is expected to have more drugs available. These drugs vary widely in their chemical composition, the way they are administered, their usefulness in treating specific forms of cancer and its side effects.

Antitumor drugs act on cell division by inhibiting or hindering, but these preparations have the disadvantage that they do not act on a selective basis and do not interact with the neoplastic cells only, but rather attack the healthy dividing ones, such as red blood cells, platelets, hair follicles, mucosal, and so on. Therefore patients receiving chemotherapy lose their hair, suffer immunosuppressive conditions, mucosal inflammation and ulcers in the mouth.

Yet the great workhorse of chemotherapists is in the ability of cancer cells to be invulnerable to drugs that interfere with the cellular division.

Since the introduction of cisplatin (**Figure 3**) in cancer therapy, metal complexes and organometallic compounds have been gaining increasing importance in oncology. The impressive clinical effectiveness of cisplatin is limited by significant side effects and the appearance of drug resistance. Therefore, new Pt (II) and Pt (IV) complexes have been introduced in therapy or are currently in advanced clinical trials. Furthermore, have been developed innovative non-platinum metal based antitumor agents whose activity are not dependent on direct DNA damage and may involve proteins and enzymes. Derivatives of gold and tin are enjoying a growing interest and look very promising as potential drug candidates.[vi]

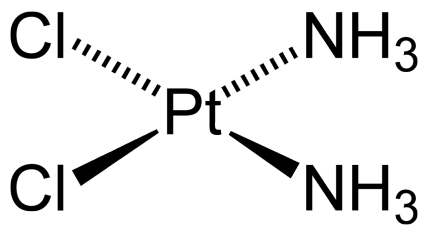



**Figure 3**.Molecular structure of cisplatin.

# 2. BACKGROUND

## 2.1 Antineoplastic Drugs.

There are many ways to induce irreparable DNA damage, which can cause the detention of its replication and create the conditions to induce cellular apoptosis.

Chemotherapy drugs can be divided into several groups based on factors such as their mechanism of action, its chemical structure and its relationship with other medicines. Since some medications work in more than one way, they may belong to more than one group. It is important to know the mechanism of action of a medicinal product to predict side effects. This helps oncologists decide which medications may work well in combination.

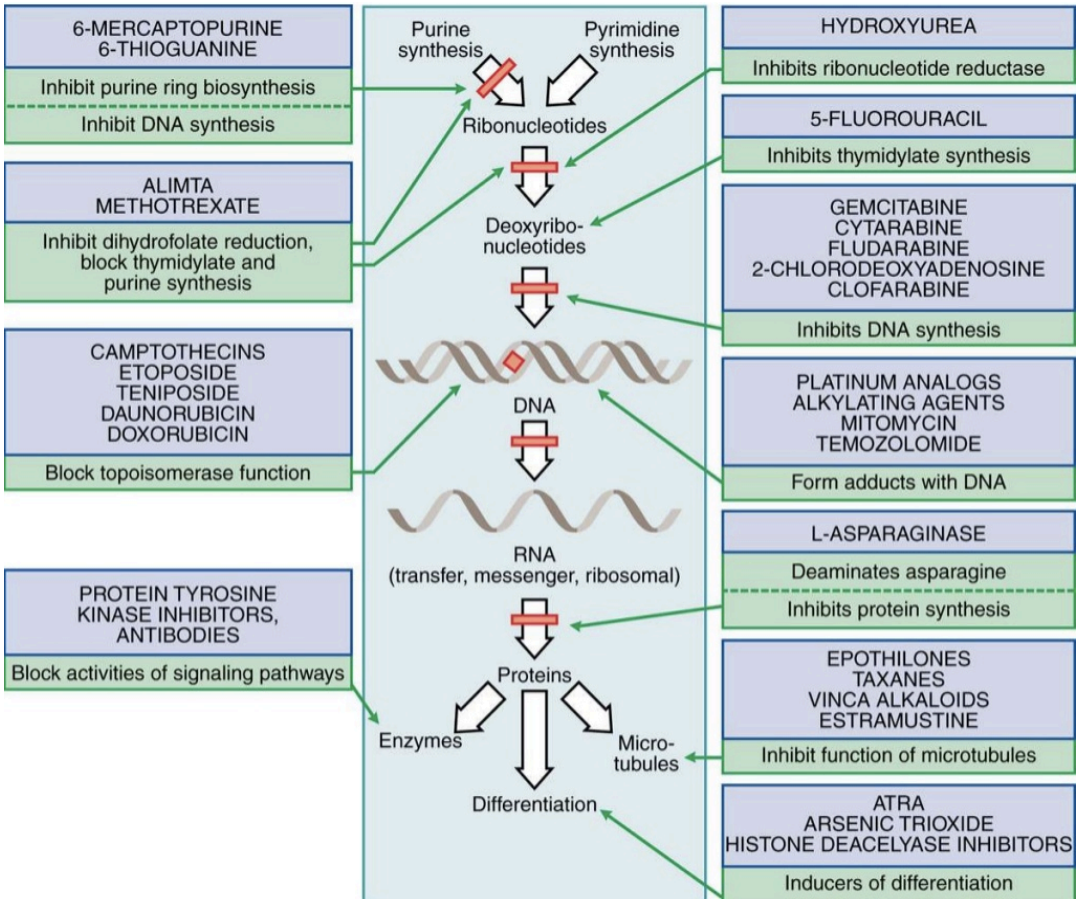
## 2.2 Types of Drug–DNA interaction.

There are primarily three different ways by which anticancer drugs interact with DNA [vii] (**Scheme 2**):

- Through control of transcription factors and polymerases. Here, the drugs interact with proteins that bind to DNA.

• Through RNA binding to DNA double helix to form nucleic acid triple helix structures or RNA hybridization (sequence specific binding) to exposed DNA single strand forming DNA–RNA hybrids that may interfere with transcriptional activity.

• Through binding of small aromatic ligand molecules to DNA double helical structures. The binding of small molecules to DNA involves electrostatic interaction, intercalation between base pairs and minor and major DNA grooves binding interaction.




**Scheme 2**. Summary of mechanisms of action of anticancer drugs [viii]

## 2.3 Metal based drugs.

The successful application of metallic complexes in the treatment of many human diseases is a rapidly expanding area in biomedical chemistry and researches, which undoubtedly help to improve the quality of life. Cancer is one of the primary objectives of scientific research in the area of drug development, as it is one of the leading causes of premature deaths around the world.

Several efforts have been carried out to find useful treatments for cancer, since 1969 Rosenberg reports on the cytostatic activity of a synthetic compound of inorganic origin that set the standard for a new series of compounds: Cisplatin (cis-diamine-dichloro platinum (II)). Many tumors are refractory to this and therefore the search for new compounds began with the following characteristics:

- Absence of cross-resistance
- Wider spectrum of activity
- Higher antitumor clinical effectiveness
- Reduction of emetic and kidney effects
- Synergism in combined therapies.

Experimental evidence has demonstrated that cisplatin coordination compounds with antineoplastic activity satisfy the following assumptions: With

the discovery of cis-diamminedichloroplatinum (II) in the sixties it has been observed that the metallic compound acts as the focal point for proteins nucleic acids of cancer cells and it has been proposed that the method of action of cisplatin is similar to an intercalating agent or alkylating and is directly linked to the nitrogen of the 7-position of guanine in DNA. [ix]

The investigation has shown that the most effective coordination compounds used as anticancer medicines, based on DNA binding, are cisplatin, oxaliplatin, carboplatin and nedaplatin, which are metalocomplexes platinum (II)[x]. These medications revolutionized chemotherapy as from the late seventies to be active against resistant tumors commercial drugs. Despite its high activity, the application of cisplatin and similar compounds has significant disadvantages which include: poor water solubility, severe side effects that are typical of the heavy metal toxicity, and the development of drug tolerance from the tumor. The last two are the main driving force behind the current research in the field of development of new anticancer agents.

The investigations of compounds with other metals (Ti, Ga, Ge, Pd, Au, Co, and Sn) are currently ongoing which may help prevent, or improve, the problems associated with the use of platinum compounds as therapeutic agents.[xi] Indirect ionizing radiation causes DNA damage by reactive oxygen species, while chemotherapy uses chemical substances that interact directly with DNA. (**Figure 4**)

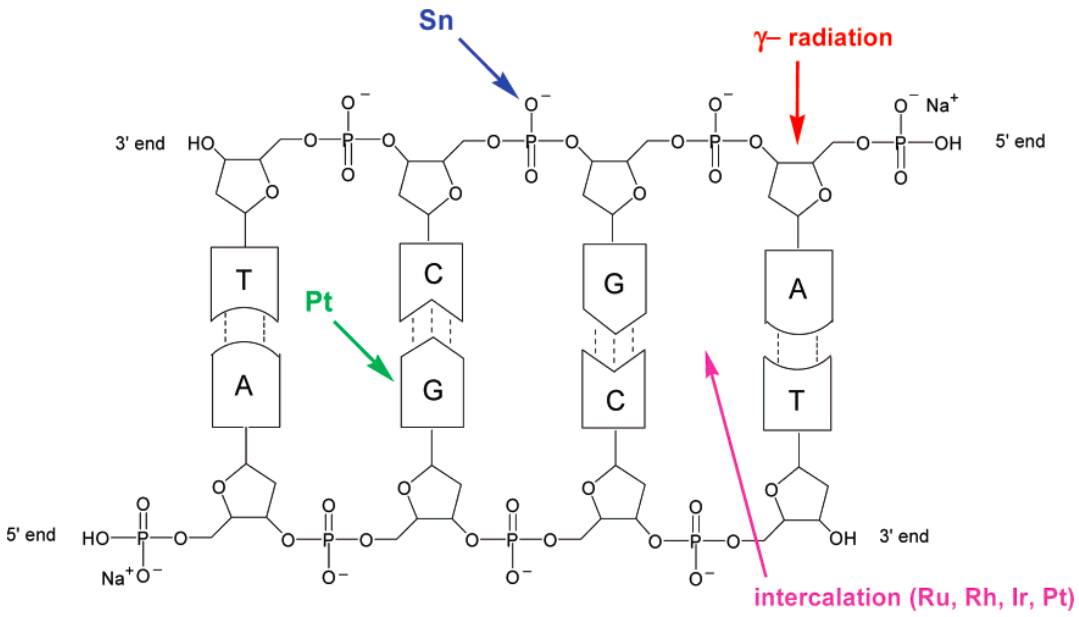



**Figure 4.** Mechanism of action proposed for organometallic compounds. [xi]

## 2.4 Organotin compounds.

The value of the organotin compounds (IV) resides in its wide range of applications such as stabilizers in PVC (polyvinyl chloride) in antifouling paints and coatings for the protection of ship hulls and as precursors of $SnO_2$ in glass coating [xii] in electro luminescence with the manufacturing of OLEDS (Organic Light-Emitting Diode),[xiii] its use in the design of sensors that selectively respond to the presence of an analyte in particular [xiv] as well as studies of biological activity of these molecules, which have proved their antifungal and antibacterial properties. [xv] Currently organometallic tin compounds are latent alternative as chemotherapeutic agents, anticancer who show acceptable antiproliferative activity in vitro and in vivo.[xvi]

The structural diversity of the compounds and their applications, particularly the cytotoxic activity, have been a major impetus for the investigation of these molecules. [xvii]

It is well known that the biochemical activity of organotin compounds is influenced by the structure of the molecule and the coordination number of tin atoms. It has been determined that the nature of the binder does not affect the toxicity with the exception that it itself is toxic. The ability to bind to DNA depends on the coordination number and nature of the groups attached to the tin atom. DNA phosphate usually acts as the anchoring site. With a coordination number of 5 tin forms a dense positive core is attracted to the negatively charged phosphate.

**2.5 Tin compounds as cytotoxic.**

The antitumor properties of tin complexes have been established since 1929. In 1980 Crowe [xviii] *et. al*. reported the anticancer characteristics of organotin complexes. Gielen published a series of research articles on this subject over the past two decades. They synthesized a series of water-soluble organotin complexes and investigated their anticancer activities. They found that the anticancer activities of these organotin complexes were 100 times higher than that of carboplatin and cisplatin used in clinical applications [xix]. Since then,

many researchers have shown great interest in this field and published reviews of progress on the detection of potential antitumor organotin compounds.

Organic tin compounds that exhibit potent anticancer activity can act through different mechanisms at the molecular level.[xx] Induced cytotoxicity of organic tin compounds has been associated with several mechanisms: inhibition of macromolecular synthesis, altered mitochondrial energy metabolism, reduction of DNA synthesis and direct interaction with the cell membrane leading to an increase calcium concentration. The promotion of oxidative damage in DNA were detected in vivo. [xxi]

The most recent use of the different organic derivatives of tin (IV) has shown an acceptable in vitro cytotoxicity and in vivo antiproliferative activity as new chemotherapy agents. [xxii] Also, organic complexes of tin (IV) have other attractive features such as increased water solubility, toxicity in general tends to be less than the platinum drugs, best possible removal of body, a reduction in the case side effects and no emetogenesis. However, the most important point of organotin compounds is the fact that these do not develop tolerance that generates cisplatin and its analogs. [xxiii] Additionally, it has been well established that organotin (IV) compounds are involved in cancer chemotherapy because of their apoptotic inducing property . [xxiv]

## 2.6 Tin complexes derivative from benzoylhydrazine.

Lately new compounds with biological activity have been developed from bezoylhidrazines. These molecules possess the azomethine fragment (-N = CH), which is the key to the design of new drugs with anticonvulsant, antidepressant, analgesic, anti-inflammatory and anti-malarial activity [xxv]. Their analysis has been a pattern to develop new hydrazones with diverse biological activity. It has been described the synthesis of tin complexes derivatives of benzoylhydrazines ligands either penta- (I-IV) [xxvi] or six-coordinated (V-VII), [xxvii] which was characterized in solution and their molecular structure was elucidated. In turn, these compounds have high thermal stability to air and moisture. (**Figure 5**)

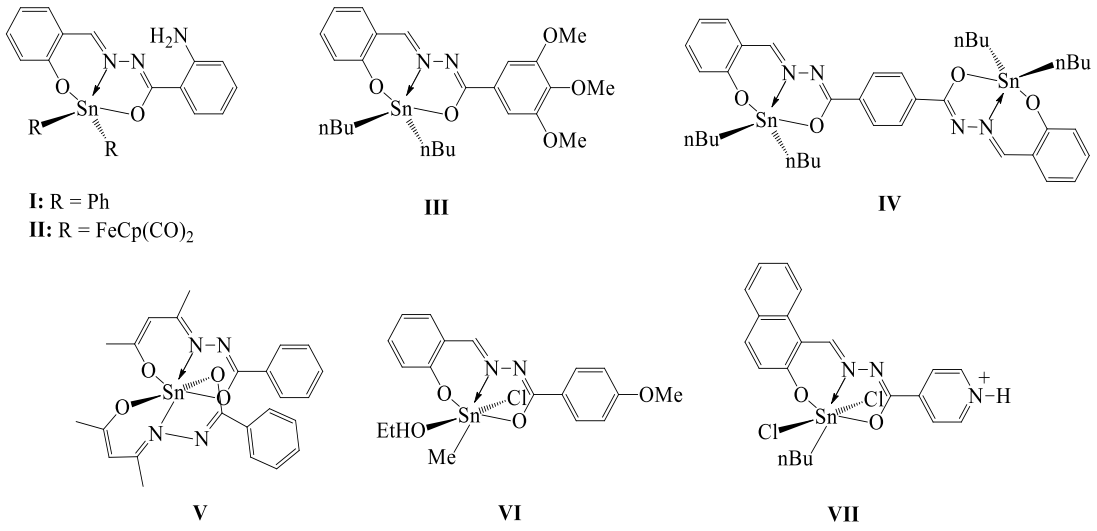



**Figure 5**. Penta and hexacoordinated organotin compounds derived from benzoylhydrazine.

# 3.HYPOTHESIS AND OBJECTIVES.

## 3.1 Hypothesis.

Pentacoordinated organotin compounds derived from benzoylhydrazine have a cytotoxic effect on neoplastic cell lines in culture.

## 3.2 General Objective.

Synthesize, characterize four new tin compounds derived from benzoylhydrazines and test its cytotoxic effect on neoplastic cell lines in culture.

### 3.2.1 Specific Objectives.

- Synthesize four new benzoylhydrazine tin compounds by a conventional method.

- Spectroscopy ($^{1}H$, $^{13}C$, $^{119}Sn$ NMR; IR) and optically (UV-vis, fluorescence) characterization of synthesized compounds.

- Obtain appropriate crystals and set the crystal structure through X-ray diffraction.

- Perform biological cytotoxicity assays in normal and cancer cell lines.

- Carry out biological cytotoxicity assays in murine melanoma line.

- Explore the possibility of using the fluorescence property of tin compounds derived from benzoylhydrazines in cell staining using a murine melanoma line.

# 4. MATERIALS AND METHODS

## 4.1 Materials.

### 4.1.1 Synthesis and characterization

All starting materials were purchased from Sigma-Aldrich Chemical Company. Solvents were used without further purification.

Melting points were determined on an Electrothermal Mel-Temp apparatus, and are not corrected. Infrared spectra were recorded using a Burker Tensor 27-FT-IR spectrophotometer equipped with a Pike Miracle $^{TM}$ ATR accessory with a single reflection ZnSe ATR cristal.

Multinuclear magnetic resonance experiments as $^{1}H$, $^{13}C$, and $^{119}Sn$- NMR spectra were recorded on a Bruker advance DPX 400. Chemical shifts (ppm) are relative to $(CH_3)_4Si$ for $^{1}H$ and $^{13}C$, and $^{119}Sn$-NMR spectra were referenced externally to $(CH_3)_4Sn$. Mass spectra were recorded on an AB Sciex API 2000 $^{TM}$ LC/MS/MS System.

Elemental analyses were carried out on a Thermo Finngan Flash EA 1112 elemental microanalyzer. High resolution mass spectra were acquired by source. UV-Vis absorption spectra were measured on a Shimadzu 2401 PC spectrophotometer. The emission spectra have been recorded with a Fluorolog 3 spectrofluorometer, by exciting 10 nm below the longer wavelength absorption band.

The X-ray data for the compounds 1-4 were covered with a layer of hydrocarbon oil that was selected and mounted with paratone-N oil on a cryo-loop, and inmediately placed in the low-temperature nitrogen stream at 100(2) K. The data for the compounds were recorded on a Bruker SMART APEX CCD area detector system equipped with a Oxford Cryosystems 700 Series Cryostream cooler, a graphite monochromator, and a Mo Kα fine-focus sealed tuve (λ=0.71037 Å). The structures were solved by direct methods using SHELXS-97 and refined against $F^2$ on all data by full-matrix least-squares with SHELXS-97. Absorption corrections were applied by using SADABS.

### 4.1.2 Bioassays

To perform the in vitro studies we used three different cell lines (**Table 1**):

**TABLE 1**

List of cell lines used in cytotoxicity assays.

| Cell line | #ATCC | Organism | Tissue | Morphology |
|---|---|---|---|---|
| **HGF1** | CRL-2014 | Human | Gingival biopsy | Fibroblast |
| **A375** | CRL-1619 | Human | Skin | Epithelial |
| **B16F10** | CRL-6475 | Mouse | Skin | Spindle-shaped and epithelial-like cells |

The culture medium used was DMEM and DMEM/F12, both supplemented with 5% fetal bovine serum and 1x antibiotic-antimycotic, all purchased from GIBCO Invitrogen Life Technologies. Other reactants used were 0.25% Trypsin-EDTA (1X), antibiotic-antimycotic (100x), purchased from GIBCO Invitrogen Life Technologies. Alamar blue used for the metabolic viability assay from Biosource Invitrogen Life Technologies. Neutral Red solution as a vital stain to stain living cells, from Sigma-Aldrich.

Equipment:

• Incubator for cell culture model 3554 Thermo Electron Corporation.

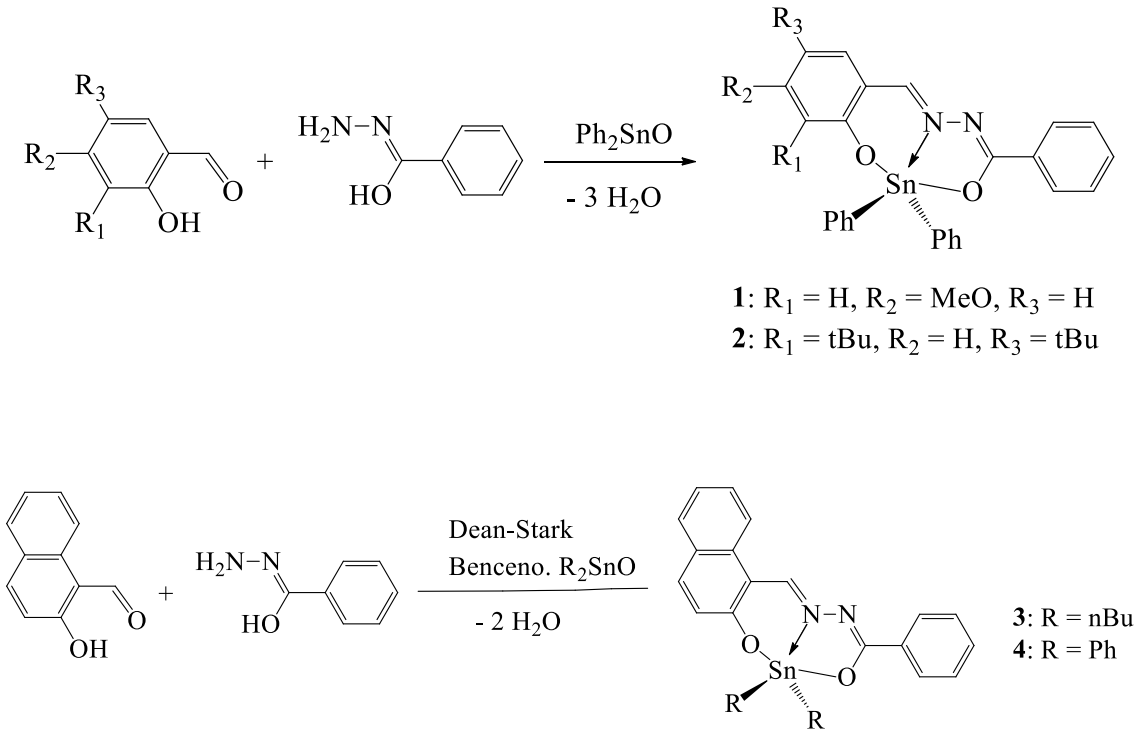
• Bell laminar flow biosafety Nuaire A-425-400 model.

• ELISA plate reader Labsystems Multiskan model ELx800.

• Confocal microscope TCS SP5 model Leica.

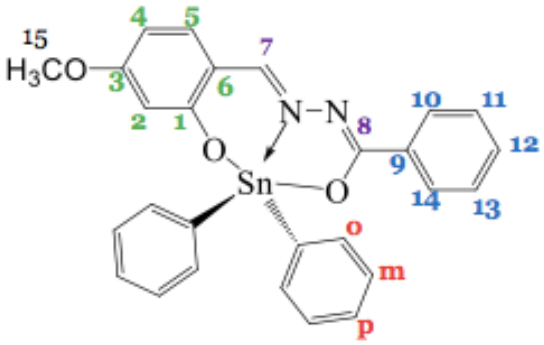
## 4.2 Methods.

### 4.2.1 Synthesis procedure of compounds 1-4.




**Scheme 3.** Synthesis procedure of compounds 1-4.

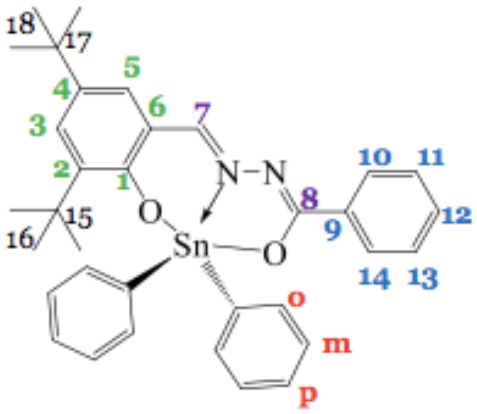
**4.2.1.1** [N-(3-methoxy-6-benzylidene)-benzyhydrazidato] diphenyltin (IV). **(1)**




A solution of 2-hydroxy-4-methoxybenzaldehyde (0.15g, 1mmol), benzoic hydrazide (0.13g, 1mmol), and di-phenyltin oxide (0.29g, 1mmol) in benzene (50 ml) was heated to reflux for 24 hr in a Dean-Stark apparatus used for the azeotropic removal of water and allowed to cool to room temperature. Then, all volatiles were removed under vacuum. Yield: 83%. Yellow solid. MP:165 ºC. $^{1}$H NMR (400.13 MHz, $CDCl_3$, 298 K) δ: 3.90 (s, 3H, H-15), 6.39 (dd, 1H, $^{3}J$ = 8 Hz, 2 Hz ,H-5), 6.58 (d, 1H, J= 2 Hz, H-2), 7.09 (d, 1H, $^{3}J$ = 8 Hz, H-4), 7.42 (m, 6H, H-*m*, H-*p*), 7.48 (m, 3H, H-11, H-12, H-13), 7.89, (m, 4H, $^{3}J$ ($^{1}$H-$^{119/117}$Sn) =84/72 Hz ,H-*o*), 8.22 (d, 2H, $^{3}J$ = 8 Hz, H-10, H-14), 8.67 (s, 1H, $^{3}J$ ($^{1}$H-$^{119}$Sn) = 54 Hz, H-7).$^{13}$C NMR (100.61 MHz, $CDCl_3$, 298 K) δ: 55.66 (C-15), 104.42 (C-2) , 107.65 (C-5), 111.05 (C-6), 127.73 (C-10,14), 128.40 (C-11,13), 128.98 (C-*m*), 130.58 (C-*p*), 131.13 (C-12), 133.54 (C-9), 135.99 (C-4), 136.36 [C-*o*, $^{2}J$ ($^{13}$C-$^{119}$Sn) = 54 Hz], 139.46 (C-*i*), 161.04 (C-7), 166.43 (C-3), 168.26 (C-8), 169.87 (C-1). COSY correlation [δH/δH]: 6.39/6.58 (H-4/H-2), 6.39/7.09 (H-4/H-5), 7.42/7.89 (H-*m*, H-*p*/H-*o*), 7.48/8.22 (H-11, H-12, H-13/H-10, H-14). HETCOR correlation [δH/δC]: 3.90/55.66 (H-15/C-15), 6.39/107.65 (H-4/C-4), 6.58/104.42 (H-2/C-2), 7.09/135.99 (H-5/C-5), 7.42/128.98 (H-*m*/C-*m*), 7.42/130.58 (H-*p*/C-*p*), 7.48/128.40 (H-11, H-13/C-11, C-13), 7.48/131.13

(H-12/C-12), 7.89/136.36 (H-*o*/C- *o*), 8.22/127.73 (H-10, H-14/C-10, C-14), 8.67/161.04 (H-7/C-7). $^{119}$Sn NMR (149 MHz, $CDCl_3$, ppm, 298 K) δ: - 330.31. IR-ATR νmax cm-1: 3090, 1600 (C=N), 1200, 1050, 770. UV/Vis: λmax (nm) = 402 ($CHCl_3$, 1x10-3 M). TOF calc. for [($C_{27}H_{23}N_2O_3Sn$+H)+] : 543.072518; Found: 543.072624 (error = 0.194677 ppm).

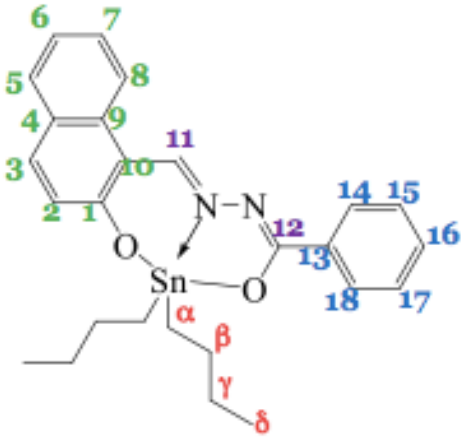
**4.2.1.2** [N-(2,4-di-tert-butyl-6-benzylidene)-benzyhydrazidato] diphenyltin(IV). **(2)**




A solution of 3,5-di-tert-butyl-2-hydroxybenzaldehyde (0.23g, 1mmol), benzoic hydrazide (0.13g, 1mmol), and di-phenyltin oxide (0.29g, 1mmol) in benzene (50 ml) was heated to reflux for 24 hr in a Dean-Stark apparatus used for the azeotropic removal of water and allowed to cool to room temperature. Then, all volatiles were removed under vacuum. Yield: 75%. Yellow solid. MP:158 ºC. $^1$H NMR (400.13 MHz, $CDCl_3$, 298 K) δ: 1.31 (s, 9H, H-18), 1.53 (s, 9H, H-16), 7.03 (s, 1H, H-5), 7.40 (m, 6H, H-*m,* H-*p*), 7.47 (m, 3H, H-11, H-12, H-13), 7.56 (d, 1H, H-3) , 7.86, (m, 4H, $^3J$ ($^1$H-$^{119/117}$Sn) =84/68 Hz ,H-*o*), 8.21 (dd, 2H, $^3$J = 8 Hz, 4 Hz, H-10, H-14), 8.83 (s, 1H, $^3$J ($^1$H-$^{119}$Sn) = 56 Hz, H-7).$^{13}$C NMR (100.61 MHz, $CDCl_3$, 298 K) δ: 30.22 (C-16), 31.43 (C-18) , 34.16 (C-17), 35.63 (H-15), 116.40 (C-6), 127.84 (C-10,14), 128.38 (C-11,13), 128.91 (C-*m*), 129.05 (C-5),

130.49 [C-*p*, $^{4}J$ ($^{13}C$-$^{119}Sn$) = 17 Hz], 131.12 (C-3), 131.23 (C-12), 133.48 (C-9), 136.32 [C-*o*, $^{2}J$ ($^{13}C$-$^{119}Sn$) = 55 Hz], 139.36 (C-*i*), 139.78 (C-2), 140.70 (C-4), 163.02 (C-7), 165.02 (C-1), 168.92 (C-8). COSY correlation [δH/δH]: 7.03/7.56 (H-5/H-3), 7.40/7.86 (H-*m,* H-*p*/ H-*o*), 7.47/8.21 (H-11, H-12,H-13/ H-10,H-14). HETCOR correlation [δH/δC]: 7.03/129.05 (H-5/C-5), 7.40/128.91 (H-*m*/C-*m*), 7.40/130.49 (H-*p*/C-*p*), 7.47/128.38 (H-11,H-13/C-11,H-13), 7.47/131.23 (H-12/C-12), 7.56/131.13 (H-3/C-3), 7.86/136.34 (H-*o*/C-*o*), 8.21/127.84 (H-10,H-14/C-10,H-14). $^{119}Sn$ NMR (149 MHz, $CDCl_3$, ppm, 298 K) δ: - 324.40. IR-ATR νmax cm-1: 3090, 2990, 1600 (C=N), 1400, 1200, 790. UV/Vis: λmax (nm) = 428 ($CHCl_3$, 1x10-3 M). TOF calc. for [($C_{34}H_{37}N_2O_2Sn$+H)+] : 625.187154; Found: 625.187862 (error = 0.759457 ppm).

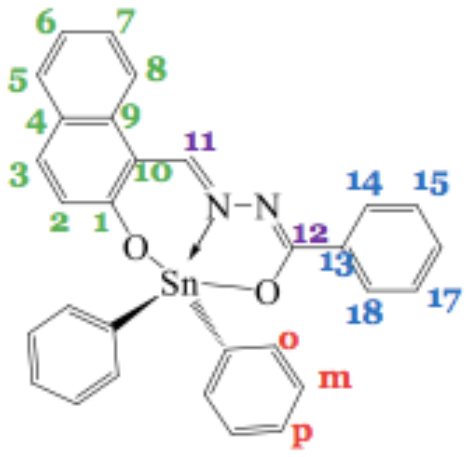
**4.2.1.3** [N-(1-oxide-10-naphthaldehyde)-benzyhydrazidato] di-n-butyltin(IV).**(3)**




A solution of 2-hydroxynaphthaldehyde (0.17g, 1mmol), benzoic hydrazide (0.13g, 1mmol), and di-n-butyltin oxide (0.25g, 1mmol) in benzene (50 ml) was heated to reflux for 24 hr in a Dean-Stark apparatus used for the azeotropic removal of water and allowed to cool to room temperature. Then, all volatiles were removed under vacuum. Yield: 85%. Brown oil.$^{1}H$ NMR (400.13

MHz, $CDCl_3$, 298 K) δ: 0.74 (t, 6H, $^3J$ = 8 Hz, CH3-δ), 1.25 (m, 4H, CH2-γ), 1.43 (t, 4H, $^3J$ = 8 Hz, CH2-α), 1.56 (m, 4H, $^3J$ = 8 Hz, CH2-β), 6.84 (d, 1H, $^3J$ = 8 Hz, H-2), 7.15 (t, 1H, $^3J$ = 8 Hz, H-6), 7.33, (m, 3H, H-15-17), 7.37 (t, 1H, $^3J$ = 8 Hz, H-7), 7.53 (d, 1H, $^3J$ = 8 Hz, H-5), 7.61 (d, 1H, $^3J$ = 8 Hz, H-3), 7.94 (d, 1H, $^3J$ = 8 Hz, H-8), 8.03 (m, 2H, H-14, H-18), 9.60 (s, 1H, $^3J$ ($^1H$-$^{119}Sn$) = 48 Hz, H-11). $^{13}C$ NMR (100.61 MHz, $CDCl_3$, 298 K) δ: 13.69 (Cδ), 22.06 [Cα, $^1J$ ($^{13}C$-$^{119/117}Sn$) = 595/576 Hz], 26.54 [Cγ, $^3J$ ($^{13}C$-$^{119}Sn$) = 82 Hz], 26.96 [Cβ, $^2J$ ($^{13}C$-$^{119}Sn$) = 35 Hz], 107.17 (C-9), 119.21 (C-8), 123.05 (C-6), 124.46 (C-2), 127.25 (C-4), 127.64 (C-14,18), 128.08 (C-7), 128.33 (C-15,17), 129.23 (C-5), 130.88 (C-16), 133.74 (C-10), 133.93 (C-13), 136. 61 (C-3), 156.97 [C-11, $^2J$ ($^{13}C$-$^{119}Sn$) = 20 Hz], 168.55 [C-12, $^2J$ ($^{13}C$-$^{119}Sn$) = 10 Hz], 169.42 [C-1, $^2J$ ($^{13}C$-$^{119}Sn$) = 33 Hz]. COSY correlation [δH/δH]: 0.74/1.25 (H-δ/H-γ), 1.56/1.25 (H-β/H-γ), 1.56/1.43 (H-β/H-α), 6.84/7.60 (H-2/H-3), 7.15/7.36 (H-6/H-7), 7.15/7.53 (H-6/H-5), 7.33/8.03 (H-15/H-14). HETCOR correlation [δH/δC]: 0.74/13.69 (H-δ/C-δ), 1.25/26.54 (H-γ/C-γ), 1.43/22.06 (H-α/C-α), 1.59/26.96 (H-β/C-β), 6.84/124.45 (H-2/C-2), 7.15/123.05 (H-6/C-6), 7.33/128.33 (H-15, H-17/C-15, C-17), 7.33/130.88 (H-16/C16), 7.36/128.08 (H-7/C-7), 7.53/129.23 (H-5/C-5), 7.60/136.61 (H-3/C-3), 7.94/119.21 (H-8/C-8), 8.03/127.64 (H-14, H-18/C-14, C-18), 9.60/156.97 (H-11/C-11). $^{119}Sn$ NMR (149 MHz, $CDCl_3$, ppm, 298 K) δ: -189.99. IR-ATR νmax cm-1: 3090, 2980, 1600 (C=N), 1410, 1200, 760. UV/Vis: λmax (nm) = 441 ($CHCl_3$, 1x10-3 M). TOF calc. for [($C_{26}H_{31}N_2O_2Sn$+H)+] : 523.140203; Found: 523.140324 (error = 0.229467 ppm).

**4.2.1.4** [N-(1-oxide-10-naphthaldehyde)-benzyhydrazidato] diphenyltin(IV).**(4)**




A solution of 2-hydroxynaphthaldehyde (0.17g, 1mmol), benzoic hydrazide (0.13g, 1mmol), and di-phenyltin oxide (0.29g, 1mmol) in benzene (50 ml) was heated to reflux for 24 hr in a Dean-Stark apparatus used for the azeotropic removal of water and allowed to cool to room temperature. Then, all volatiles were removed under vacuum. Yield: 80%. Yellow solid. MP:200ºC. $^{1}$H NMR (400.13 MHz, DMSO-$d_6$, 298 K) δ: 7.19 (d, 1H, $^{3}$J = 8 Hz, H-2), 7.30-7.39 (m, 7H, H-6, H-m, H-p), 7.50-7.55 (m, 4H, H-7,H-15, H-16, H-17), 7.70 (d, 4H, $^{3}$J = 8 Hz, H-o), 7.81 (d, 1H, $^{3}$J = 8 Hz, H-5), 7.93 (d, 1H, $^{3}$J = 8 Hz, H-3), 8.19 (d, 2H, $^{3}$J = 8 Hz, H-14, H-18), 8.26 (d, 1H, $^{3}$J = 8 Hz, H-8), 9.62 (s, 1H, $^{3}$J ($^{1}$H-$^{119}$Sn) = 52 Hz, H-11). $^{13}$C NMR (100.61 MHz, DMSO-$d_6$, 298 K) δ: 107.51 (C-9), 119.97 (C-8), 123.01 (C-6), 124.48 (C-2), 126.85 (C-4), 127.27 (C-14,18), 128.02 (C-7), 128.40 (C-15,17), 128.47 (C-*p*), 128.91 (C-5), 129.24 (C-*m*), 131.09 (C-16), 133.28 (C-10), 133.42 (C-13), 134.88 [C-*o*, 2J ($^{13}$C-$^{119}$Sn) = 55 Hz], 135.99 (C-3), 154.16 (C-*i*) , 158.46 (C-11), 166.60 (C-12), 167.92 (C-1). COSY correlation [δH/δH]: 7.19/7.94 (H-2/H-3), 7.30-7.39/7.56 (H-*m*,H-*p*/H-*o*), 7.30-7.39/7.81 (H-6/H-5), 7.30-7.39/7.50-7.55 (H-6/H-7), 7.50-7.55/8.26 (H-7/H-8), 7.50-7.55/8.19 (H-15, H-17/ H-14, H-18). $^{119}$Sn

NMR (149 MHz, DMSO-d6, 298 K) δ: - 406.72 ppm. IR-ATR ῡmax cm-1: 3090, 1610 (C=N), 1200, 790. UV/Vis: λmax (nm) = 437 ($CHCl_3$, 1x10-3 M). Anal. calcd. for $C_{30}H_{22}N_2O_2Sn$: C 64.20 %, H 3.95 %, N 4.99 %; Found: C 64.14 %, H 4.10 %, N 4.72 %.

### 4.2.2 Bioassays

#### 4.2.2.1 Cell culture.

B16F10 cell line was cultured in 25 $cm^2$ flasks, DMEM / F12 was used as culture medium supplemented with 5% fetal bovine serum (FBS). Cell passage was performed twice a week and the culture was maintained at 37 ° C in an atmosphere of 5% CO2 and 95% humidity.

For the manipulation of cells and culture medium the trypsin was heated at 37º C. When the culture plates reach a confluence of 80% or more, is necessary to pass the cells to have enough cells to work.

For the first pass of cells, the culture medium was removed from the bottle and subsequently 1 ml trypsin was added, ensuring that the trypsin completely covers the floor of the plate, after 5 minutes, when the cells are detached, the trypsin was inactivated with 3 ml of culture medium and carefully shaken with a pipette several times to make sure there are no clumps of cells in the culture.

8 ml of medium was added in an empty flask properly labeled and subsequently added 3-5 drops of cell culture (depending on the junction) and stirred gently before narrow plate, and allowed to incubate at 37ºC. The remaining cell culture is counting, for your later use in the bioassays.

#### 4.2.2.2 Cytotoxicity.

**Part A**

The neutral red assay system is a way of measuring living cells via the uptake of the vital dye neutral red (**Figure 6**). Viable cells will take up the dye by active transport and incorporate the dye into lysosomes, whereas non-viable cells will not take up the dye. After the cells have been allowed to incorporate the dye they are briefly washed or fixed. The incorporated dye is then liberated from the cells in an acidified ethanol solution. An increase or decrease in the number of cells or their physiological state results in a concomitant change in the amount of dye incorporated by the cells in the culture. This indicates the degree of cytotoxicity caused by the test material. [xxviii]

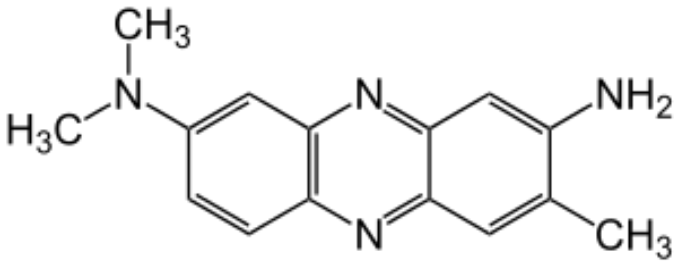


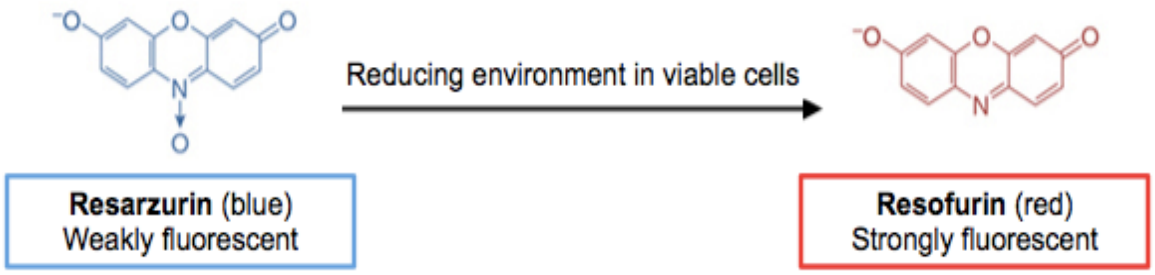

**Figure 6**. Molecular structure of Neutral red.

HGF1 (gingival biopsy) and A-375 (melanoma cells) cells were seeded in 96 wells plates at a density of $1x10^4$ per well in 100 ml of media and incubated for 24 hours at 37°C. Media was exchanged and compounds were added at concentrations ranging from 3 µg/ml to 50 µg/ml. Twenty four hours later, media was removed, and 100 µl of Neutral Red (Sigma-Aldrich) were added to each well, and after 2 hours of incubation, the medium was carefully removed and the cells quickly rinsed with PBS, and a fade solution was added. Viability of the cultures was measured by spectrophotometry on an ELISA Microplate Reader (Biotek Multiskan ELX800, BioTek Instruments, Inc, Winooski, VT) at wavelength of 540 nm.The data obtained were then analyzed by computer.

**Part B**

The cell viability assay Alamar Blue is widely used for measurement of proliferation and cytotoxicity in several cell lines (human, animals, fungi and of bacteria strains) [xxix , xxx , xxxi] This nontoxic and highly permeable membrane

reactive, uses the advantage of the reducing environment within healthy cells to reduce resarzurine (blue reagent) to resorufin (red reactive). (**Scheme 4**) The color change is directly proportional to the number of living cells. Can thus relate the change in the color of the cell culture with the viability and cytotoxicity.




**Scheme 4.** Reduction of Alamar blue reagent within metabolically active cells.

B16F10 murine melanoma cells were seeded in 96 wells plates at a density of $1x10^4$ per well in 100 ml of media and incubated for 24 hours at 37°C. Media was exchanged and compounds were added at concentrations ranging from 0.01 µg/ml to 10 µg/ml. Untreated cells or treated with dimethyl sulfoxide (DMSO, Sigma-Aldrich Co, St. Louis, MO) were used as controls. Twenty four hours later 10 µl of alamarBlue (Biosource Invitrogen Life Technologies, Carlsbad, CA) were added to each well. Viability of the cultures was measured as reagent colour change by spectrophotometry on an ELISA Microplate Reader (Biotek Multiskan ELX800, BioTek Instruments, Inc, Winooski, VT) at dual wavelength of 570 and 600 nm. The data obtained were then analyzed by computer.

#### 4.2.2.3 Cell images.

B16F10 murine melanoma cells were seeded in 6 wells plates on sterile coverslips at a density of 2x105 cells/well in 2 ml of DMEM/F12 media and maintained at 37°C in a controlled humid atmosphere of 5% CO2 and 95% air. 24 hours later media was exchanged and cells were exposed to the compounds at concentration of 10 µg/ml, for two hours. Untreated cells or treated with DMSO were used as controls. Supernatants were removed and coverslips were washed once with 2 ml of PBS, mounted on microscope slips using glycerol 75% and imaged using confocal laser microscopy (Leica TCS SP5 Confocal System). Samples were excited at 405 and 458 nm and the fluorescence emission was measured at 478-612 nm.

Compounds that showed good cell staining were selected. And stains at lower concentrations of 1 µg/ml and 0.1 µg/ml were performed. Each experiment was performed in duplicate and repeated 3 times.

Each compound was excited to a specific wavelength and reading the emission wavelengths according to the absorption and emission values of each compound used was performed. (**Table 2**)

**TABLE 2**

Parameters used with each compound.

| Compound | Excitaton (nm) | Emission (nm) |
|---|---|---|
| **1** | 458 | 478-612 |
| **2** | 458 | 475-550 |
| **3** | 405 | 420-500 |
| **4** | 458 | 475-550 |

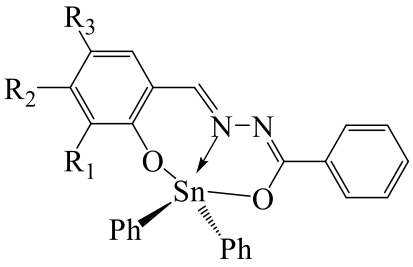
### 4.3 Disposal of hazardous waste.

The organic waste generated in the reactions like benzene, as well as for the work up: hexane, acetone, ethyl acetate were collected in the container C, which is assigned for halogen free-solvents. Waste of solvents like chloroform and dichloromethane were collected in container D, which is assigned for halogen containing solvents. Biological waste was disposed in the appropriate containers.

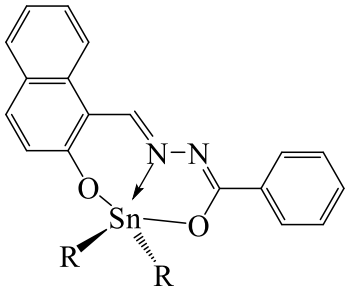
# 5. RESULTS AND DISCUSSION

## 5.1 Synthesis.

We carry out the synthesis of Schiff bases 1-4 (**Figure 7**) by condensation reaction of the appropriate salicylaldehyde or napthaldehyde with the corresponding benzohydrazides in benzene for 48 hours. The Schiff bases were isolated with good yields (75-85%).




**1**: $R_1$ = H, $R_2$ = MeO, $R_3$ = H (83%)
**2**: $R_1$ = tBu, $R_2$ = H, $R_3$ = tBu (75%)




**3**: R = nBu (85%)
**4**: R = Ph (80%)

**Figure 7**. Schiff bases 1-4

The products were obtained after filtration of the crude reaction and precipitation using hexane, as yellow powders and one of the compounds as brown oil, where all compounds were soluble in common organic solvents such as dichloromethane, chloroform and acetone.

## 5.2 Chemical structure elucidation.

Organotin complexes were characterized by UV-vis, spectroscopic methods (IR, 1 and 2D-NMR), mass spectrometry and powder X-ray single crystal.

### 5.2.1 UV Analysis

The UV-Vis spectra of the compounds and its ligands were obtained in chloroform (**Figure 8**). As we can see, the free ligands present a main absorption peak in the UV region (260-390 nm), but after the coordinated bond is formed N ➔ Sn, the bands are red-shifted (400-450 nm) due to the larger electronic delocalization.

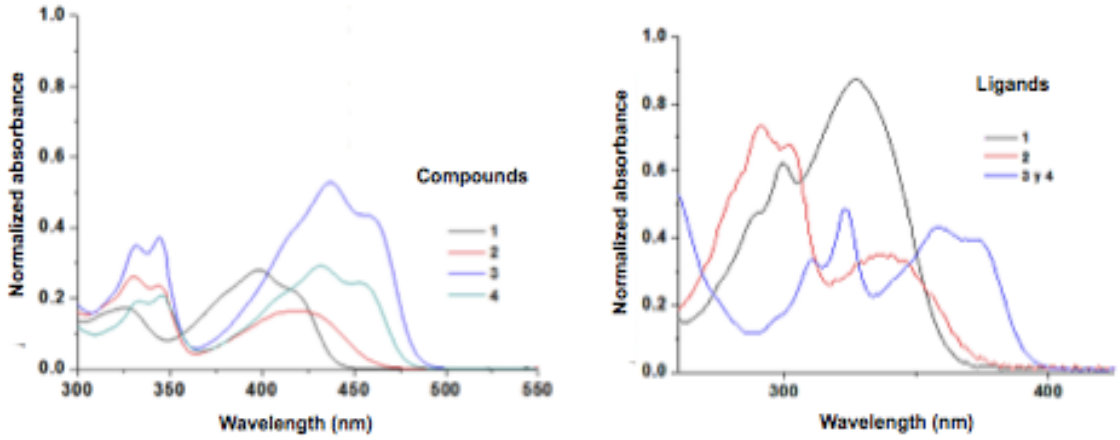



**Figure 8.** Absorption spectrum of compounds **1-4** (left) and its ligands (right), in chloroform.

The emission spectrum, showed wavelengths for compounds 1-4 between 468 to 513 nm (**Figure 9**), being compounds 3 and 4 the most red-shifted, attributed to the presence of naphthalene group. The data are represented in **Table 3**.

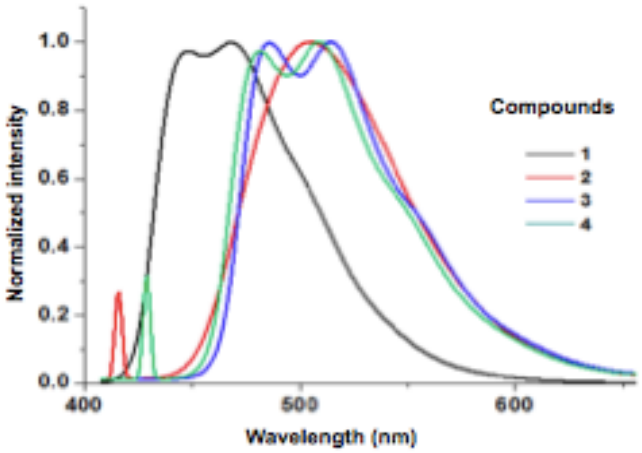



**Figure 9.** Emission spectra of compounds **1-4** in chloroform.

**TABLE 3**

Absorption and emission data of compounds 1-4

| Compound | Max. Absorption (nm) | Emission (nm) |
|---|---|---|
| 1 | 402 | 468 |
| 2 | 428 | 505 |
| 3 | 441 | 513 |
| 4 | 437 | 509 |

### 5.2.2 Analysis of NMR data

NMR spectroscopy is a reliable and powerful tool to obtain information about the structure, $^{1}H$, $^{13}C$ and $^{119}Sn$ NMR analyses, collectively provide highly valuable information and hence are used for the characterization of organotin compounds.

$^{1}H$-NMR spectra confirmed the formation of the Schiff bases **1-4** (see appendix), with signals for H-7 (compounds **1 and 2**) and H-11 (compounds **3 and 4**) in the range 8.67 to 9.62 ppm, typical of an imine proton, which are according to reported by Santillan et al. [xxxii]

In the same manner it is possible to see satellite signals for this protons ($^{n}J^{1}H$-$^{119}Sn$: 48-56 Hz) due coupling with $^{119}Sn$, which are according to reported by Smith for organotin compounds. [xxxiii] In addition to the signals of the aromatic rings of the molecule, it is also possible to notice the signals for the substituents. For compounds 1 and 2 the coupling of the ortho protons due to the proximity of tin is appreciated. (**Table 4**).

**TABLE 4**

$^{1}$H-NMR data of Sn substituents (ppm).

| Comp. | $CH_2$-α | $CH_2$-β/$CH_o$ | $CH_2$-γ/$CH_m$ | $CH_3$-δ/$CH_p$ |
|---|---|---|---|---|
| **1** | | 7.89 [84/72] | 7.42 | 7.42 |
| **2** | | 7.86 [84/68] | 7.40 | 7.40 |
| **3** | 1.43 | 1.56 | 1.25 | 0.74 |
| **4** | | 7.56 | 7.35 | 7.35 |

In the $^{13}$C NMR spectra of tin compounds, the principal signals correspond to C-11/C-7 (C=N) between 156.97 to 163.02 ppm, C-1 (C-O) with shifts between 165.02 to 169.87 ppm and C-12/C-8 (N=C-O) that appear between 166.60 to 168.92 ppm.

The existence of the N ➔ Sn coordination bond was evidenced by $^{119}$Sn-NMR spectra for the compounds 1-4, observed values for the chemical shifts confirm that the compounds are pentacoordinated. Five- and six-coordinate organotin compounds show 119Sn signals, which occur at much lower frequencies than those of the four-coordinate derivatives. Has been reported that signals between -204 to -395 ppm are indicative of pentacoordinated tin atoms, which is consistent with the chemical shifts obtained. (**Table 5**)

**TABLE 5**

Selected $^{1}H$ ($^{n}J^{1}H$-$^{119}Sn$), $^{13}C$ [$^{n}J^{13}C$-$^{119}Sn$], and $^{119}Sn$ (ppm).

| Comp. | $^{1}H$ | $^{13}C$ | | | | $^{119}Sn$ |
|---|---|---|---|---|---|---|
| | H-11/H-7 | C-11/C-7 | C-$\alpha$/$C_i$ | C-1 | C-12/C-8 | |
| **1** | 8.67 (54) | 161.04 | 139.46 | 169.87 | 168.26 | -330.31 |
| **2** | 8.83 (56) | 163.02 | 139.36 | 165.02 | 168.92 | -324.40 |
| **3** | 9.60 (48) | 156.97 [20] | 22.06 [595/576] | 169.42 [34] | 168.55 [10] | -189.90 |
| **4** | 9.62 (52) | 158.46 | 154.16 | 167.92 | 166.60 | -406.73 |

### 5.2.3 Analysis of IR data

The IR spectral analysis showed that the C=N stretching vibration bands for compounds 1-4 (1600-1610 $cm^{-1}$) were shifted to lower wavenumbers in comparison with the ligands (1635 $cm^{-1}$), demonstrating a decrease in strength as the new dative bond is formed. Main bands are shown in **Table 6**:

**TABLE 6**

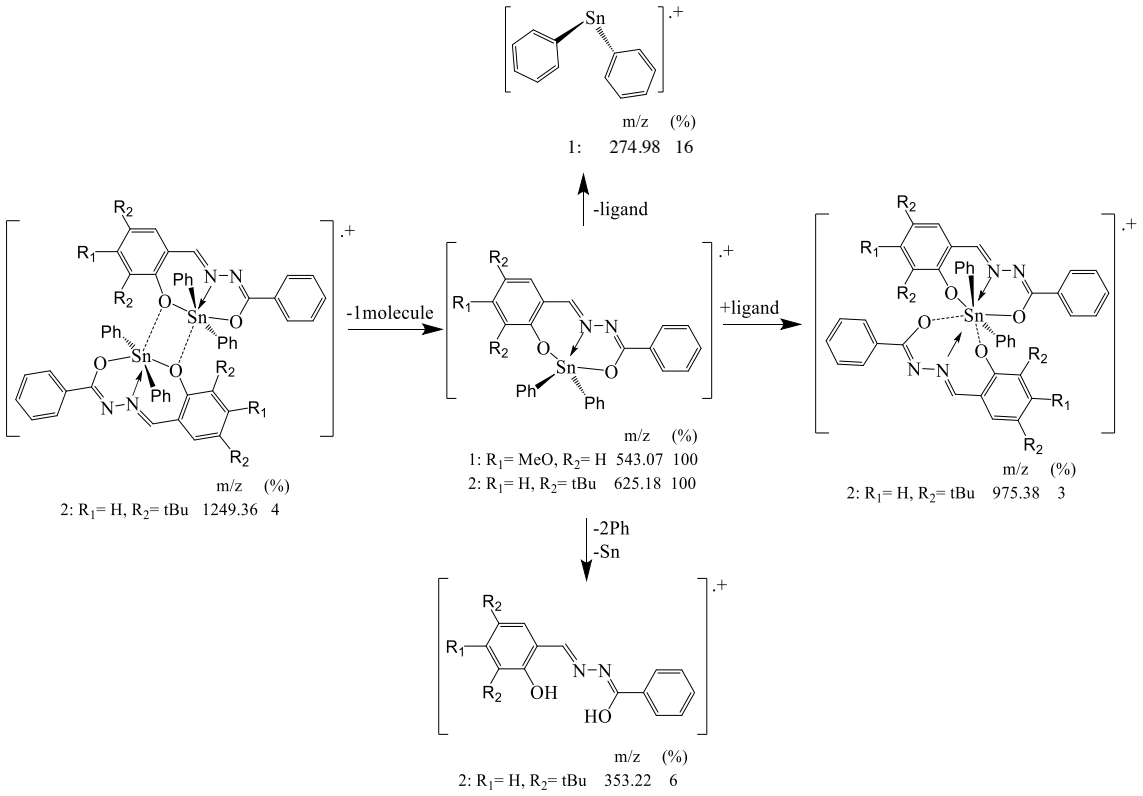
Main IR bands of the four compounds.

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **C-H arom.** | 3090 $cm^{-1}$ | 3090 $cm^{-1}$ | 3090 $cm^{-1}$ | 3090 $cm^{-1}$ |
| **C-H sp3 stre.** | 2990 $cm^{-1}$ | 2990 $cm^{-1}$ | 2980 $cm^{-1}$ | - |
| **C=N** | 1600 $cm^{-1}$ | 1600 $cm^{-1}$ | 1600 $cm^{-1}$ | 1610 $cm^{-1}$ |
| **C-H sp3 bend.** | 1400 $cm^{-1}$ | 1400 $cm^{-1}$ | 1400 $cm^{-1}$ | - |
| **C-H arom. IP** | 1200 $cm^{-1}$ | 1200 $cm^{-1}$ | 1200 $cm^{-1}$ | 1200 $cm^{-1}$ |
| **C-H arom. OP** | 770 $cm^{-1}$ | 790 $cm^{-1}$ | 760 $cm^{-1}$ | 790 $cm^{-1}$ |

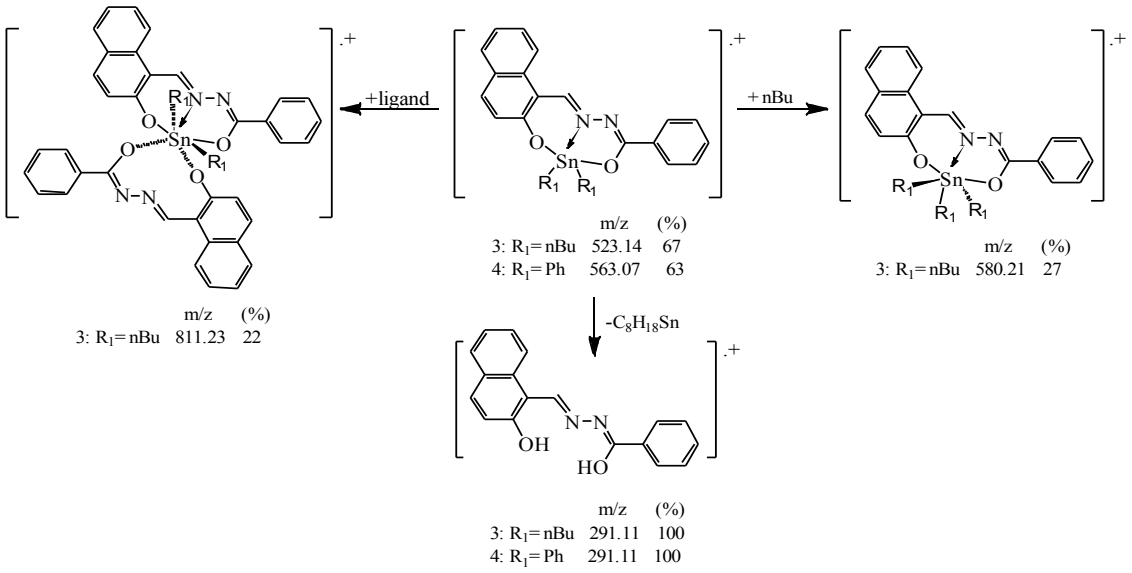
### 5.2.4 Mass spectra. Molecular ion and fragmentation.

The mass spectra of the four molecules, was obtained by the TOF method. The spectra of organotin compounds 1 and 2 showed that the base peak correspond to the molecular ion peak (**Scheme 5**), while compounds 3 and 4 showed a base peak after loss of the di-n-butyltin and di- phenyltin fragment (**Scheme 6**).

The presence of the base peak of the tin compounds is consistent with the theoretical molecular mass.




**Scheme 5.** Proposed fragmentation of tin compounds (1 and 2).




**Scheme 6.** Proposed fragmentation of tin compounds (3 and 4).

### 5.2.5 Elemental analysis.

The elemental analysis data of the compound 4 ($C_{30}H_{22}N_2O_2Sn$) is given in **Table 7**. The results obtained for C, H and N are in good agreement with those calculated for the suggested formula, indicating the purity of the tin compound.

**TABLE 7**

Elemental analysis of compound 4 ($C_{30}H_{22}N_2O_2Sn$).

| Element | Calc. (%) | Found (%) |
|---|---|---|
| **C** | 64.20 | 64.14 |
| **H** | 3.95 | 4.10 |
| **N** | 4.99 | 4.72 |

### 5.2.6 X-ray analysis

The structures of compounds 1,2 and 4 are represented by the thermal ellipsoid plots in figures **10, 11** and **12**. Selected bond distances and angles are listed in **Table 9** and **10**. Compounds 1 and 2 belongs to triclinic space group *P*-1 and compound 4 to monoclinic space group $P2_1/c$ (**Table 8**).

The crystal structures of the compounds, (**Figures 8, 9 and 10**) shows that the molecules contain a pentacoordinated tin atom, and the formation of five-ring-fused skeletons whit N ➔ Sn coordination bond with lengths of 2.143 Å,

2.137 Å and 2.147 Å respectively, characteristic of this type of bonds. The C-C bond lengths of the aromatic systems correspond to the data previously reported for similar molecules.[xxxiv]

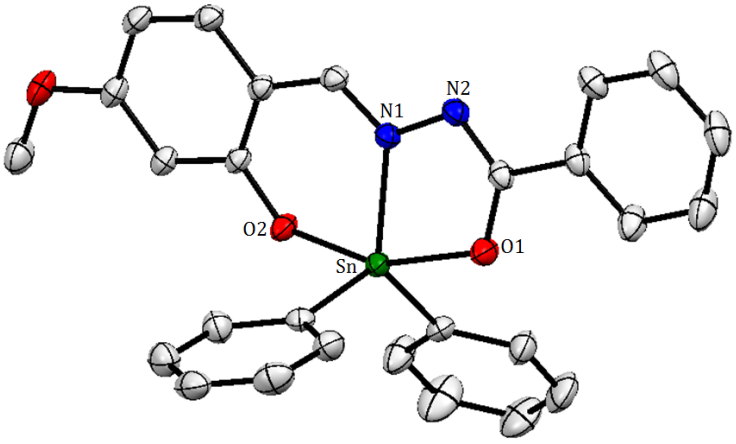



**Figure 10.** X-ray molecular structure of compound **1.**

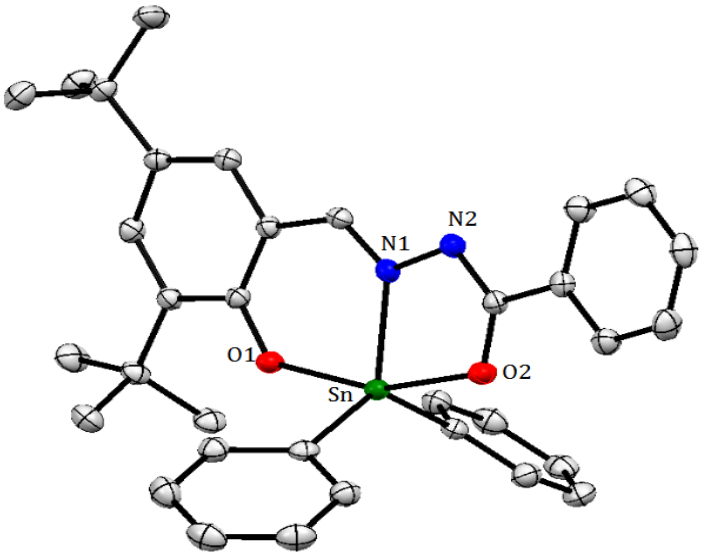



**Figure 11.** X-ray molecular structure of compound **2.**

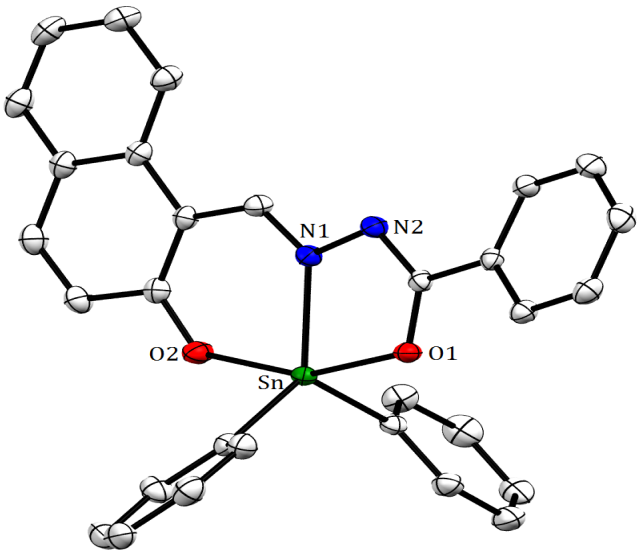



**Figure 12.** X-ray molecular structure of compound **4**.

As we can see in every molecule, the tin atom adopts a distorted trigonal bipyramid geometry, where the apical position is occupied by oxygen atoms (Sn-O1 2.080(2) Å and Sn-O2 2.123(2) Å compound 1, Sn-O1 2.082(1) Å and Sn-O2 2.138(1) Å compound 2, Sn-O1 2.0773(13) Å and Sn-O2 2.1203(13) Å compound 4) and the equatorial by nitrogen and the ipso-carbons (Sn-C16 2.123(3) Å and Sn-C22 2.126(2) Å compound 1, Sn-C23 2.120(2) Å and Sn-C29 2.111(2) Å compound 2, Sn-C19 2.1101(19) Å and Sn-C25 2.1164(18) Å compound 4).

In the three compounds, the bond angle O-Sn-O is in the interval of 156.90º (7) to 158.23º (5), far from the ideal angle (O-Sn-O 180º) due to the tension generated by the formation of 5-membered plane rings.

Another important aspect observed by X-ray analysis is the intermolecular interactions; pi interactions and hydrogen bonds are the principals, as we can see in **figures 13, 14 and 15**,

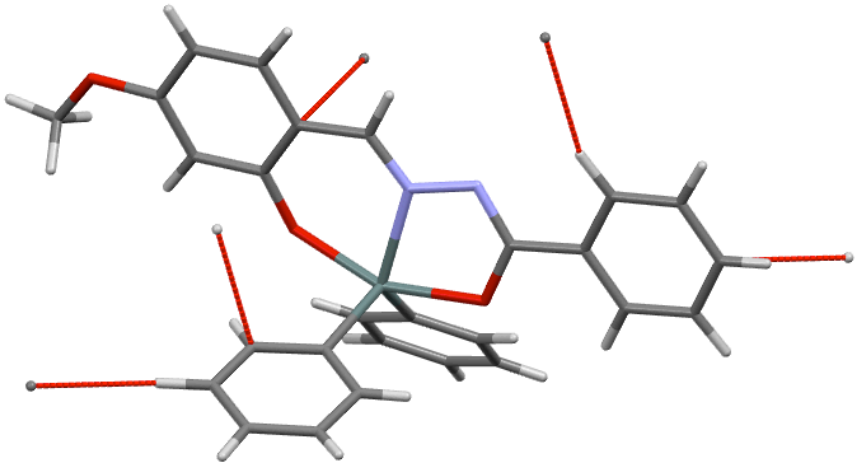


**Figure 13.** Intermolecular interactions of compound **1.**

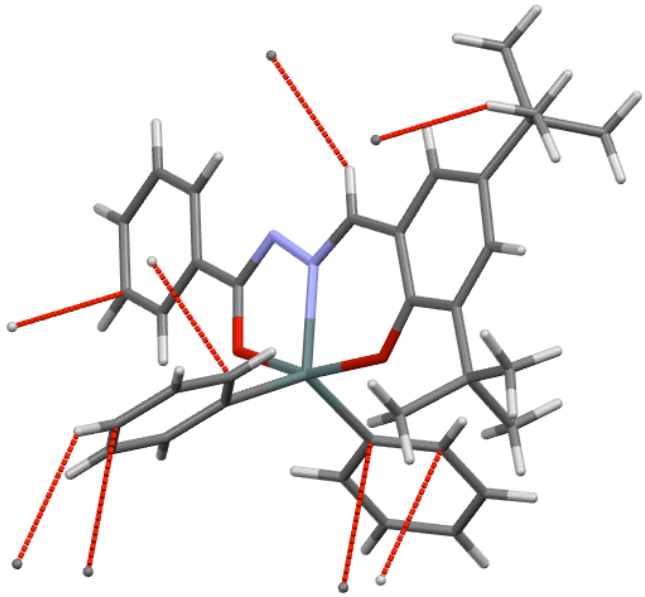


**Figure 14.** Intermolecular interactions of compound **2.**

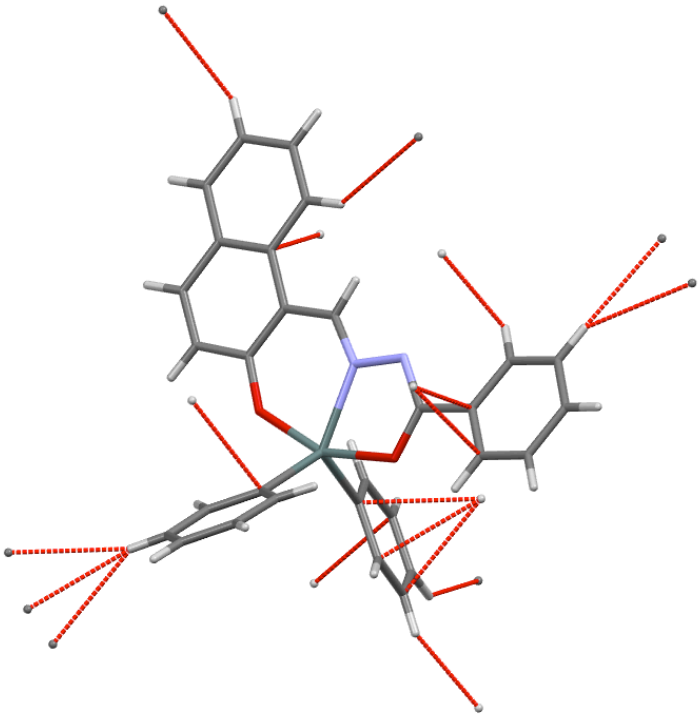


**Figure 15.** Intermolecular interactions of compound **4.**

**TABLE 8**

Crystal data for compounds **1**, **2** and **4**.

| | **1** | **2** | **4** |
|---|---|---|---|
| **Empirical formula** | $C_{27}H_{22}N_2O_3Sn$ | $C_{34}H_{36}N_2O_2Sn$ | $C_{30}H_{22}N_2O_2Sn$ |
| **Formula weight** | 541.15 | 623.34 | 561.19 |
| **Temperature, K** | 293(2) | 100(2) | 100(2) |
| **Wavelength** | 0.71073 | 0.71073 | 0.71073 |
| **Cryst size, mm$^3$** | 0.15x0.07x0.05 | 0.15x0.05x0.04 | 0.12x0.11x0.10 |
| **Crystal system** | Triclinic | Triclinic | Monoclinic |
| **Space group** | *P*-1 | *P*-1 | *P*21/*c* |
| ***a*, Å** | 8.7687(2) | 10.3043(12) | 9.8450(12) |
| ***b*, Å** | 11.4801(2) | 11.1764(13) | 8.6454(10) |
| ***c*, Å** | 13.2600(2) | 12.8993(15) | 28.744(3) |
| $\alpha$ | 64.2670(10) ° | 85.3470(10) ° | 90 ° |
| $\beta$ | 81.3370(10) ° | 87.3890(10) ° | 93.0780(10) ° |
| $\gamma$ | 90.3440(10)° | 83.9970(10) ° | 90 ° |
| **V,Å$^3$** | 1184.90(4) | 1471.5(3) | 2443.0(5) |
| **Z** | 2 | 2 | 4 |
| $\rho_{calc,Mg.cm-3}$ | 1.517 | 1.407 | 1.526 |
| **$\mu$, mm$^{-1}$** | 1.109 | 0.901 | 1.076 |
| **2$\theta$ range for data collection** | 1 – 14 ° | 2.33 – 28.33 ° | 1.42 – 28.3 ° |
| **Index ranges** | -10 ≤ *h* ≤ 11, -14 ≤ *k* ≤ 14, -17 ≤ *l* ≤ 17 | -13 ≤ *h* ≤ 13, -14 ≤ *k* ≤ 14, -16 ≤ *l* ≤ 16 | -13 ≤ *h* ≤ 13, -11 ≤ *k* ≤ 11, -38 ≤ *l* ≤ 38 |
| **No. reflns collected** | 26003 | 14585 | 23387 |
| **No. indep reflns** | 4712 | 6954 | 6086 |
| **[$R_{int}$]** | 0.0268 | 0.0282 | 0.0266 |
| **Goodness of fit** | 1.062 | 1.081 | 1.065 |
| ***R*1, w*R*2 (I>2$\sigma$(I))** | 0.0345<br>0.0662 | 0.0293<br>0.0727 | 0.0287<br>0.0730 |
| ***R*1, w*R*2 (all data)** | 0.0268<br>0.0627 | 0.0282<br>0.0718 | 0.0310<br>0.0748 |
| **$\Delta\rho_{min}$ (e Å$^{-3}$)** | - 0.602 | - 0.653 | - 0.656 |
| **$\Delta\rho_{max}$ (e Å$^{-3}$)** | 0.528 | 1.140 | 1.552 |

**TABLE 9**

Selected bond distances (Å) for **1**, **2** and **4**.

| | **1** | **2** | | **4** |
|---|---|---|---|---|
| **Sn-O(1)** | 2.080(2) | 2.082(1) | **Sn-O(1)** | 2.0773(13) |
| **Sn-O(2)** | 2.123(2) | 2.138(1) | **Sn-O(2)** | 2.1203(13) |
| **Sn-N(1)** | 2.143(2) | 2.137(1) | **Sn-N(1)** | 2.1478(15) |
| **Sn-C(16)** | 2.123(3) | | **Sn-C(19)** | 2.1101(19) |
| **Sn-C(22)** | 2.126(2) | | **Sn-C(25)** | 2.1164(18) |
| **Sn-C(23)** | | 2.120(2) | **C(1)-O(1)** | 1.305(2) |
| **Sn-C(29)** | | 2.111(2) | **C(10)-C(11)** | 1.426(3) |
| **C(1)-O(1)** | 1.326(2) | 1.330(2) | **C(11)-N(1)** | 1.303(2) |
| **C(8)-O(2)** | 1.311(3) | 1.306(2) | **N(1)-N(2)** | 1.403(2) |
| **C(6)-C(7)** | 1.420(4) | 1.435(2) | **N(2)-C(12)** | 1.309(2) |
| **C(7)-N(1)** | 1.299(2) | 1.303(2) | **C(12)-O(2)** | 1.305(2) |
| **N(1)-N(2)** | 1.397(3) | 1.398(2) | | |
| **N(2)-C(8)** | 1.304(2) | 1.311(2) | | |
| **C(15)-O(3)** | 1.413(5) | | | |

**TABLE 10**

Selected angles (°) for **1**, **2** and **4**.

| | **1** | | **2** | | **4** |
|---|---|---|---|---|---|
| **C(16)-Sn-C(22)** | 125.28(9) | **C(23)-Sn-C(29)** | 129.95(7) | **C(19)-Sn-C(25)** | 120.13(7) |
| **C(16)-Sn-N(1)** | 108.06(8) | **C(23)-Sn-N(1)** | 117.60(6) | **C(19)-Sn-N(1)** | 123.30(6) |
| **C(16)-Sn-O(1)** | 96.31(8) | **C(23)-Sn-O(1)** | 94.43(6) | **C(19)-Sn-O(1)** | 96.80(6) |
| **C(16)-Sn-O(2)** | 98.08(8) | **C(23)-Sn-O(2)** | 92.64(6) | **C(19)-Sn-O(2)** | 94.06(6) |
| **C(22)-Sn-N(1)** | 126.48(8) | **C(29)-Sn-N(1)** | 112.11(6) | **C(25)-Sn-N(1)** | 116.30(6) |
| **C(22)-Sn-O(1)** | 93.11(8) | **C(29)-Sn-O(1)** | 95.78(6) | **C(25)-Sn-O(1)** | 95.43(6) |
| **C(22)-Sn-O(2)** | 93.21(8) | **C(29)-Sn-O(2)** | 95.38(6) | **C(25)-Sn-O(2)** | 96.50(6) |
| **O(1)-Sn-O(2)** | 156.90(7) | **O(1)-Sn-O(2)** | 158.23(5) | **O(1)-Sn-O(2)** | 157.04(5) |
| **N(1)-Sn-O(1)** | 84.56(7) | **N(1)-Sn-O(1)** | 74.17(5) | **N(1)-Sn-O(1)** | 74.23(5) |
| **N(1)-Sn-O(2)** | 73.85(7) | **N(1)-Sn-O(2)** | 84.32(5) | **N(1)-Sn-O(2)** | 82.90(5) |

## 5.3 Bioassays

### 5.3.1 Cytotoxic selectivity

Organotin compounds were used on two different cell lines in order to compare the behavior in normal and cancer cells. HGF1 (gingival biopsy) and A-375 (melanoma cells) human cells were treated with five different concentrations of the compounds (50, 25, 12, 6, and 3 µg/ml) for 24 hours and then viability was determined using Neutral Red Technique (**Figure 16 and 17**).

As we can appreciate, the compounds at different concentrations, follow the same tendency in both of the cell lines, this behavior indicate that there is no selectivity for melanoma cancer cells with none of the compounds. Studies with other cell lines are required to evaluate the selectivity for other cancer cells. These results cannot indicate the real cytotoxicity of the compounds, because the experiment was performed only once by duplicated, more number of experiments are required to perform in order to determinate the cytotoxic effect of each compound.

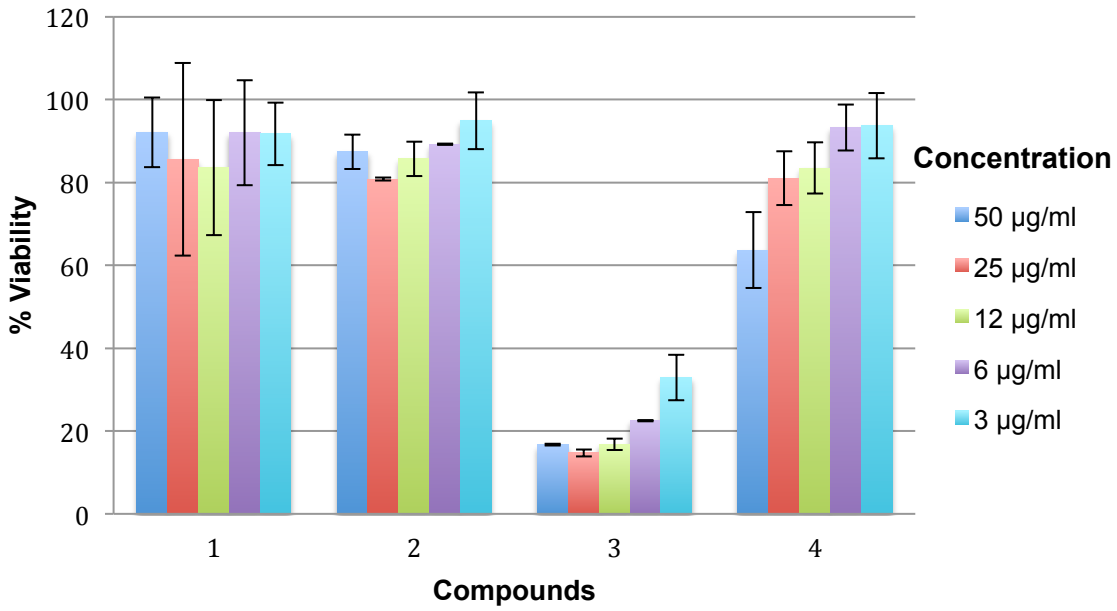



**Figure 16**.Cytotoxic effect of organotin compounds. Gingival cells HGF1 were treated with 50 µg/ml (blue bars), 25 µg/ml (red bars), 12 µg/ml (green bars), 6 µg/ml (purple bars), 3 µg/ml (light blue bars), for 24 hours. Untreated cells were used as controls. (Data represents media +/- SD of the duplicated of one experiment).

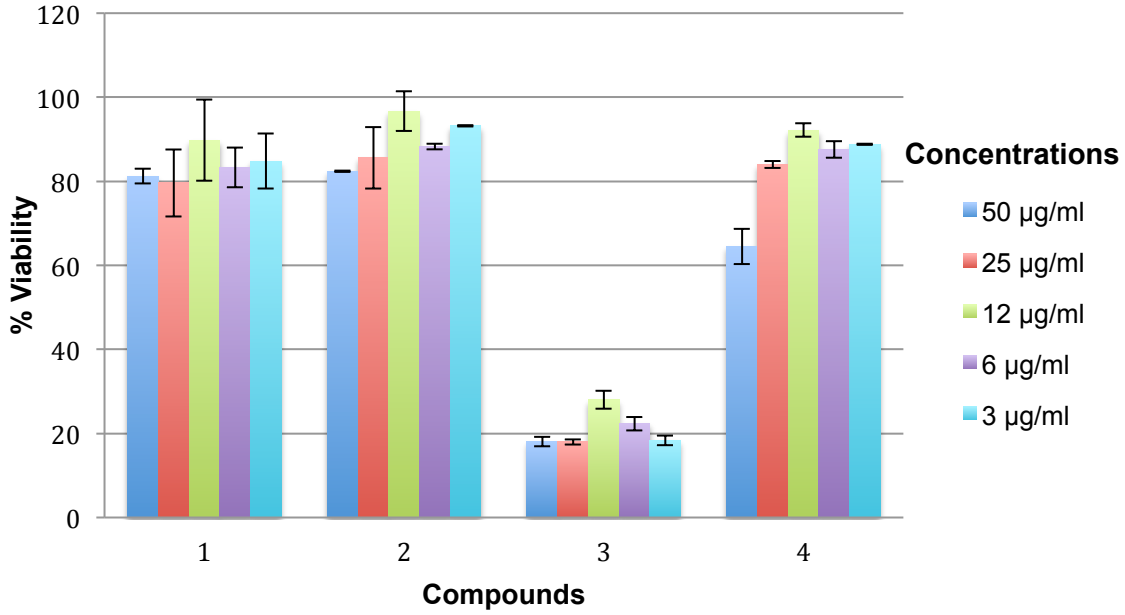



**Figure 17.**Cytotoxic effect of organotin compounds. Melanoma cells A-375 were treated with 50 µg/ml (blue bars), 25 µg/ml (red bars), 12 µg/ml (green bars), 6 µg/ml (purple bars), 3 µg/ml (light blue bars), for 24 hours. Untreated cells were used as controls. (Data represents media +/- SD of the duplicated of one experiment).

### 5.3.2 Cytotoxic capacity evaluation

Organotin compound, were used on tissue culture in order to assess their cytotoxic effects. B16F10 murine melanoma cells were treated with three different concentrations of the compounds (10, 5, and 2.5 µg/ml) for 24 hours and then viability was determined by Alamar Blue. As DMSO was the solvent used for the compounds, its toxicity was analyzed as well (**Figure 18**). The

viability of the cells was greatly affected by the higher concentrations tested, being compounds 1 y 4 the more toxic (showing 0% of viability a concentration of 10 µg/ml), and also compound 3 presents considerable toxicity, while compound 2 did not present toxic activity. When lower concentrations of compounds were used, a dose-response effect tendency was observed for all compounds, except for compound 2 which remained non-toxic at all tested concentrations.

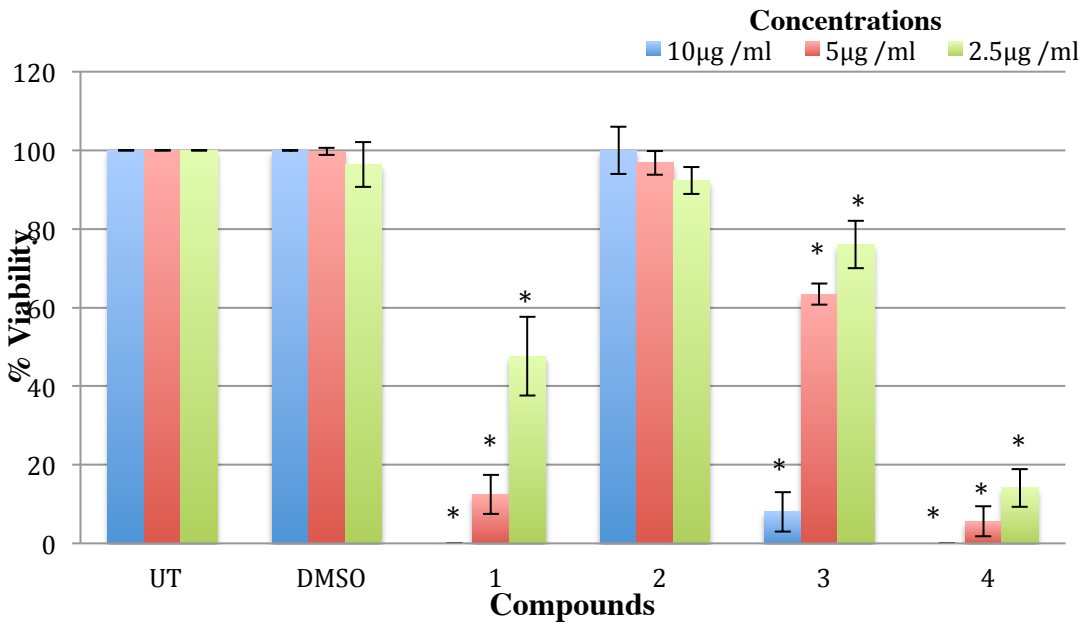



**Figure 18.** Cytotoxic effect of organotin compounds. Murine melanoma cells B16F10 were treated with 10 µg/ml (blue bars), 5 µg/ml (red bars), 2.5 µg/ml (green bars) for 24 hours. As controls, cells treated with DMSO and untreated cells were used. (Data represents media +/- SD of three independent experiments). Significant difference from the DMSO control is represented by an asterisk ($P < 0.05$).

In order to corroborate that the toxic effects are due to the organotin compounds and not to their metabolites, the same cytotoxic assay was performed with the ligands of each compound. B16F10 cells were treated with three different concentrations of the ligands (10, 5, and 2.5 µg/ml) for 24 hours and then viability was determined (L-1: ligand of compound 1, L-2: ligand of compound 2, L-3&4: ligand of compound 3 and 4) . As we can see (**Figure 19**), none of the ligands presents significant cytotoxic effect with the different concentrations, which suggest that the ligand has no influence on the cytotoxicity. While the organotin moiety is crucial for cytotoxicity, the ligand design also plays a key role in transporting and addressing the molecule to the target. [xxi]

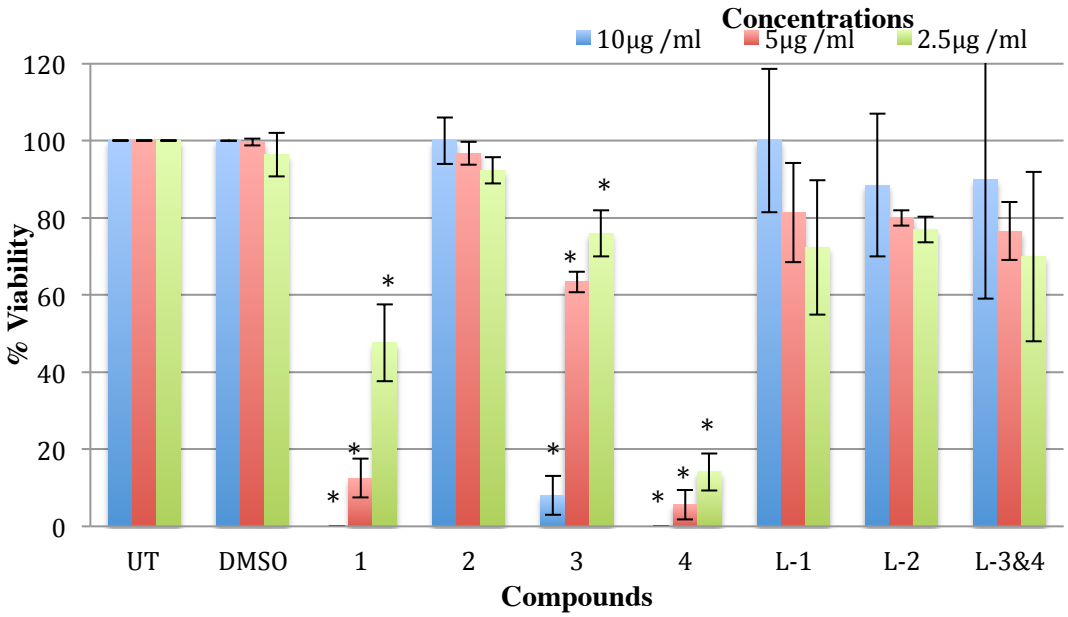



**Figure 19**. Cytotoxic effect of organotin compounds and its ligands. Melanoma cells B16F10 were treated with 10 µg/ml (blue bars), 5 µg/ml (red bars), 2.5

µg/ml (green bars) of each compound for 24 hours. Cells treated with DMSO and untreated cells were used as controls. (Data represents media +/- SD of three independent experiments). Significant difference from the DMSO control is represented by an asterisk ($P < 0.05$).

As compounds, 1, 3 and 4 showed the best cytotoxic effect, we determined their toxicity effects at lower concentrations (1 µg/ml and 0.1 µg/ml) (**Figure 20**). A dose-response effect tendency was observed for compounds1 and 4, both compounds at 0.1 µg/ml are practically innocuous to cells. As we can see, compound 3 is very cytotoxic at concentration of 10 µg /ml, and a concentration of 5 µg /ml and lower showed not significant change.

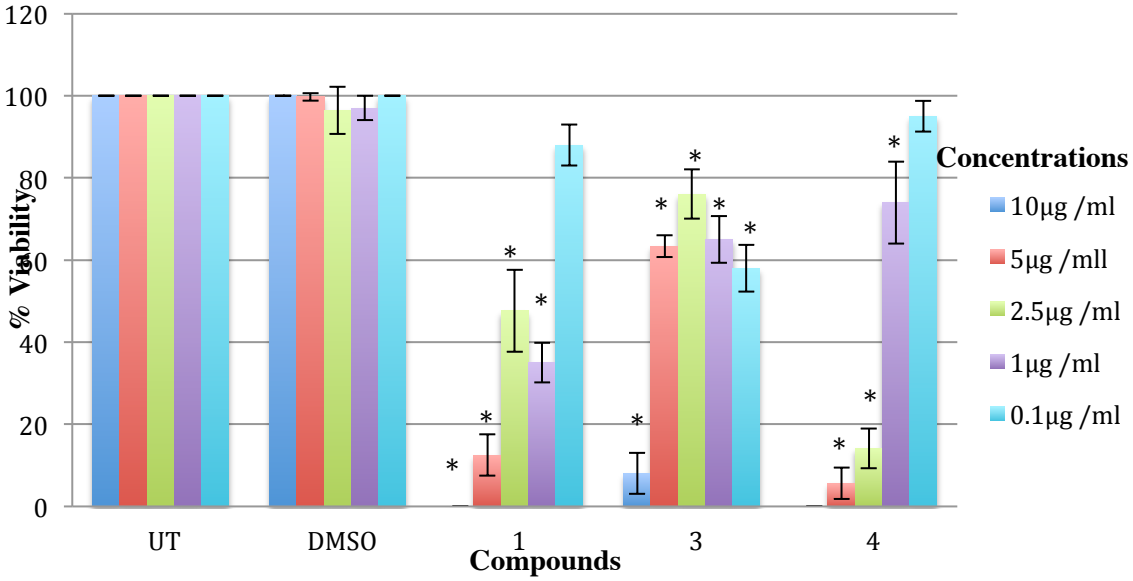



**Figure 20**. Cytotoxic effect of organotin compounds. Melanoma cells B16F10 were treated with 10 µg/ml (blue bars), 5 µg/ml (red bars), 2.5 µg/ml (green bars), 1 µg/ml (purple bars) and 0.1 µg/ml (light blue bars) of each compound for

24 hours. Cells treated with DMSO and untreated cells were used as controls. (Data represents media +/- SD of three independent experiments). Significant difference from the DMSO control is represented by an asterisk ($P < 0.05$).

Silva et. al, [xxxv] reported the Inhibitory concentration of 50% ($IC_{50}$) of cell growth using the cell line B16F10 for cisplatin and carboplatin, 4.2 and 6.3 µmol/L respectively, this values converted to µg/ml are represented in **Table 11**:

**TABLE 11**

Inhibitory concentration of 50% of cell growth for cisplatin and carboplatin.

| | **B16F10** |
|---|---|
| **Cisplatin** | 1.26 µg/ml |
| **Carboplatin** | 2.33 µg/ml |

Compound 1 at concentration of 2.5 and 1 µg/ml maintains similar cytotoxicity under 50% of cell growth, this result is favorable if we compare with the $IC_{50}$ from the cisplatin, with concentration of 1 µg/ml we can observe almost the same toxicity that cisplatin has at 1.26 µg/ml, and it is almost the half amount that carboplatin needs to inhibit the 50% of cell growth.

Compound 3 only has good cytotoxicity at high concentration of 10 µg/ml, at concentrations of 5, 2.5, 1 and 0.1 maintains the same behavior with viability

up to 50%, if we compare with cisplatin and carboplatin, this compound is not a good cytotoxic agent, because it requires a lot of concentration to observe a toxic effect.

Finally, compound 4 has a very good toxic capacity at concentration of 5 and 2.5 µg/ml, with less than 20% of viability, but at lower concentration of 1 µg/ml the viability increments almost to 70%. This means that the $IC_{50}$ of this compound is between 2.5 and 1 µg/ml. And the value will be similar to those reported for cisplatin and carboplatin (1.26 and 2.33 µg/ml respectively), considering this compound like a good cytotoxic agent for this cell line.

### 5.3.3 Bioimages

To evaluate the behavior of the luminescent organotin compounds in cells, confocal fluorescence microscopy measurement was carried out. Briefly, melanoma cells B16F10 were seeded at 2 x $10^5$ cells/2 ml media per well in 6 wells plates on sterile coverslips, 24 h later media was removed and fresh media and the compounds at 10 µg/ml were added. Two hours later media and compounds were removed and cells were washed once with PBS. Coverslips were recovered and mounted on microscope slides using 75% glycerol. Cells were analyzed by confocal microscopy in a Leica SP5 system using a 458 nm laser to excite the luminescence while emission was registered between 478 and 612 nm. These experiments were performed three times in duplicate.

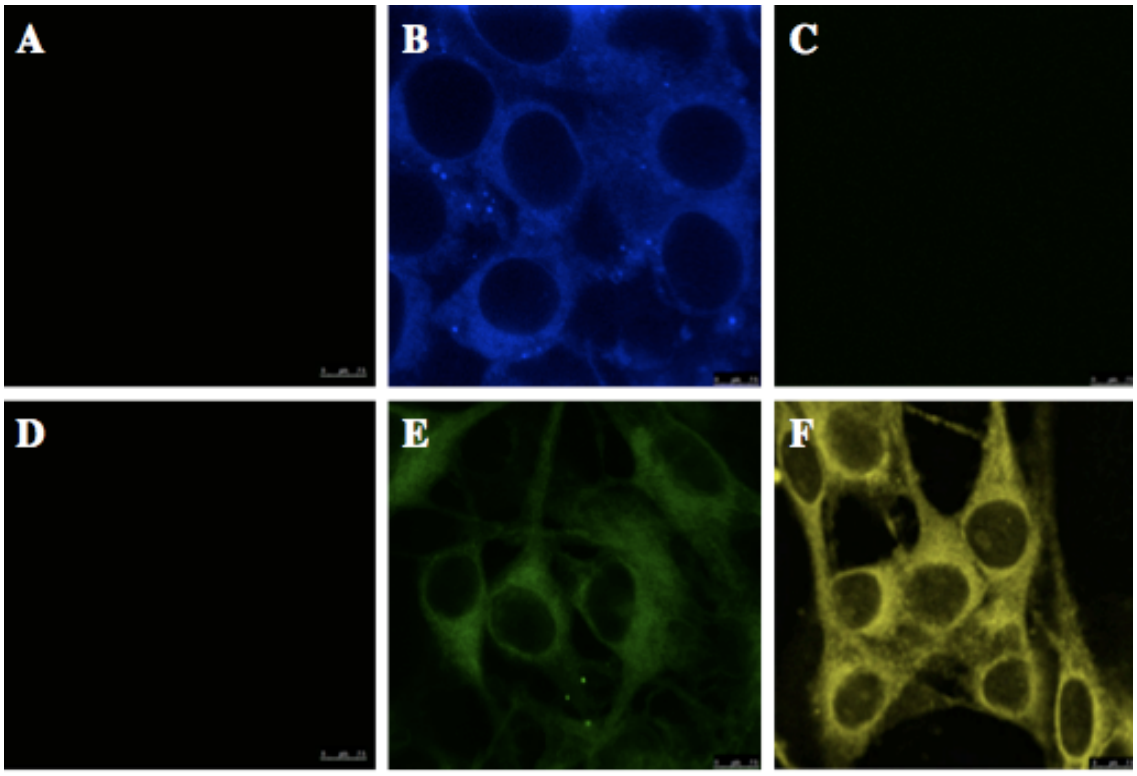



**Figure 21.** Staining of cells with organotin compounds. Confocal microscopy of melanoma cells B16F10 treated with 10 µg/ml of each compound for 2 h. A, Untreated cells; D, DMSO control; B, Compound 1; C, Compound 2; E, Compound 3; F, Compound 4. (Scale bar shown represents 7.5 µm).

Cells exposed to each organotin compound at 10 µg/ml showed different staining patterns ranging from lack of stain to very strong staining (**Figure 21**).

Treatment of cells with compound **2** did not produce staining (**Figure 21-C**). Compound **1** produces a good staining, but it quickly presents photobleaching and its fluorescence disappear after a few seconds (**Figure 21-**

**B**). Cells exposed to compound **3** showed a strong green cytoplasmic staining with focalized vesicle-like structures showing stronger staining (**Figure 21-E**). Compound **4** produced the stronger effect with yellow cytoplasmic staining showing as well brighter focalized vesicle-like structures (**Figure 21-F**). It is important to notice that neither compound is able to stain the nucleus.

The three compounds that present cell staining are the most cytotoxic, unlike the compound 2 which does not exhibit luminescence nor cytotoxicity, proving that its no toxicity is caused by the inability of the compound to penetrate the cell, probably because the molecule has tert-butyl groups which make this section largest, which prevents the passage into the cell.

The staining capacity of the ligands was analyzed as well at the highest concentration (10 μg/ml), but none of the three ligands produced cell staining. Images with the ligands of the compounds 2, 3 and 4 presented the same pattern of extracellular crystals formation with intense fluorescence, but no one was capable of penetrate the cell. Crystal formation suggests precipitation of the ligands due the low solubility, reaffirming that Sn may increase the solubility of the compounds.

#### 5.3.3.1 Dose-response study.

We analyzed the capacity of compounds 3 and 4 to stain the cells at lower concentrations due its good staining capacity (Compound 1 was not used due to

the photobleaching showed). Similar to the previous experiments, B16F10 cells were treated now with 1 µg/ml or 0.1 µg/ml of each compound for two hours and then analyzed by confocal microscopy. As shown in **Figure 22**, compound 3 is able to produce both cytoplasmic and focalized green staining even when we lower two magnitude orders its concentration (**Figure 22 B-D**). At 1 µg/ml produced a staining pattern similar to that at the higher concentration and the brighter vesicle-like structures are still visible (**Figure 22-C**), however at the lower concentration of 0.1 µg/ml, the cytoplasmic staining is almost undetectable (**Figure 22-D**). On the other hand, lowering the concentration of compound 4 resulted in a very weak staining at 1 µg/ml and a complete lack of staining at 0.1 µg/ml (**Figure 22-G and 22-H**, respectively).

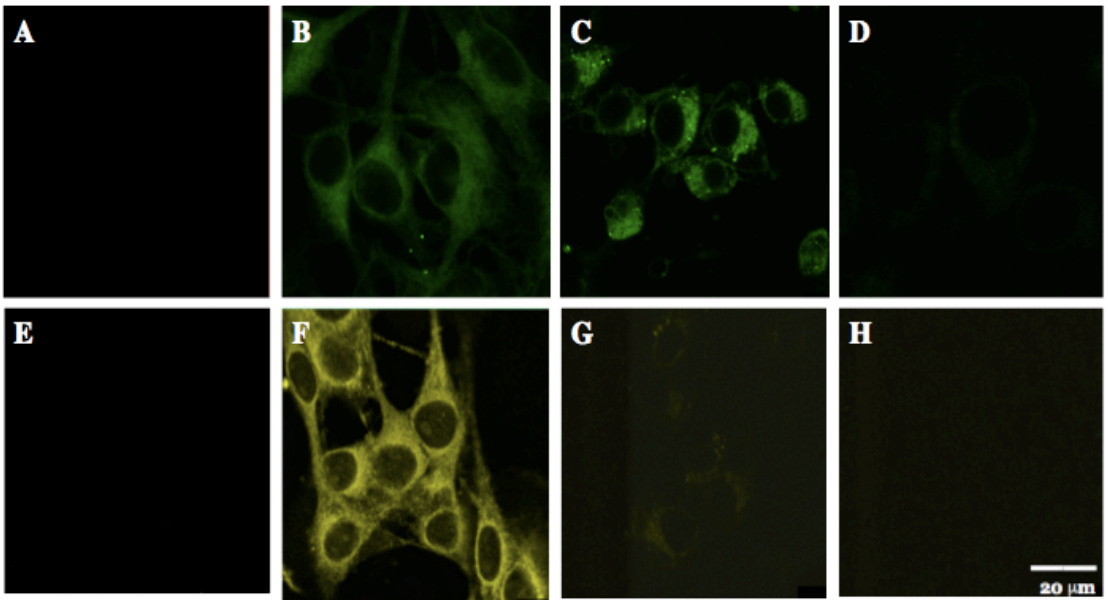



**Figure 22.** Staining of cells with organotin compounds 3 and 4. Confocal microscopy of melanoma cells B16F10 treated with compound 3 (B-D) or compound 4 (F-H) at three different concentrations: 10 µg/ml (B and F), 1 µg/ml

(C and G), or 0.1 μg/ml (D and H) of each compound for 2 h. A, Untreated cells; E, DMSO control. (Scale bar shown represents 20 μm).

The very strong cytoplasmic staining in the cells with numerous vesicle-like structures showing a stronger staining, suggests that the possible internalization mechanism of the organotin compounds is endocytosis. We hypothesize that after endosome processing the compound and/or its metabolites are released to the cytosol. Once in the cytoplasm they are not able to get into the nucleus. Vesicle formation may be influenced by the solubility of the molecule, if not completely solubilized microprecipitates are formed which lead to the internalization of the compound by endosomes.

Another possible mechanism is that the compounds are being recognized by cell surface receptors inducing in this way endocytosis. Important to mention, high intensity luminescence from the vesicles might be explained on the basis of the acidic internal pH. The cytosol luminescence is unambiguously due to the internalization of the whole organotin complex; we infer that because the free ligand showed lack of staining under the same condition.

Interestingly, none of the compounds is able to get into and stain the nucleus, which suggests the dissociation of the chelating ligand and then producing the intermediate R2Sn2+,[xxxvi] which causes the high cytotoxicity. The role of ligands is of considerable importance in tuning the cytotoxic characteristics of the complex. Ligands can modify the reactivity, lipophilicity, oral/systematic

bioavailability of metal ions, stabilization of the oxidation state and substitutional inertness depending on the requirements for chemotherapy. [xxxvii] ,[xxxviii]

Now it is generally accepted that organotin(IV) moieties may bind to glycoproteins or to cellular proteins, and directly interact with DNA, causing cell death by apoptotic mechanisms. [xxxix]

# 6. CONCLUSIONS

In summary, we have synthesized four new organotin compounds derivative from Schiff bases in good yields.

The results of the cytotoxic selectivity assay, indicates that the compounds are not selective when using two human tissue cultures of normal (HGF1, gingival biopsy) and cancer cells (A-375, melanoma cells), in both of the cultures follows the same toxicity tendency.

Cytotoxic assays with melanoma murine cells B16F10, shows that compound 2, does not have any toxic activity, even with the highest concentration. Compounds 1, 3 and 4 has a good cytotoxic capacity at 10 µg/ml, but a lower concentrations compound 1 and 4 presents better cytotoxicity.

The cytotoxicity comparison between the ligands and compounds, suggest that the ligand has no influence on the toxic capacity, while the organotin moiety is crucial for cytotoxicity.

Comparing compound 1 and 4 with metal based commercial drugs, like cisplatin and carboplatin proved in the same tissue culture (B16F10, melanoma murine cells), compound 1 at concentration of 1 µg/ml, almost has the same toxicity that cisplatin ($IC_{50}$: 1.26 µg/ml), and it is nearly the half amount that

carboplatin needs to inhibit the 50% of cell growth. Compound 4 has a very good toxic capacity at 2.5 µg/ml, with less than 20% of viability, compared with carboplatin ($IC_{50}$: 2.33 µg/ml).

The cell images shows very strong cytoplasmic staining in the cells with numerous vesicle-like structures which suggests that the possible internalization mechanism of the organotin compounds is endocytosis.

The cytosol luminescence is unambiguously due to the internalization of organotin complex; we infer that because the free ligand showed lack of staining under the same condition.

No one of the compounds is able to get into and stain the nuclei, which suggests the dissociation of the chelating ligand and then producing the intermediate $R_2Sn^{2+}$, which causes the high cytotoxicity. The intermediate $Ph_2Sn^{2+}$ , (compound 1 and 4 )has the best cytotoxic behavior.

The results obtained in this work contribute to the tin cytotoxic capacity knowledge, and know some more about the behavior in cells.

## 6.1 Perspectives

As part of the knowledge generated in this research work was demonstrated that tin compounds derivative from salicylidene bezoylhydrazones have good cytotoxic properties, and that the ligand plays an important role in the behavior of the molecule. Studies with other cells lines are required to evaluate if this compounds have selectivity for other kind of cancer cells.

To continue with this work, we are going to carry out the synthesis of new molecules, whit some structural variations, to improve the cellular internalization. Also we could continue the study of the behavior of the molecule and the tin directly with the DNA using electrophoresis.

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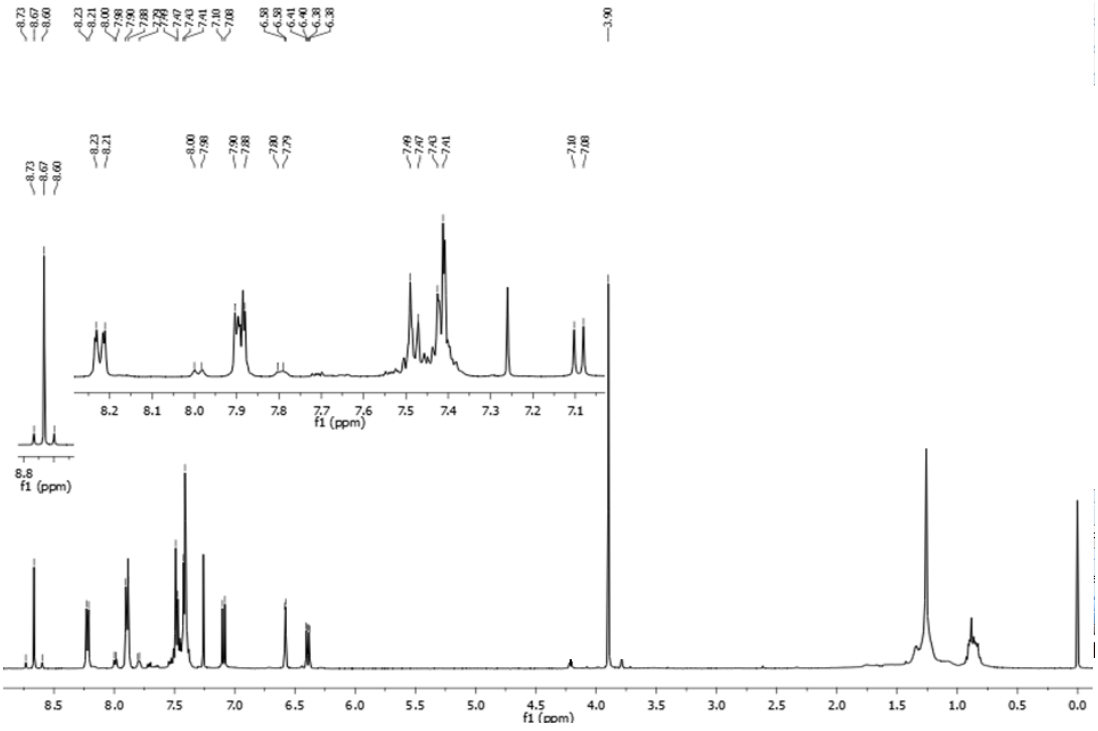
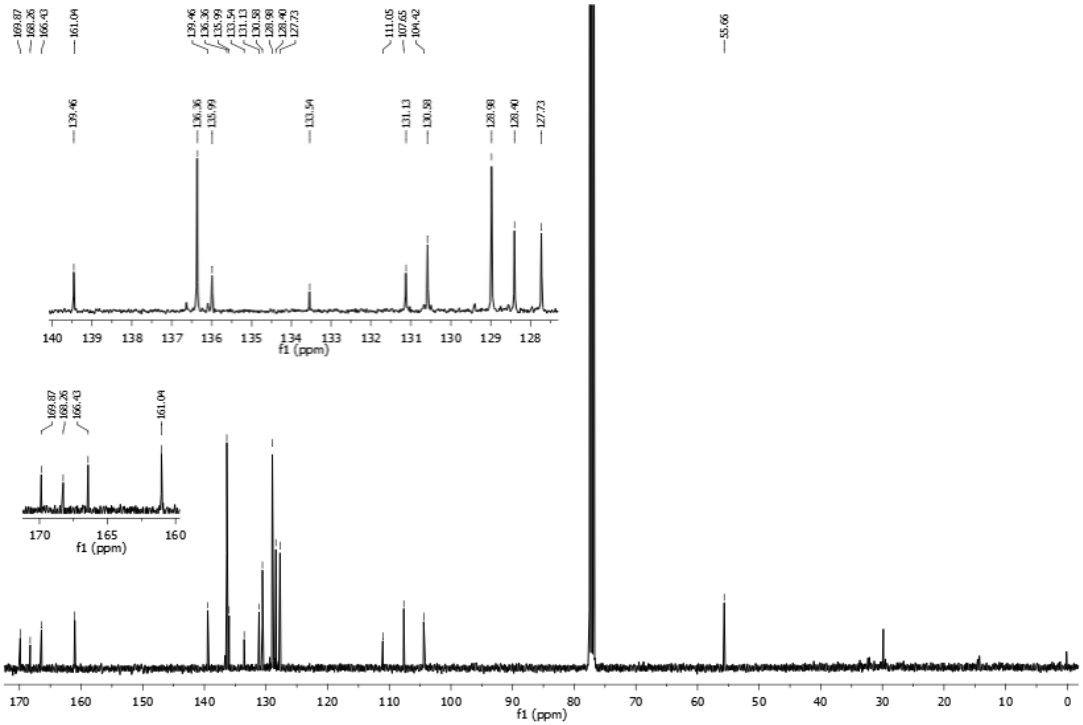
# 8. APPENDIX

APENDIX A

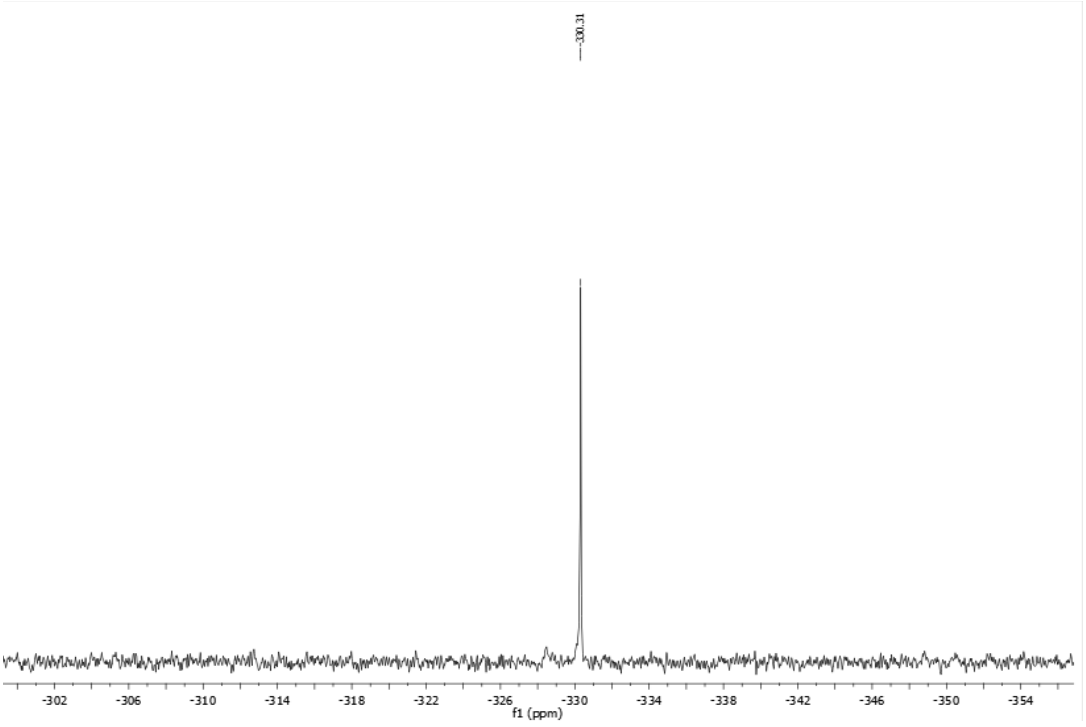
CHARACTERIZATION



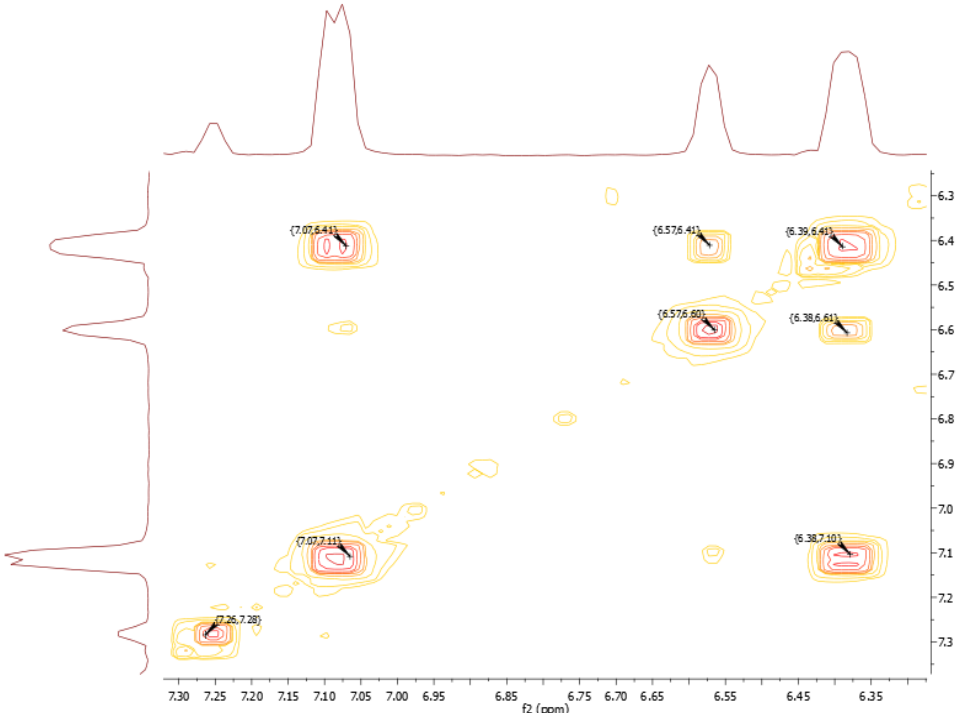

$^{1}$H -NMR (400 MHz, $CDCl_3$) spectrum of compound **1.**



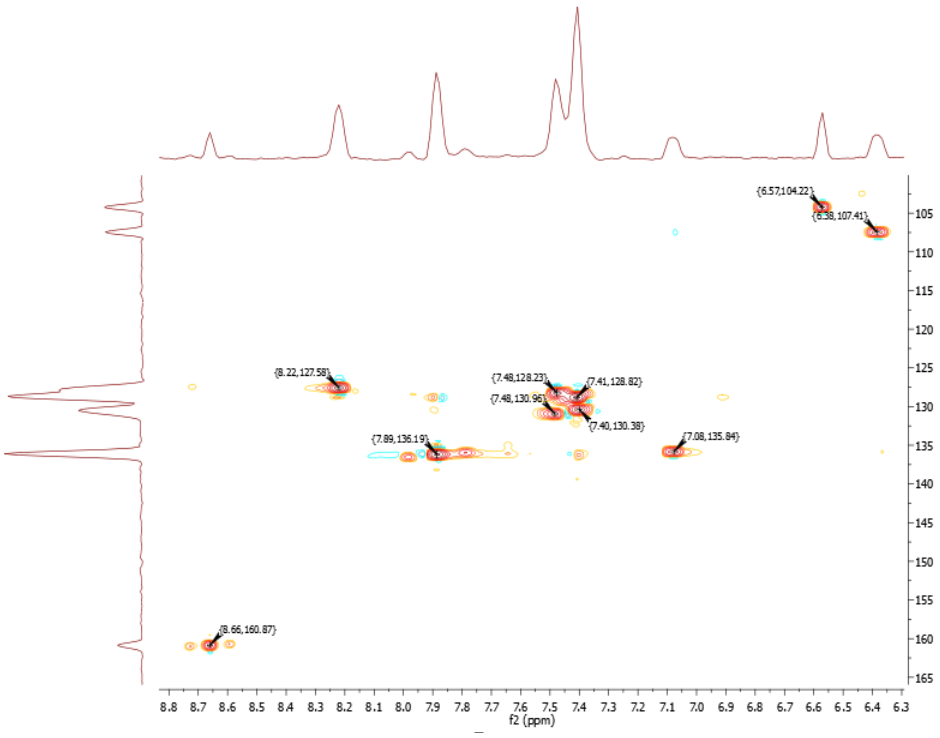

$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum of compound **1.**




$^{119}$Sn-NMR (149.14 MHz, $CDCl_3$) spectrum of compound **1.**




$^{1}H/^{1}H$ COSY spectrum of compound **1** (aromatic region).

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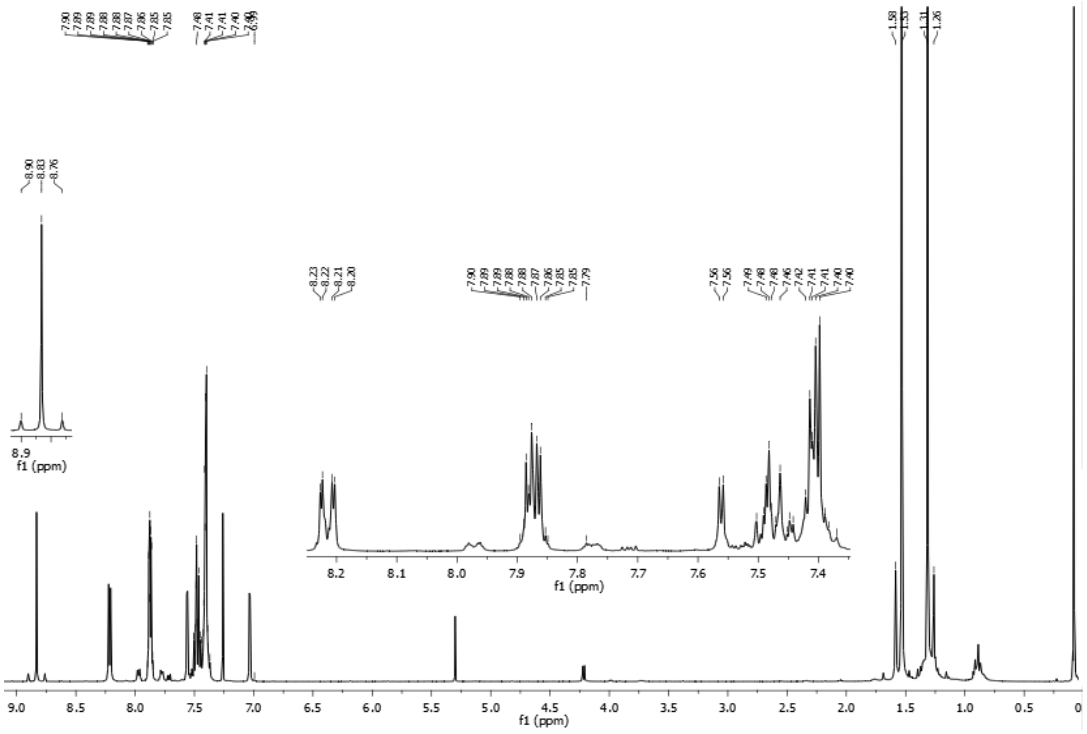
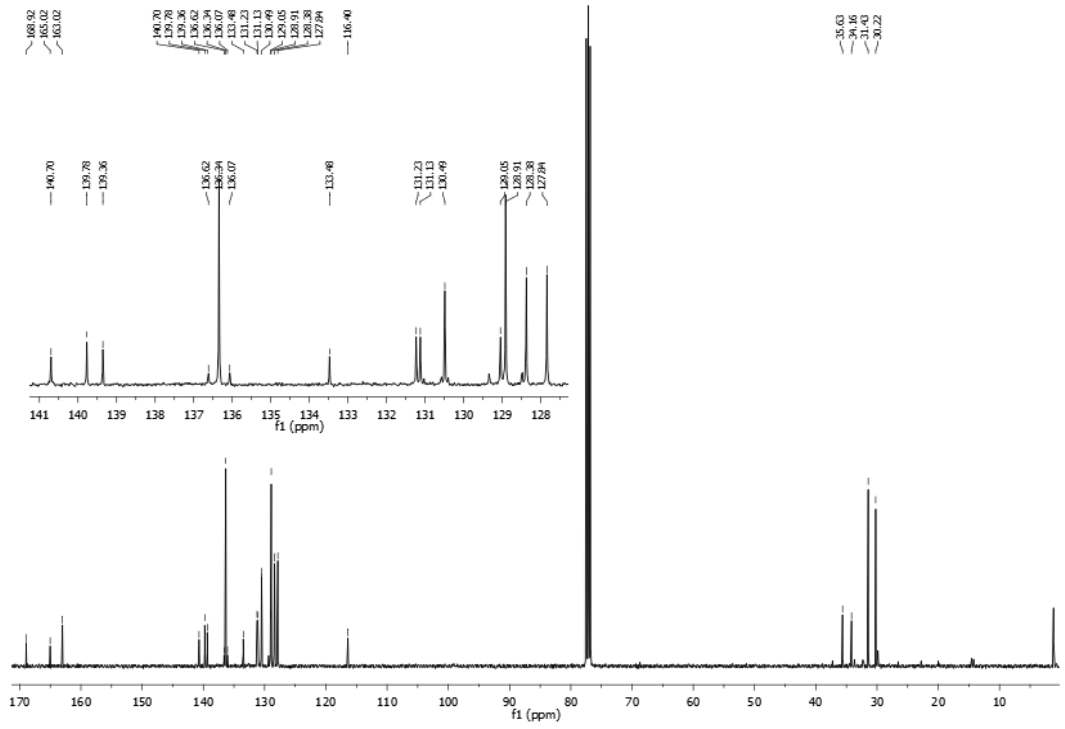
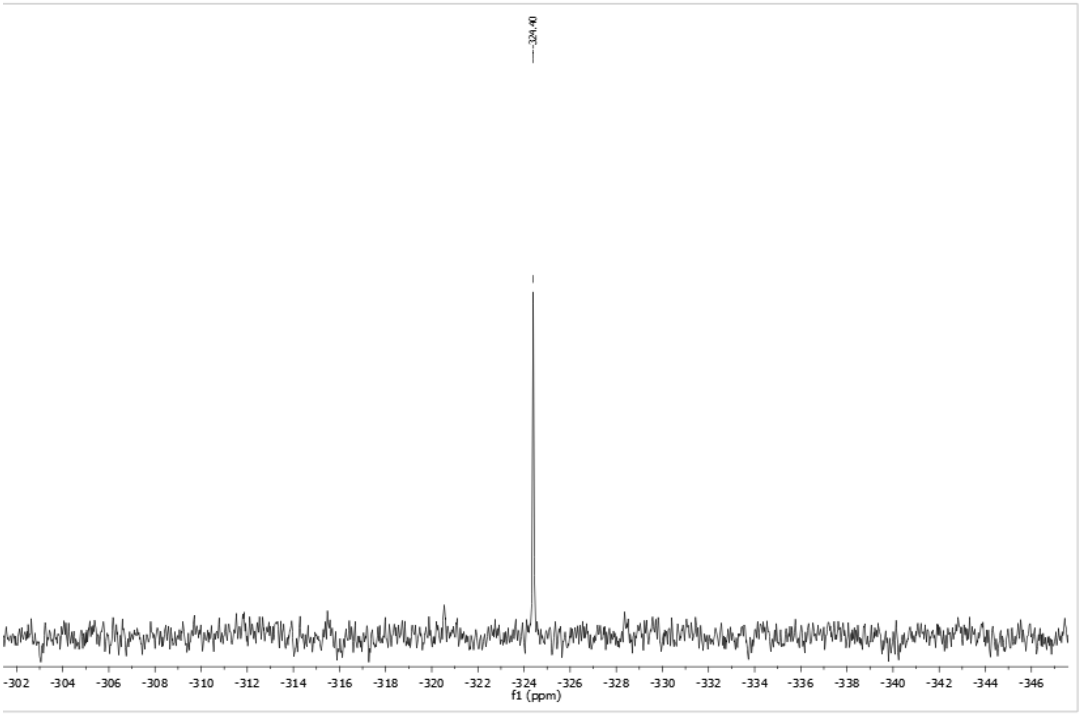


$^{1}$H-NMR (400 MHz, $CDCl_3$) spectrum of compound **2.**



$^{13}$C-NMR (100 MHz, $CDCl_3$) spectrum of compound **2.**

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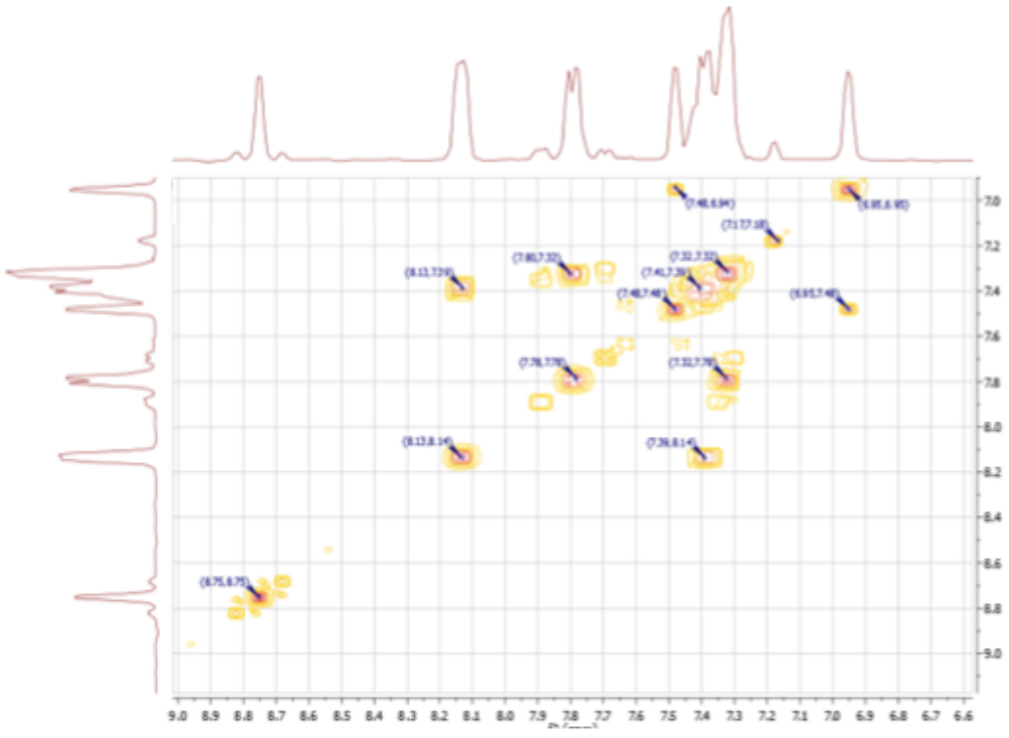


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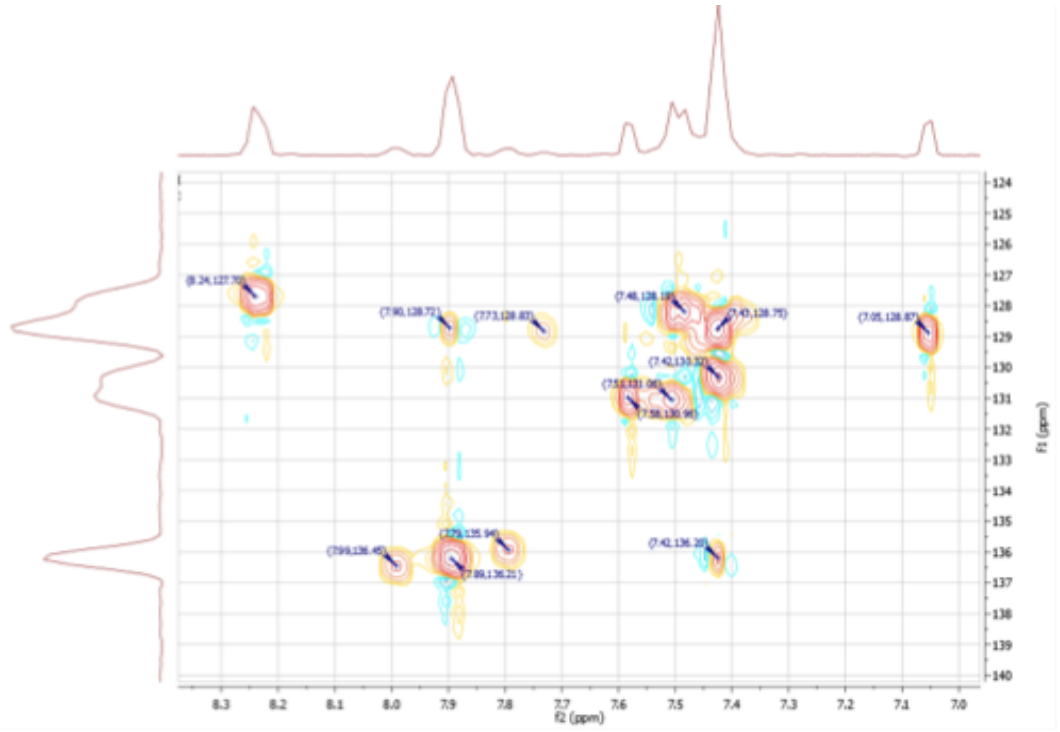


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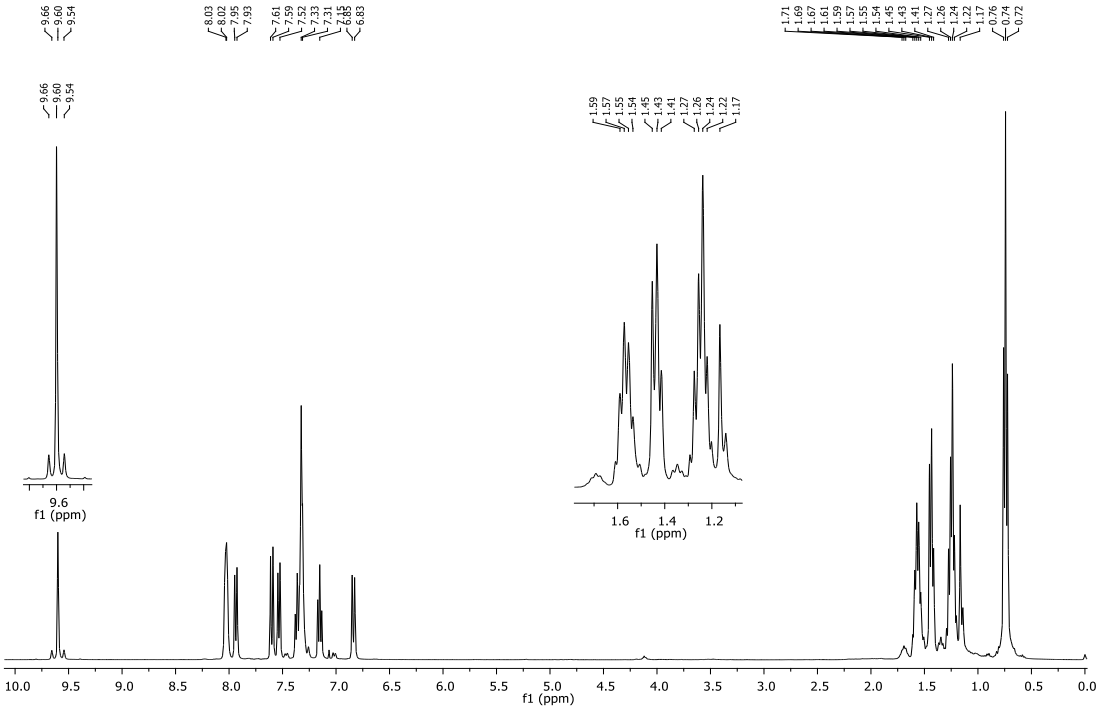

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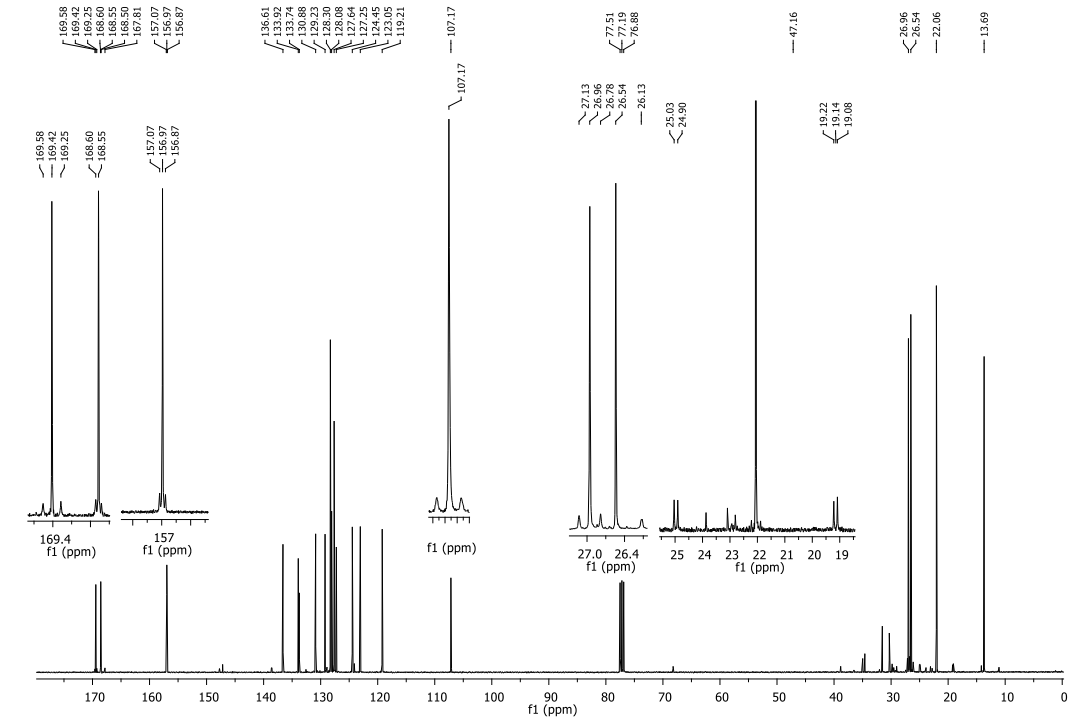

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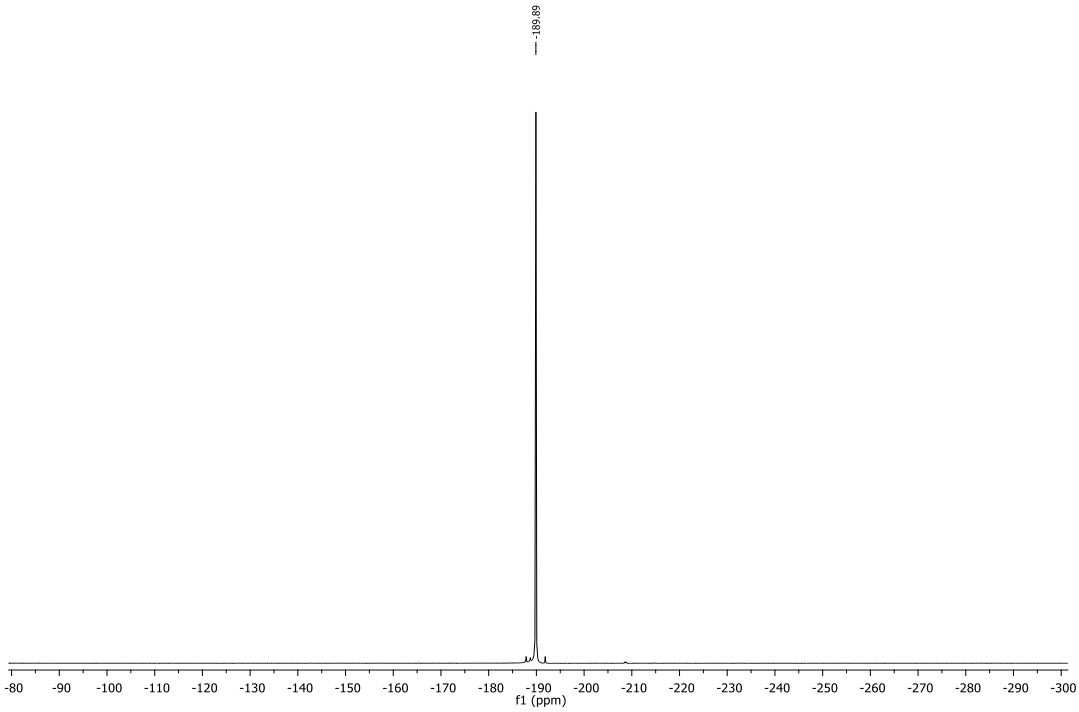


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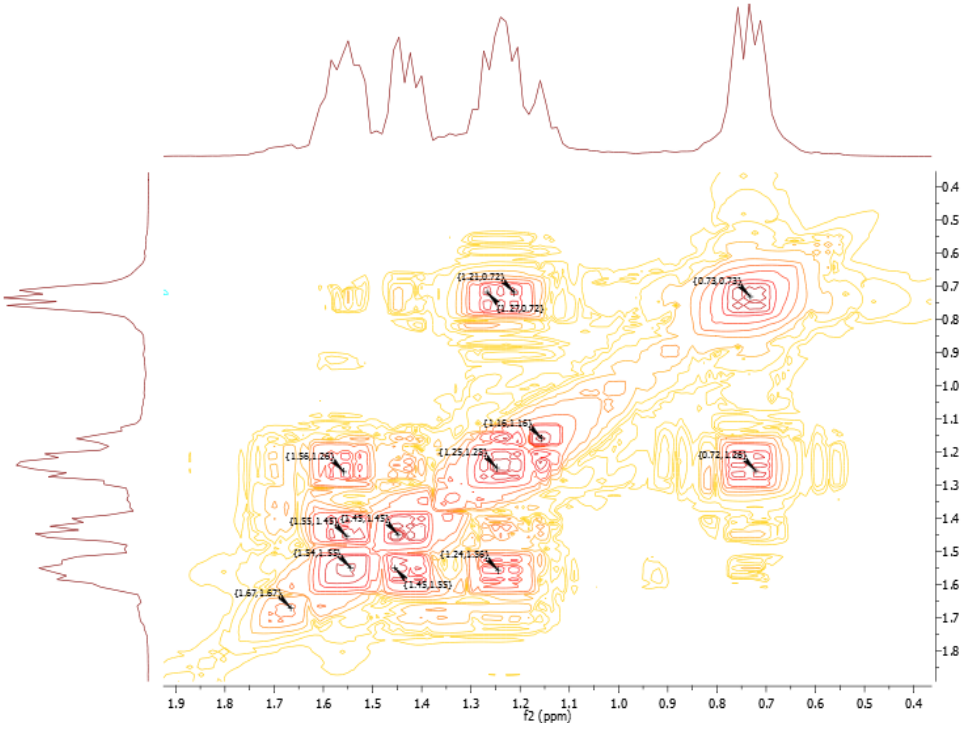
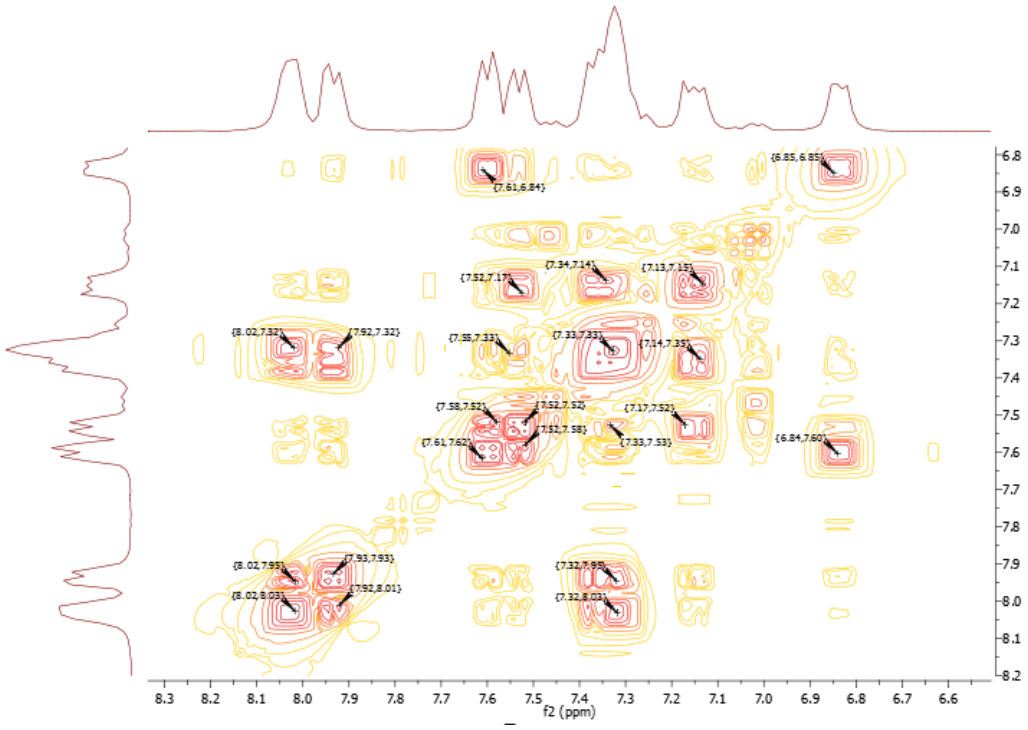



$^{1}$H/$^{1}$H COSY spectrum of compound **3** (alifatic region).

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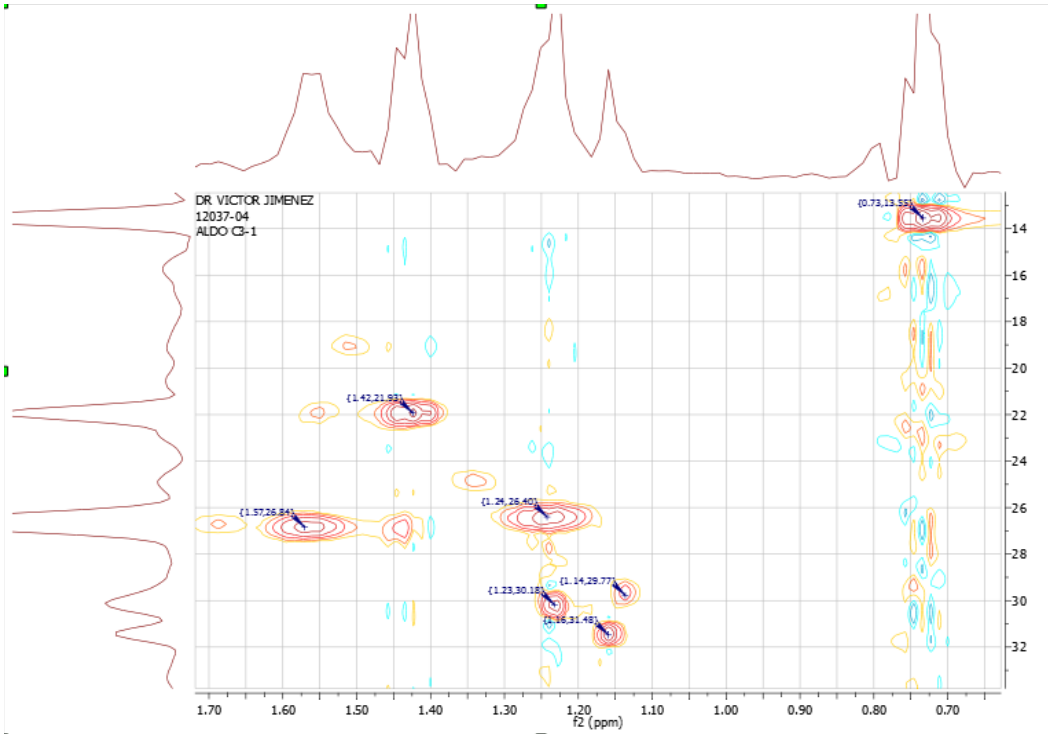


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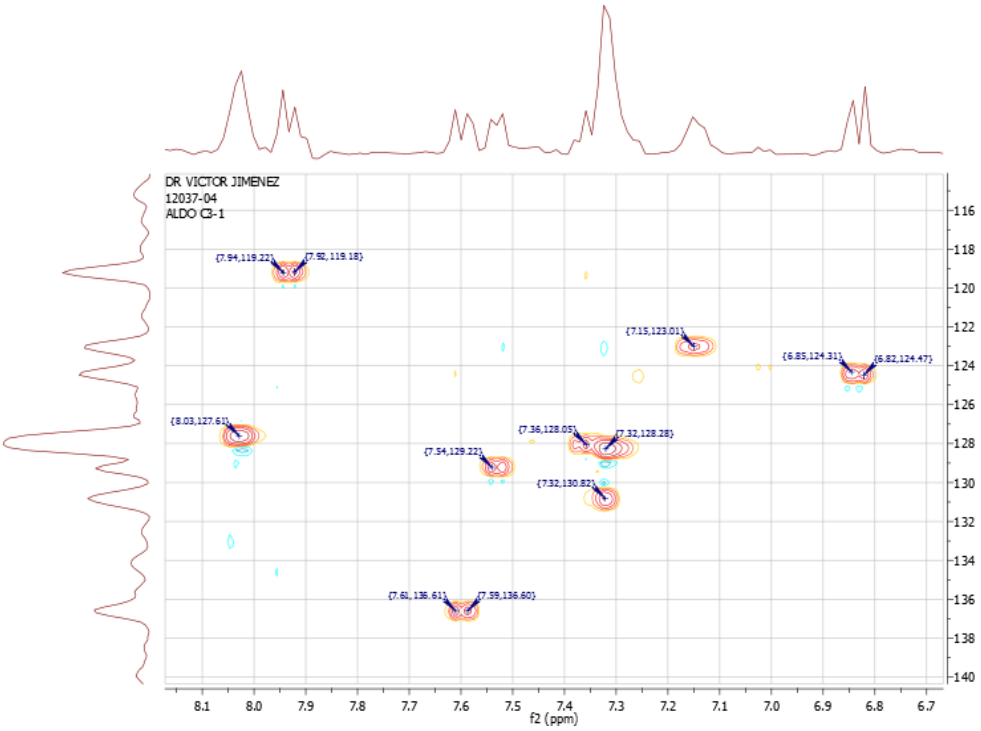


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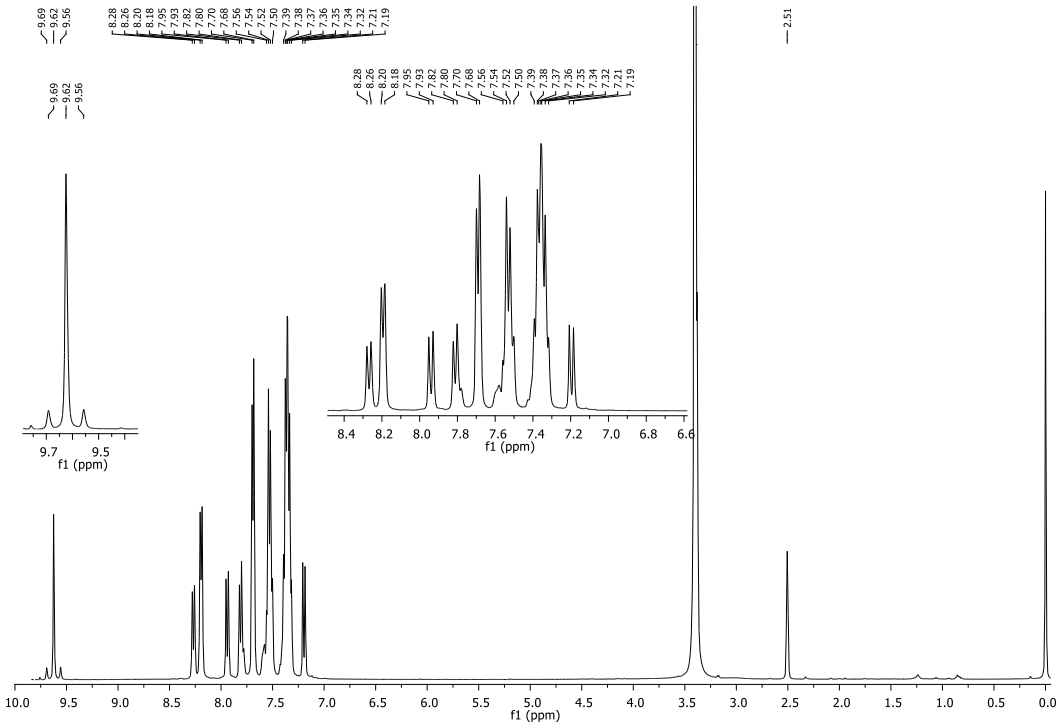
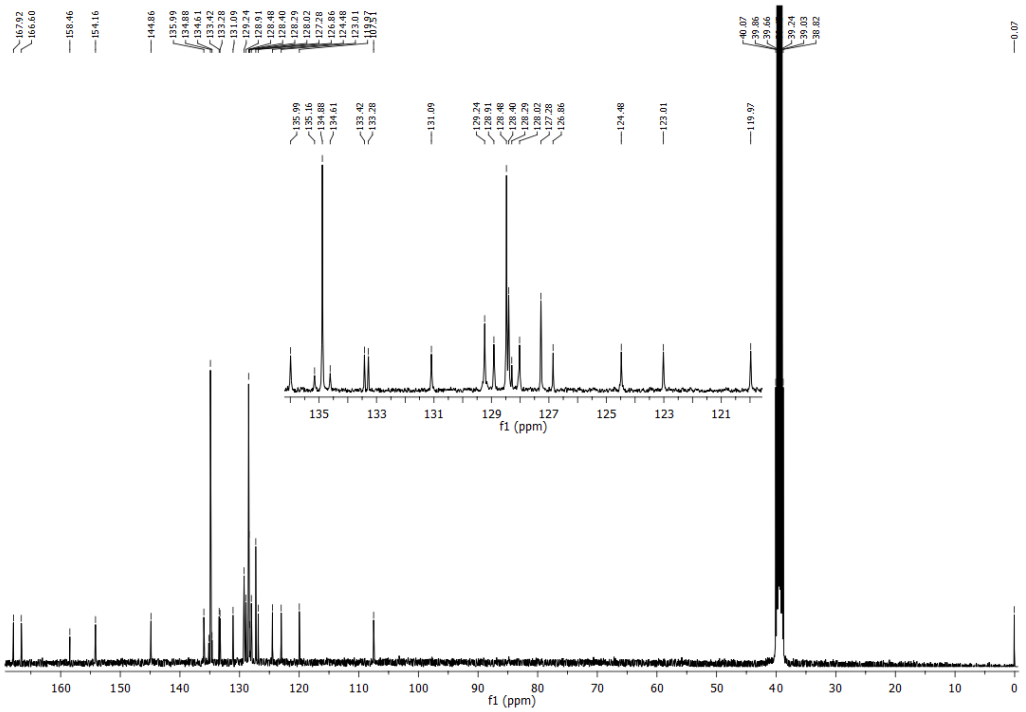
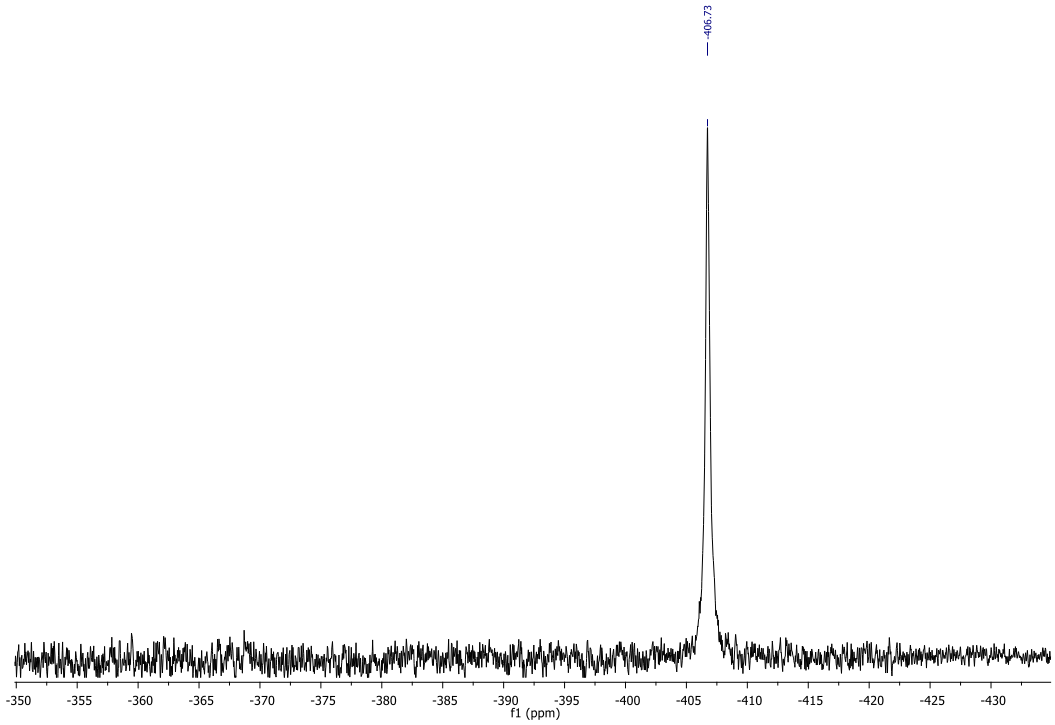


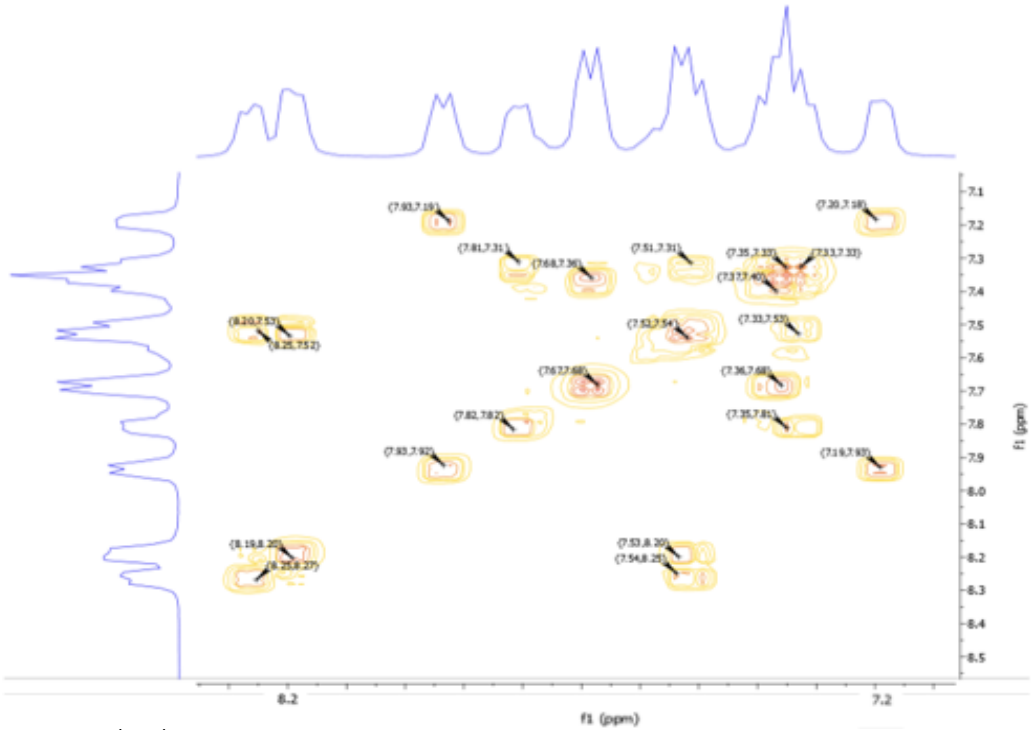
$^{1}$H-NMR (400 MHz, DMSO-$d_6$) spectrum of compound **4.**



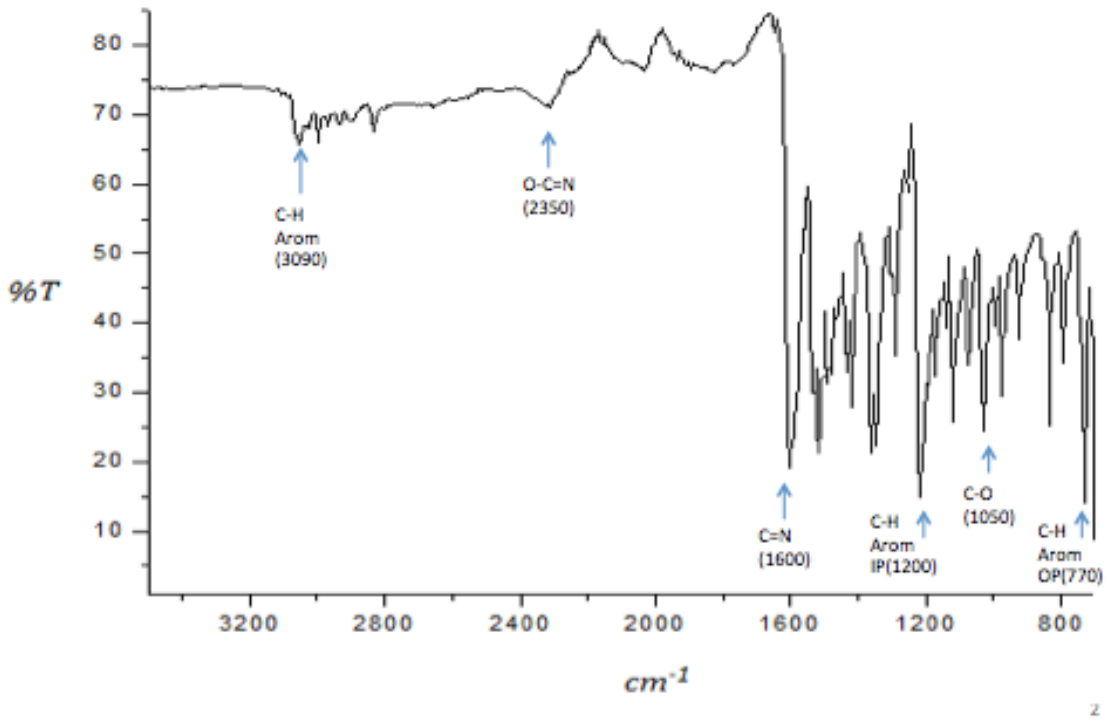
$^{13}$C-NMR (100 MHz, DMSO) spectrum of compound **4.**



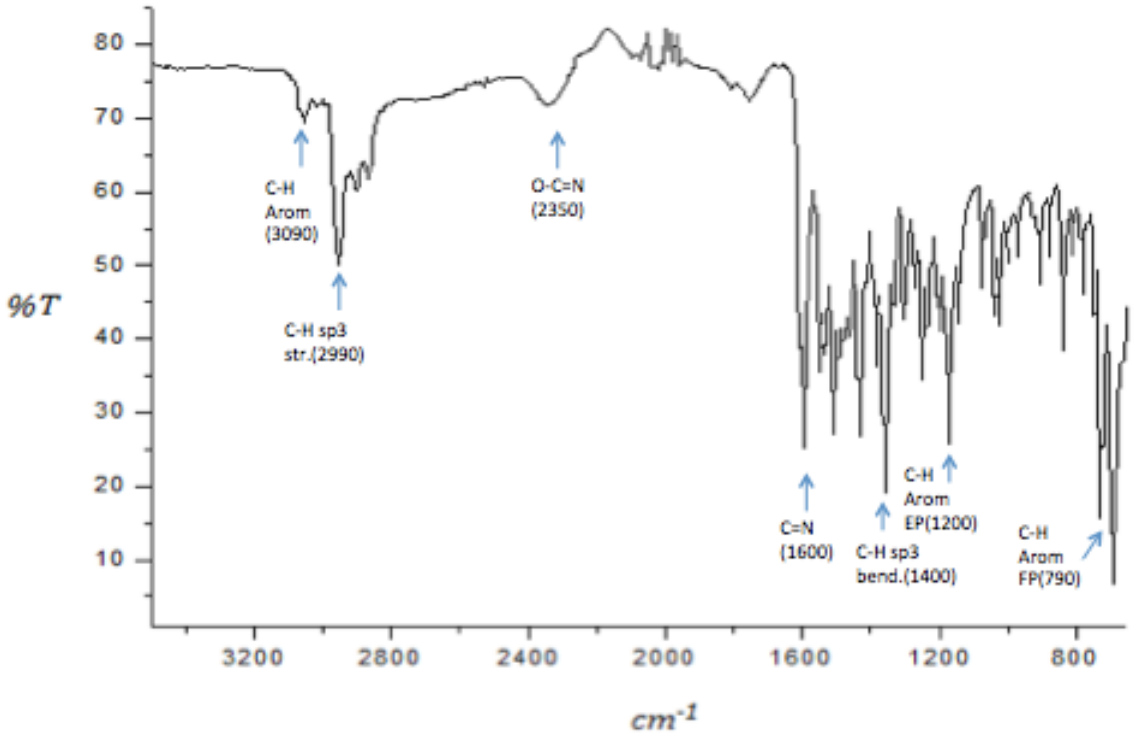

$^{119}$Sn -NMR(149.14 MHz, DMSO) spectrum of compound **4.**



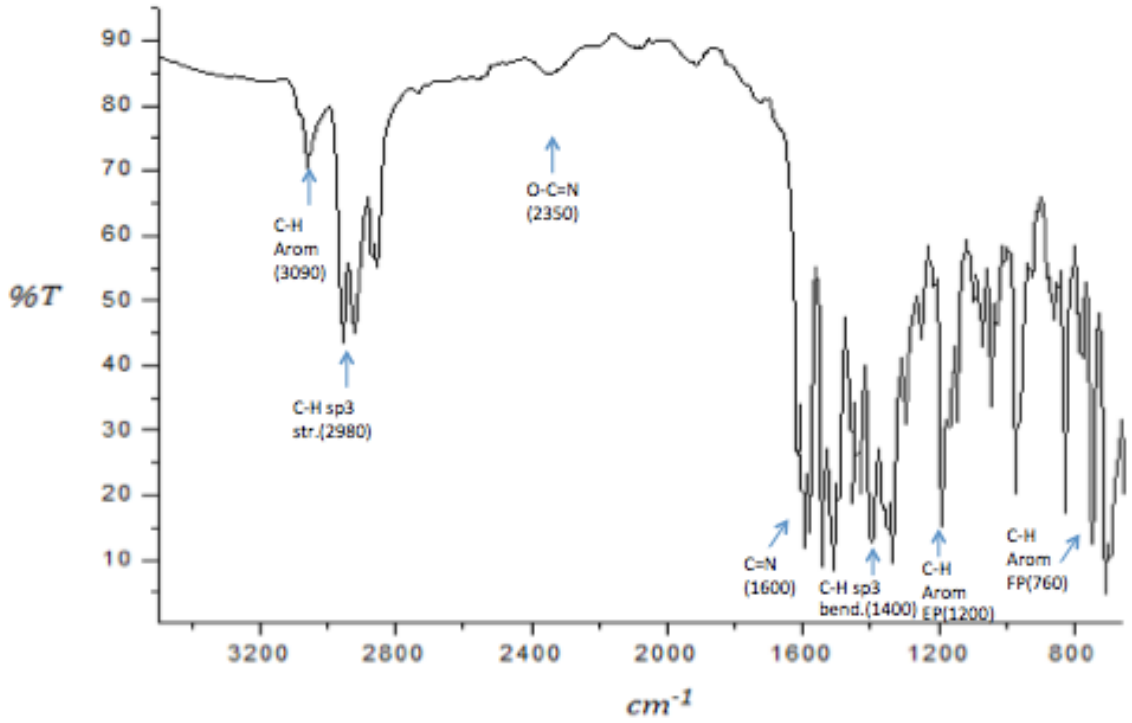

$^{1}$H/$^{1}$H COSY spectrum of compound **4** (aromatic region).



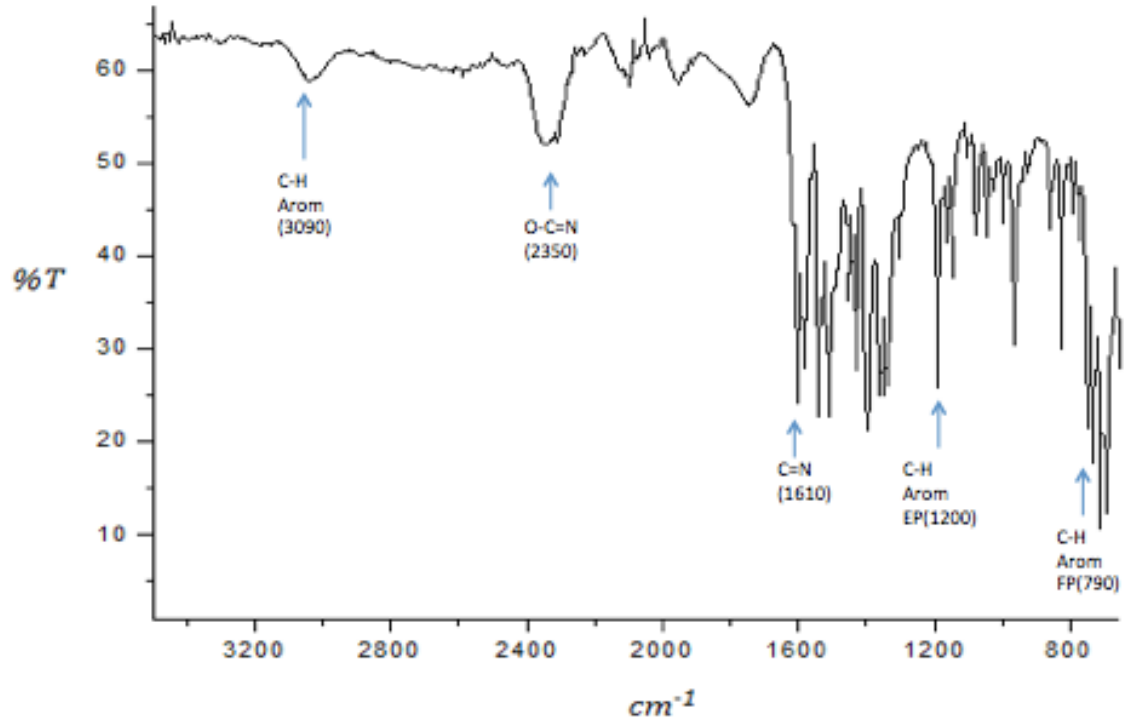

IR spectrum of compound **1**



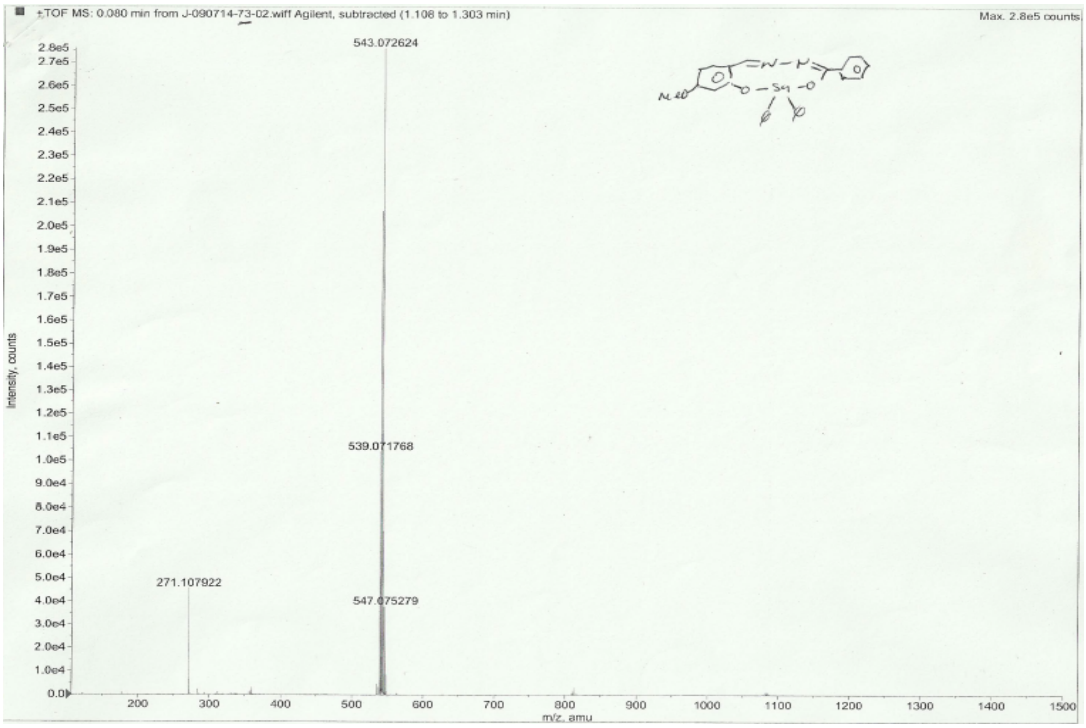

IR spectrum of compound **2**



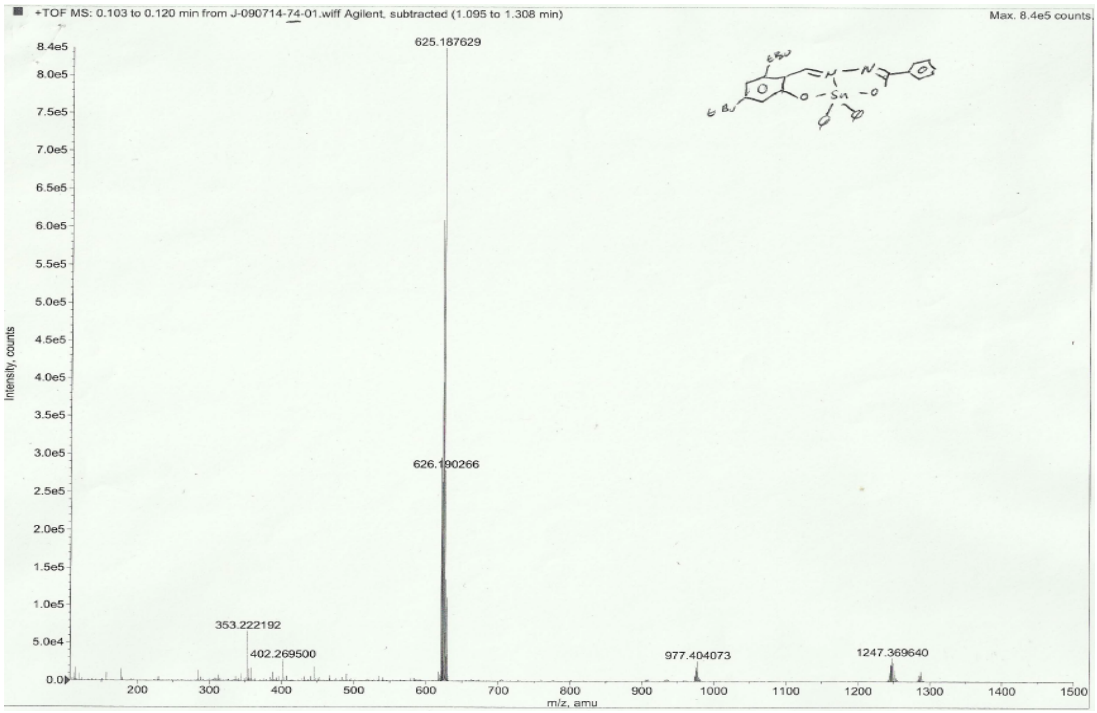

IR spectrum of compound **3**



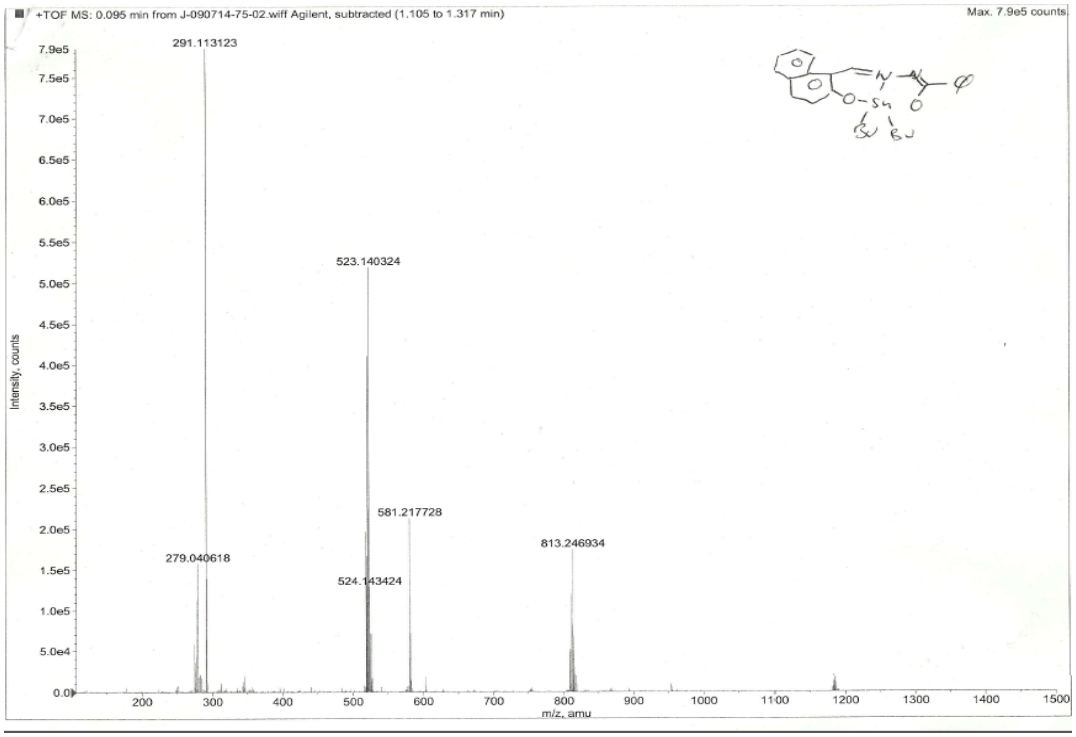

IR spectrum of compound **4**



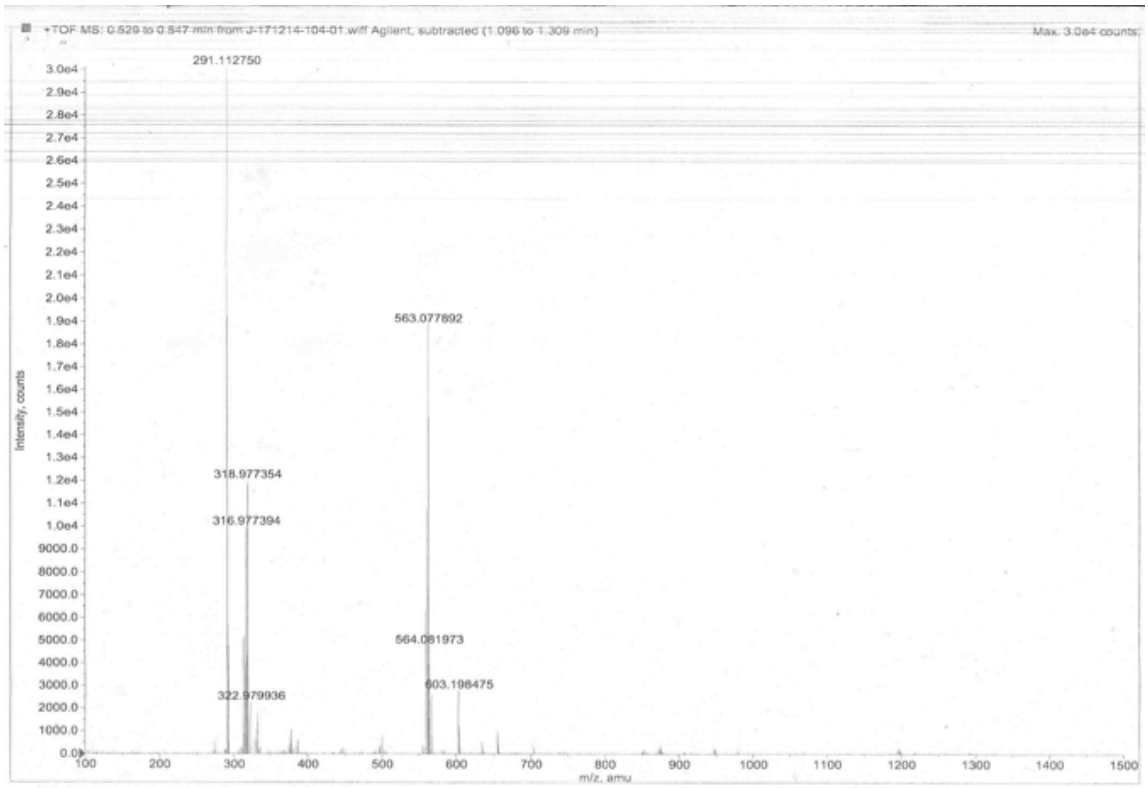

Mass spectrum of compound **1**



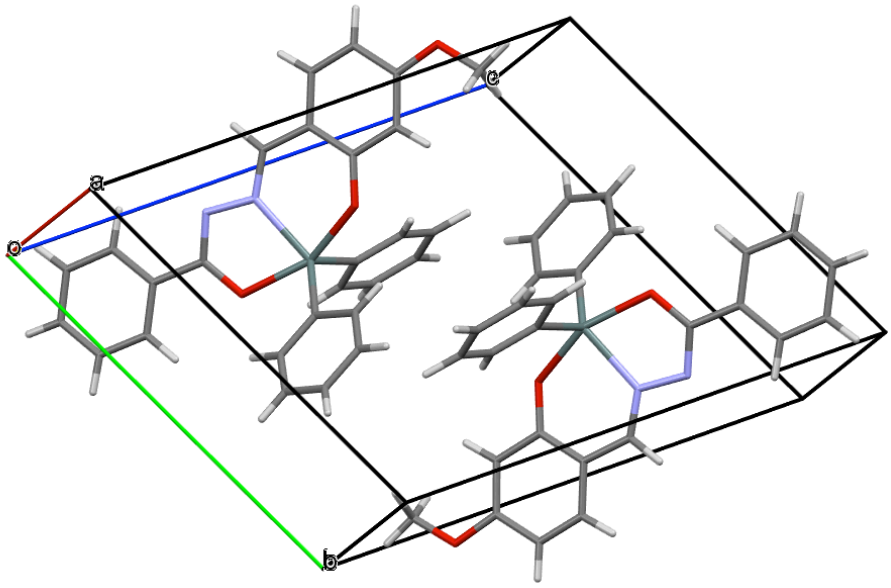

Mass spectrum of compound **2**



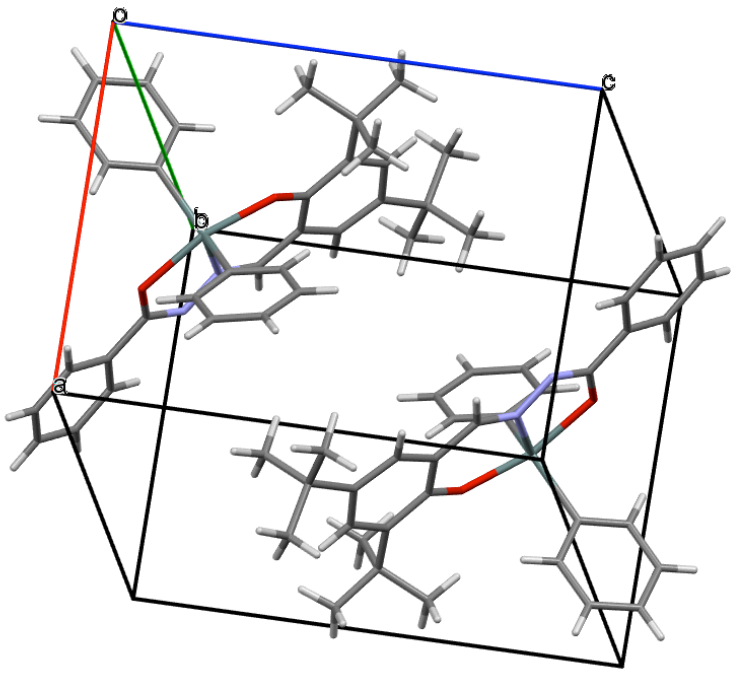

Mass spectrum of compound **3**



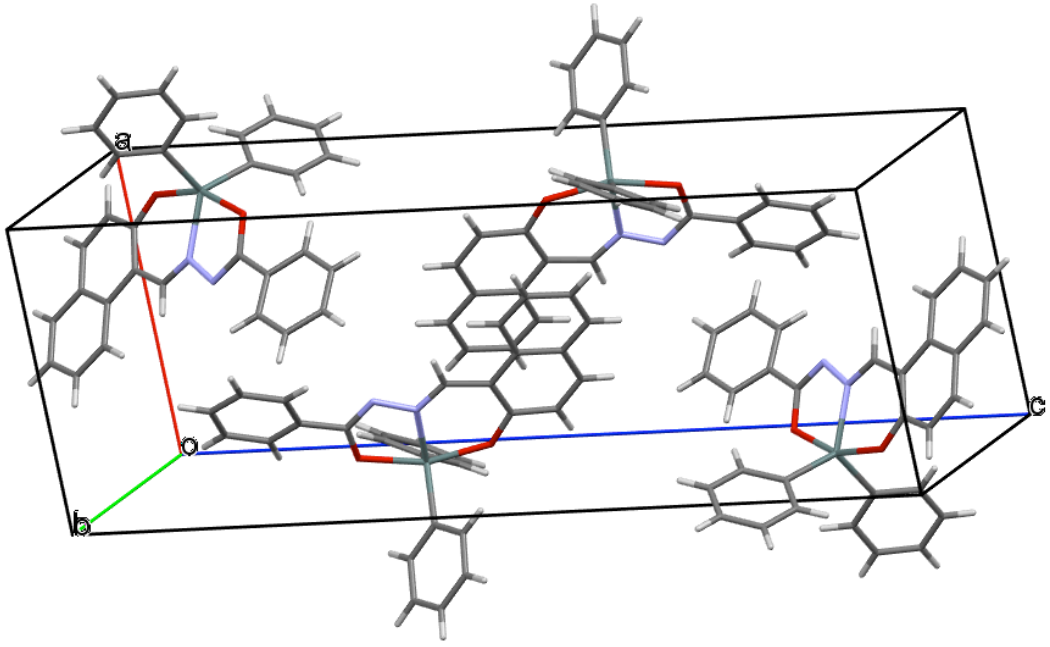

Mass spectrum of compound **4**




Packing structure of compound **1**



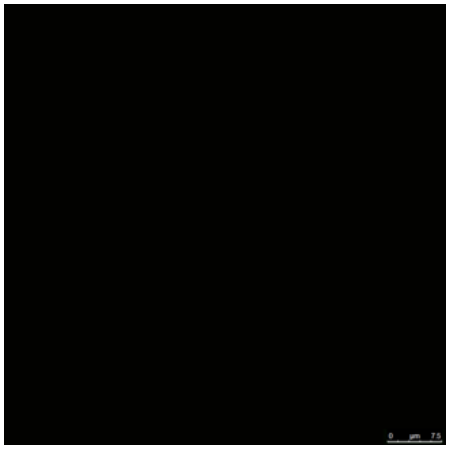

Packing structure of compound **2**



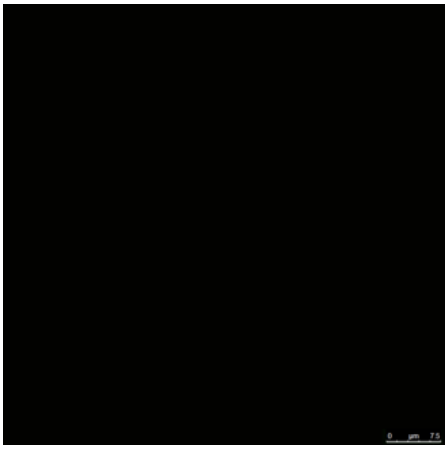

Packing structure of compound **4**

# APENDIX B

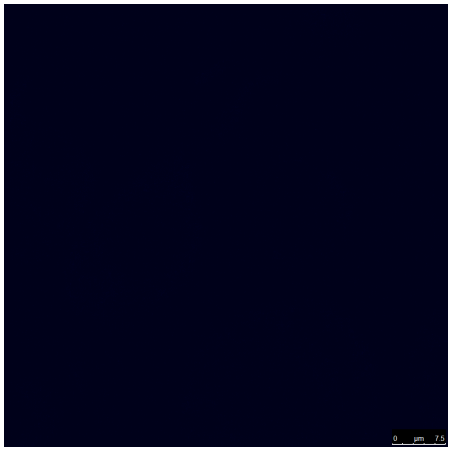
IMAGES



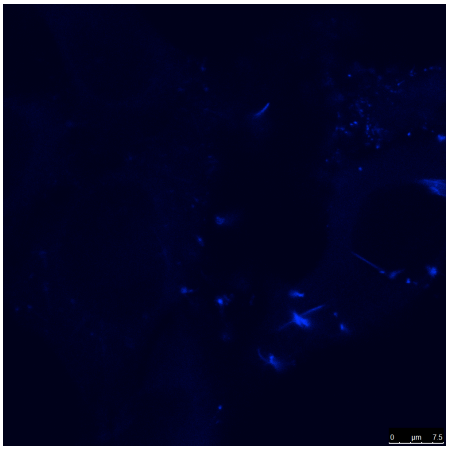
Confocal microscopy of melanoma cells B16F10 **untreated** (control).



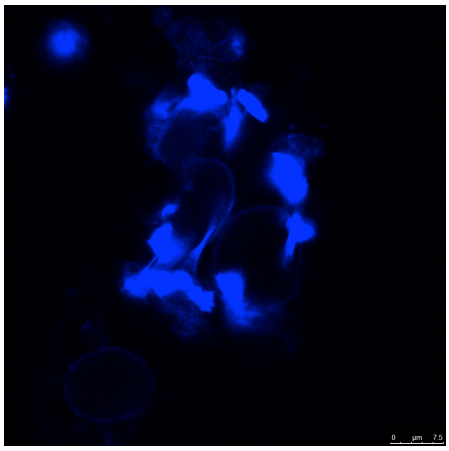
Confocal microscopy of melanoma cells B16F10 with **DMSO** (control).



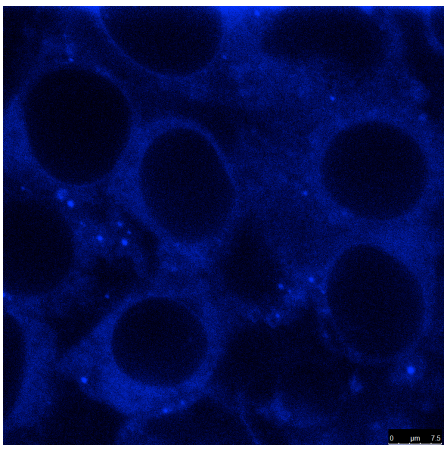
Confocal microscopy of melanoma cells B16F10 treated with **Ligand 1** (10 µg /ml).



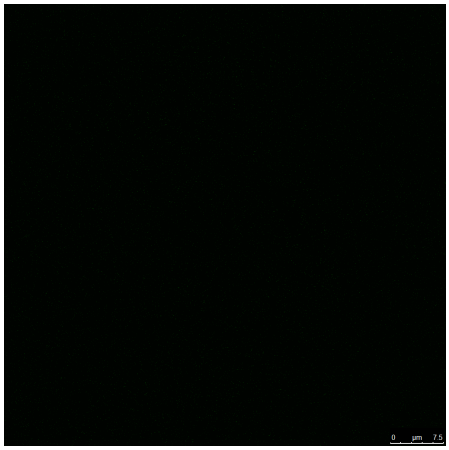
Confocal microscopy of melanoma cells B16F10 treated with **Ligand 2** (10 µg /ml).



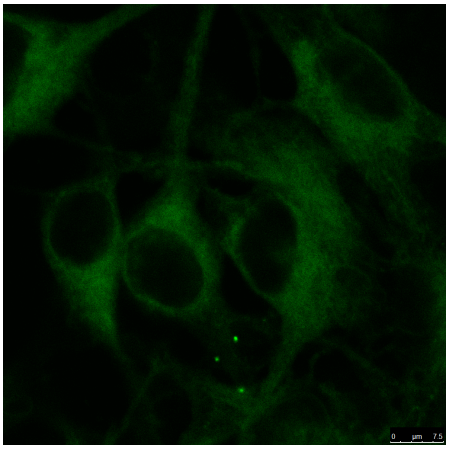
Confocal microscopy of melanoma cells B16F10 treated with **Ligand 3 y 4** (10 µg /ml).



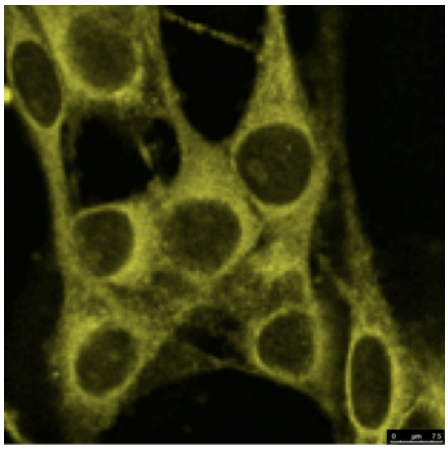
Confocal microscopy of melanoma cells B16F10 treated with **compound 1** (10 µg /ml).



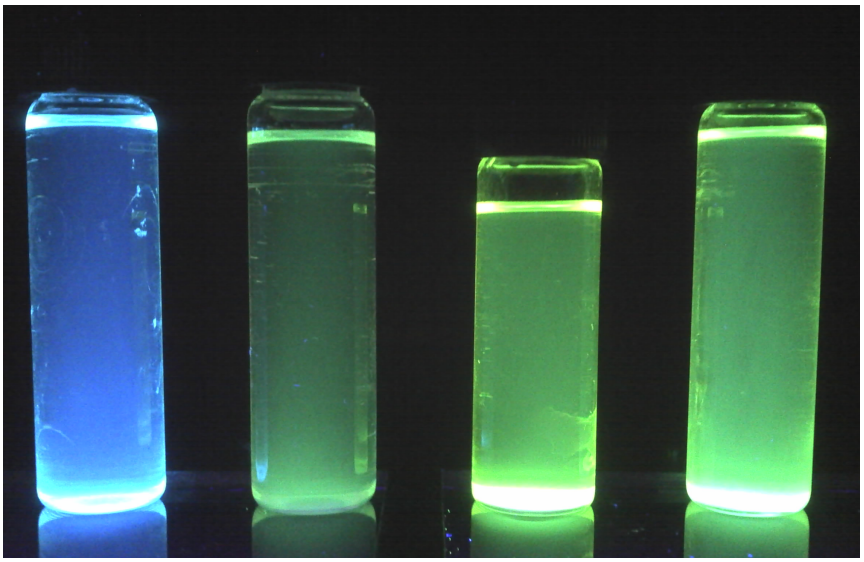
Confocal microscopy of melanoma cells B16F10 treated with **compound 2** (10 µg /ml).



Confocal microscopy of melanoma cells B16F10 treated with **compound 3** (10 µg /ml).



Confocal microscopy of melanoma cells B16F10 treated with **compound 4** (10 µg /ml).



Compounds 1-4 in chloroform. (UV-365nm)

# 9. AUTOBIOGRAPHIC SUMMARY

## QFB JESSICA CECILIA BERRONES REYES

Candidate for the degree of

Master of Science with Orientation in Pharmacy

**Thesis:**

DETERMINATION OF THE CYTOTOXIC CAPACITY OF ORGANOTIN COMPOUNDS DERIVATIVES FROM SALICYLIDENE BENZOYLHIDRAZONE.

**Field of study:** Pharmacy

**Biography:** Born in Cd. Victoria Tamaulipas, México, on September 28th of 1990.

**Education:** Graduated from the Universidad Autónoma de Nuevo León, degree obtained: Chemistry Pharmaceutical Biologist in 2013, second place of the generation.

**Professional experience:** Professional practices in the department of controlled drugs Hospital Universitario Dr. José Eluterio González, UANL, 2012.