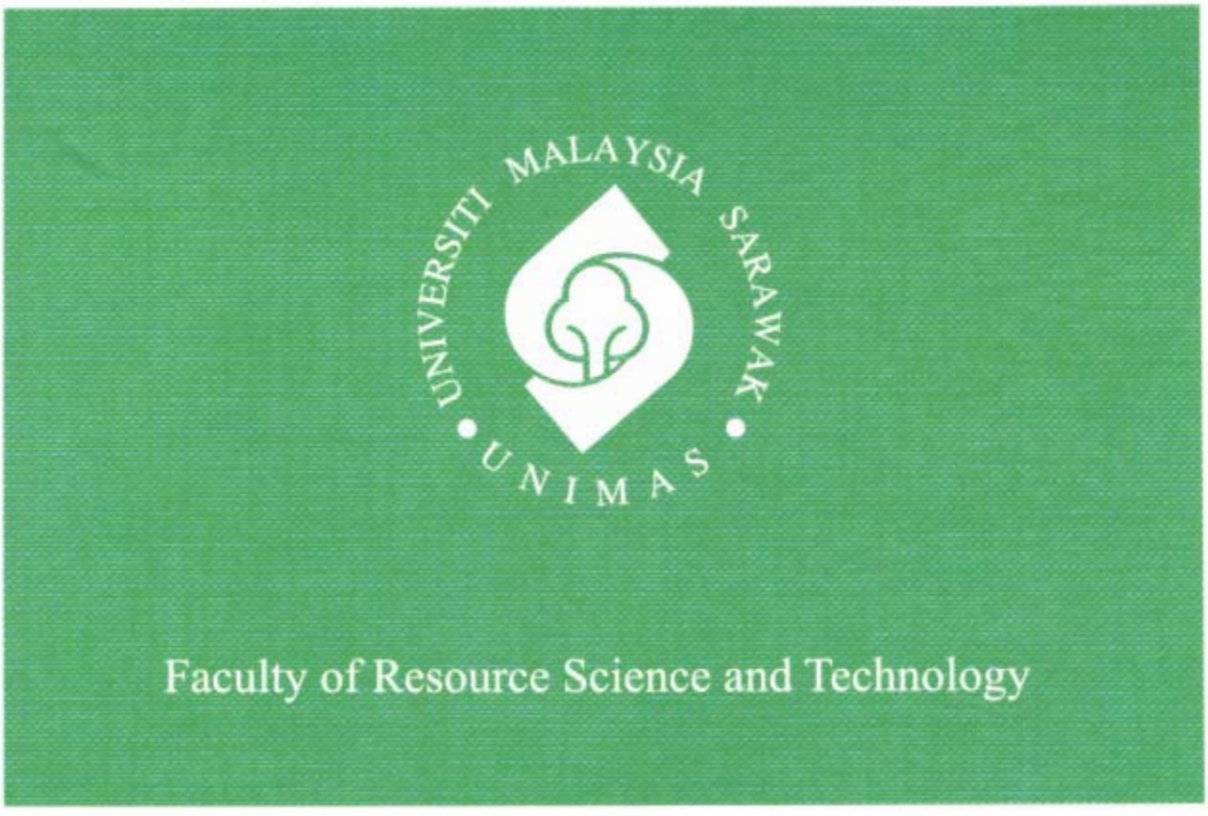

SCREENING FOR ANTIMICROBIAL ACTIVITIES OF ORGANOTIN (IV) DERIVATIVES OF THIOCARBOHYDRAZINE AND VITAMIN K_3-2-HYDRAZINOPYRIDINE

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List of Abbreviations

AIDS: Acquired Immune Deficiency Syndrome

CFU: Colony Forming Unit

DMF: Dimethyl fluoride

DMSO: Dimethyl sulfuroxide

HIV: Human Immunodeficiency Virus

MHA: Mueller Hinton Agar

MHB: Mueller Hinton Broth

MIC: Minimum Inhibition Concentration

MRSA: Methicillin Resistant *Staphylococcus aureus*

SARS: Severe Acute Respiratory Syndrome

TBTO: Tributyltin (IV) oxide

VISA: Vancomycin Intermediate *Staphylococcus aureus*

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Screening for Antimicrobial Activities of Organotin (IV) derivatives of Vitamin K_3-2-hydrazinopyridine and Thiocarbohydrazine

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ABSTRACT

Organotin (IV) compounds have been known for their biological properties. such as antibacterial, antitumour and antifungal activities. The present study examines the antibacterial activities of fourteen new organotin (IV) complexes, which are derived from thiocarbohydrazine and vitamin K_3-2-hydrazinopyridine ligands. Antibacterial assays done on these compounds were accomplished by applying the disc diffusion method. The results showed that the organotin (IV) derivatives of thiocarbohydrazine, which were diphenyltin (IV), dibutyltin (IV) complexes and the ligand, thiocarbohyrazine were able to inhibit the growth of the Gram-positive bacteria, *Staphylococcus aureus*, but not the Gram negative bacteria, *Salmonella typhi* and *Escherichia coli*. On the other hand, the addition of vitamin K_3-2-hydrazinopyridine ligand to the organotins was found to modify its antibacterial activities. In comparison, monophenyltin (IV), monobutyltin (IV) and dibutyltin (IV) compounds of vitamin K_3-2-hydrazinopyridine were found to inhibit the growth of Gram-positive bacteria, *Staphylococcus aureus*. In contrast, diphenyltin (IV) complex of vitamin K_3-2-hydrazinopyridine exerted antibacterial effect against Gram-negative bacteria, *Salmonella typhi* and *Escherichia coli*. However, upon storage vitamin K_3-2-hydrazinopyridine and its organotin (IV) derivatives will lost their antibacterial effect. As the study was preliminary, further investigation of organotin (IV) with thiocarbohydrazine derivatives on other Gram-positive bacteria is required to confirm its unique ability to inhibit Gram-positive bacteria. Moreover, anti-tumour effects of these novel compounds should be studied in detail.

Keywords: Organotin (IV) compounds, vitamin K_3-2-hydrazinopyridine, thiocarbohydrazine, disc diffusion, antibacterial, cytotoxicity

ABSTRAK

Kompleks organotin (IV) telah dikenali secara umum, kerana ia mempunyai sifat-sifat biologi seperti aktiviti anti-bakteria, aktiviti anti-kanser dan aktiviti anti-fungi. Projek telah menjalankan ujian antimikro ke atas bakteria dengan empat belas kompaun organotin (IV) yang dihasilkan oleh Jabatan Kimia, FSTS, UNIMAS. Monobutyltin (IV) dan monophenyltin (IV) dengan vitamin K_3-2-hydrazinopyridine dapat merencatkan pertumbuhan bakteria Gram-positif, Staphylococcus aureus. Selain itu, kompleks diphenyltin (IV) juga mempunyai kesan perencatan ke atas bakteria Gram- negative, Salmonella typhi dan Escherichia coli. Tetapi, penyimpanan kompleks yang berasal daripada vitamin K_3-2-hydrazinopyridine dari semasa ke semasa didapati tidak stabil. Walaubagaimanapun, organotin (IV) yang dihasilkan daripada thiocarbohydrazine didapati mengandungi kesan perencatan yang spesifik terhadap Staphylococcus aureus. Dengan itu, penyelidikan kompaun tersebut secara lanjut haruslah dijalankan ke atas bakteria Gram-positif yang lain untuk menentukan keupayaannya untuk merencat pertumbuhan bakteria Gram-positif. Di samping itu, saringan organotin (IV) kompleks dengan thiocarbohydrazine terhadap sel normal dan sel kanser perlu dijalankan dan mechanism untuk membunuh sel kanser harus dikajikan.

Kata kunci: Kompaun organotin (IV), vitamin K_3-2-hydrazinopyridine, thiocarbohydrazine, disk diffusi, antibakteria, sitotoksiksiti

1.0: Introduction

An organotin (IV) compound is an organometallic compound. It is characterized by the presence of at least one covalent C-Sn bond. Organotin compounds can be classified as mono-, di-, tri- and tetraorganotin, depending on the number of alkyl (R) or aryl (Ar) moieties attached to the tin metal (Hadi *et al.*, 2009). The organotin (IV) compounds continue to be of interest in research, due to their biological properties such as antibacterial, antitumor and antifungal activities. Besides, it is also able to accommodate functional groups such as carboxylate and amides, exhibit different coordination number various from four to six, which can accept electrons that are donated by the ligand (Bhatti *et al.*, 2005).

Organotins have found applications in various fields or human activities. In industry and agriculture, the uses of organotins have been increasing steadily in the last 20 years. Organotins are used as stabilizers for plastics and paints. Moreover, it also acts as industrial catalysts in the manufacture of polyurethane foam such as dibutyltin diacetate. It is also play crucial role in textile and wood preservation such as tributyltin oxide, TBTO (Gitliz *et al.*, 1991). Organotins also being used as agrochemical to combat numerous types of fungal diseases in plant crops especially leaf spot on the sugar beat and celery (Blunden *et al.*, 1987). For instance, triphenyltin hydroxide and triphenyltin acetate have been successfully utilized in specified agriculture fields. Both of these compounds are class specific, hence they are less harmful to non-targeted organisms and easily degraded in the environment when exposed to UV light.

In addition to industry and agriculture, organotins do have also applications in biomedicinal areas, such as amaebicide, fungicide and bactericide and some of them are potential candidates for cancer chemotherapy (Saxena, 1987).

attributed the attachment of organic groups, R or Aryl to the tin atom as the determining factor of biological activity. In contrast, the inorganic radicals, X have been found to have very little contribution in enhancing their toxicity. Thus, the biological role of X is unclear and needed to be further studied.

Based on previous studies, it had suggested that the biological activity of organotin (IV) compounds may depend on the number of organic groups that are attached to the tin. Besides, the interaction of ligand that usually consists of N-donor or S-donor with the tin such as Sn-N, Sn-O and Sn-S bonds, or termed as metallation also able to enhance the toxicity of organotins. Metallation may contribute to the detachment of active bioorganotin, $RnSn^{2+}$ from the complex and across the membrane to the interior of an organism (Prof Ismail bin Ahmad, *pers com*).

Recently, new series of synthetic organotin (IV) have been synthesized by the Chemistry department of FRST, UNIMAS (Dr. Md Abu Affan, *pers com*). The organotin compounds contain either vitamin K_3-2-hydrazinpyridine or thiocarbohydrate ligand. The present work will focus on the coordinating behaviour of vitamin K_3-2- hydrazinopyridine and thiocarbohydrate in connection with the peculiar properties of their metal complexes, and subsequently examining antimicrobial activities of different organotin (IV) complexes. Therefore, the **main objectives** of this project are:

1) To screen for antibacterial activity of series of organotin (IV) complexes that are derived from thiocarbohyrazine on bacterial strains
2) To screen for antibacterial activity of series of organotin (IV) compounds with vitamin K_3-2- hydrazinopyridine on bacterial strains

2.0 Literature Review

2.1: Infectious diseases

Infectious diseases are one of the leading causes of death worldwide, accounting for 13.3 million deaths (25% of all deaths) in 1998 (WHO, 1999). Since 1973, malaria and cholera that in more virulent form have spread geographically (Center for Disease Control and Prevention, 1998). By the same time, more than 30 new diseases agents have been identified. In 1980s, *Escherichia coli* 0157 have caused numerous diseases outbreak and deaths associated with contaminated food and water. The World Health Organization estimated in the year 2000, 1.9 million children died worldwide of respiratory infections with 70% of these deaths occurring in Africa and Asia. The same organization also estimated that tuberculosis was responsible for about two million deaths in 2002 and that one in three of the world's population was infected (Graham,2005).

While in early 2003, severe acute respiratory syndrome (SARS) is an epidemic pneumonia that has been spread worldwide (Poutanen *et al.*, 2005) and it is caused by a novel human coronavirus, SARS-CoV (Rota *et al.*, 2003). Nevertheless, the reasons for the increase in incidence of infectious diseases are not fully understood. This may due to antibiotic resistant, changes in human demographics and behavior, international travel and breakdown of public health measures (Institute of Medicine, 1992).

2.2: Antibiotic Resistance

During the past two decades, new infectious diseases have appeared and old ones previously thought could be controlled also have reemerged. New and reemerged infectious agents continue to pose serious health threats into the 21st century. The danger of new and reemerging infections is compounded by the increase in antibiotic-resistant bacteria (Institute of Medicine, 1998). Antibiotics discovery and development had been

exponential since the 1940s, but no new clinically useful structures were discovered after 1961, and almost the drugs are only undergone chemically modification.

Basically, there are five biochemical mechanisms that can protect bacteria from being killed by antimicrobials. The mechanisms are target receptors of the antimicrobials in the bacterial cells are altered, the ability of antibiotics entry into the cells decreases and increasing removal of the antimicrobials from the cells. The antimicrobials are also being destructed or inactivated and synthesis of new metabolic pathways in the bacterial cells which are not able be inhibited by antibiotics (Neu *et al.*, 1996).

Both Gram-positive and Gram-negative bacteria produce β-lactamase. The β-lactamase able to enzymatically cleaves the four membered β lactam rings, rendering the antibiotic inactive (Livermore, 1995). For instance, during the 1980s and 90s, the methicillin resistant *Staphylococcus aureus,* MRSA which has been emerged and increased in frequency as the hospital pathogen in many countries (Voss et al., 1994). Besides, this bacterium also shows trend of decreased susceptibility to vancomycin (Vancomycin Intermediate *S.aureus*, VISA) in Japan and United States recently.

Staphylococcus aureus is Gram-positive and coagulase-producing cocci. It is responsible for cutaneous infections such as boils and carbuncles, food poisoning, scalded skin syndrome and toxic shock syndrome. In addition to destruction of antibiotics, clinical staphylococci also shows elevated degree of resistance towards marcolide-lincomycin. This phenomenon is due to the biochemical changes such as methylation in the 50s ribosomal subunit RNA of the bacteria, which reduces the binding efficiency of lincomycin to the ribosome.

2.3: Causes of antibiotic resistance

Usually, development of antimicrobial resistance in bacteria is due to acquisition and spread of the resistance genes that are carried by the chromosomal or plasmid DNA, or known as plasmid-mediated resistance mechanism (Neu *et al.*, 1996). Bacteria able to transfer those DNA containing resistance genes to other bacteria through conjugation, transduction or transformation processes. Transferable resistance has been recognized since 1959, when resistance genes found in *Shigella* spp. transferred to *E. coli* via plasmids (Neu *et al.*, 1996).

However, the range of bacteria that are adopting plasmid-mediated mechanism is often limited, thus transposons are important in spreading resistance genes across such boundaries. Transposons are the genes which contain insertion sequences at each end. The insertion sequences allow the genes to move to different locations such as from plasmid to plasmid or from chromosome to plasmid (Manus, 1997). The *mecA* gene found in MRSA may well have been acquired by transposition (Grubb, 1998).

Microbial development of resistance towards antibiotics has also been postulated to be due to the widespread use of antibacterial drugs. According to Working Party of the British Society (1994), doctors in hospitals often encountered with difficulty of selecting the proper anti-infective drugs empirically, insufficient use of microbiological information and fear of litigation will prompt the use of broad spectrum of drugs. Furthermore, patients often fail to finish the full course of antibiotic treatment. This has lead to failure of eliminating the disease agent completely. As a result, those bacteria with some insensitivity may survive and grow as resistant population (Grubb, 1998).

Since there are many factors have been contributed to the rapid mechanisms of acquiring resistance in bacteria, thus there is a need to discover new antimicrobial agents with higher potency towards antibiotic resistant bacteria.

2.4: High rate of cancer occurrence

Cancer is one of the diseases that have been contributed to the elevated death globally and it is one of the most challenging research fields. Cancer is the world's second biggest killer after cardiovascular disease (WHO, 2009). WHO also states that, at least seven million people die from cancer, more than HIV/AIDS, malaria and tuberculosis combined each year. Death from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030 (WHO, 2009).

Occasionally, cancer disease is characterized by the uncontrolled and abnormal division of eukaryotic cells to form tumour or neoplasm. They become "rogue cells" and often lose the specialized characteristics that distinguish one type of cell from another, termed as loss of differentiation. Tumours are of two types, which are benign and malignant. If the cancer is localized, it is categorized to be benign. If the cancer cells invade other parts of the body and set up secondary tumours, a process known as metastasis or defined as malignant (Graham, 2005).

Treatments that are used to combat cancer usually include surgery, chemotherapy and radiation therapy. However, due to development of tumor cell resistance, some chemothepeutic agents are unsuccessful to inhibit the growth of tumours. Moreover, antitumor drugs such as cisplatin usually are accompanied with a set of severe side effects (Galanski *et al.*, 2007). Therefore, discovery of new series of synthetic compounds with minimum side effect and with high potency to kill cancer cells, are in demand.

2.5: Drug design for optimizing target interactions

Drug design has a crucial role in the field of medical chemistry. There are various aims in drug design such as the designed drug should has a good selectivity and level of activity for its target, and has a minimal side effect. Besides, it should be easily synthesized and

chemically stable. Finally, the drug should has acceptable pharmacokinetic properties and be non-toxic (Graham, 2005). Pharmacokinetic properties are referred to the drug's ability to reach its target and to have an acceptable lifetime.

There are many strategies in drug design that recently are applied in medical chemistry industries to optimize the interactions of a drug with its target to allow higher activity and selectivity. For instance, variation of substituents that includes alkyl and aromatic substituents is a common method of tuning the binding interactions of a drug. Usually, organotin (IV) compounds possess alkyl groups that are interacting with a hydrophobic pocket in the binding site of the particular organism. Hence, by varying the length and bulk of the alkyl groups allow them to probe the depth and width of the pocket (Acharya, 2003).Choosing a suitable substituent that will definitely fill the pocket will then increase the binding interaction (Figure 1).

On the other hand, if a drug contains an aromatic ring, the position of substituents can be varied to find better binding interactions, resulted in increased activity. Position of a substituent is subjected to changes such as at the *para* position and *meta* position on the aromatic ring able to vary the biological activity of the particular drug in figure 2 (Graham, 2005).

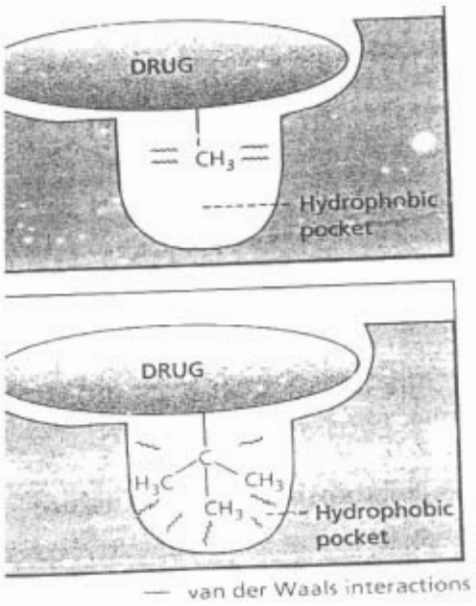

Figure 1: Variation of alkyl chain to fill a hydrophobic pocket (Graham, 2005)

For example, the OH group that is attached to the *para* position will make the phenyl ring a weaker base and the aromatic ring is less liable to be protonated. Thus, it decreases the aromatic ability to interact with ionic binding groups in the binding site and subsequently decreases activity. However, if the OH group is attached to the *meta* position of the aromatic ring, it able to form a strong hydrogen bond with the phenyl. Thus the binding interaction is vice versa. Besides, the effectiveness of a drug also depends on the steric, hydrophobic and electronic properties of substituents that are bonded with it. The therapeutic property of a synthetic compound can be improved by having a more electron withdrawing substituent, such as a chloro substituent (neutrophilic group) is replaced by a methyl substituent (electrophilic group) (Meyer *et al.*, 1995).

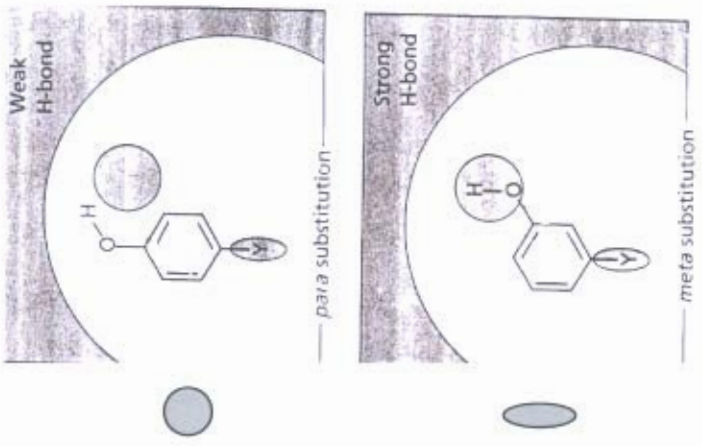

Figure 2: Aromatic substituents at *para* and *meta* position (Graham, 2005)

Furthermore, extension of the structure is also one of the popular strategies in drug design. This strategy involves the addition of other functional groups to the lead compound in order to probe for extra binding interactions with the target in figure 3 (Acharya, 2003). For example, the addition of alkyl or aryl groups to the Tin, Sn^{4+} and organotin (IV) compounds are formed. Consequently, such compounds are added to functional groups such as amide that present in the vitamin K_3-2-Hydrazinopyridine ligand to form therapeutic agents.

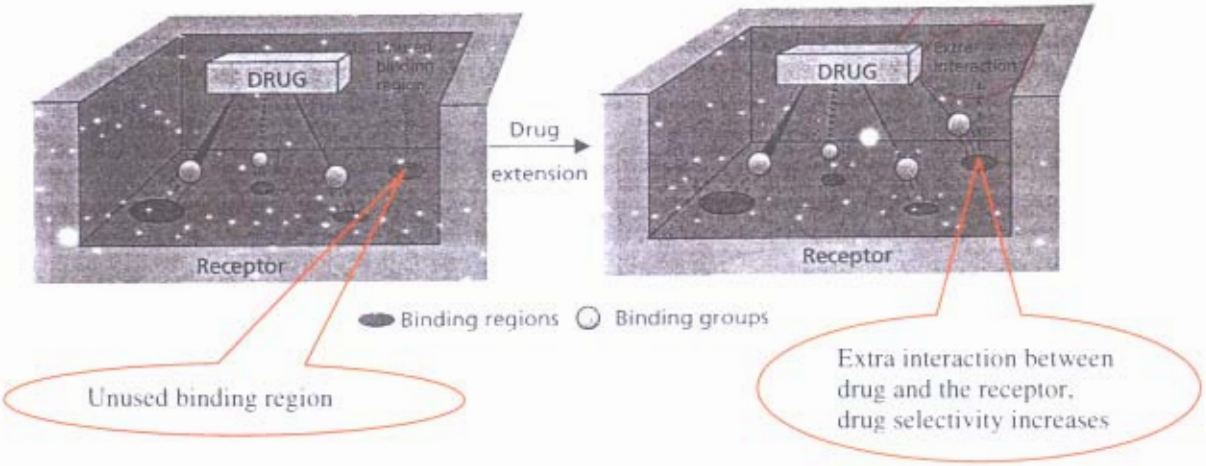

Figure 3: Extension of a drug to provide a fourth binding group (Graham, 2005)

2.6: Biological active components of organotin (IV) compounds

Based on previous studies, various interaction of Schiff base ligand with organotin (IV) compounds can exhibit useful biological activity (Akhbar *et al.*, 1999). Thus, organotin (IV) compounds can be categorized as pharmaceutical agent. The toxicity of organotin (IV) compounds is determined by the number and nature of the organic substituents, because the organic group, R can determine the potential activity (Snoeij *et al.*, 1987).

In general, triorganotins and diorganotins (IV) compound consist of highest toxicity. Triorganotins are exploited for their fungicidal, bactericidal and algicidal properties in manufacture and agriculture industries. Tributyltin is usually used in antifouling paints that coats structures exposed to aquatic environment including ships, sea walls and pleasure boats (Cooney *et al.*, 1994).Whereas, triphenyltins act as fungicides to protect crops, including potato, celery and rice (Fent, 1996b).

Researches had shown that there are certain dialkyltin compounds may have a role in cancer chemotherapy (Barbieri *et al.*, 1989). A series of diorganotin dihalides complexes with monodentate or bidentate such as N-donor ligands, are resemblance to the cis-platin and has been screened against P388 lymphocytic leukaemia in mice (Gilelen, 1996). The N-ligand donor able to enhance transportation of the drug into cells and that antitumour activity arises from the R_2Sn species (Barbieri *et al.*, 1989). In the active Sn complex, the average Sn-N bond length of the organotin (IV) complex is more than 2.39 Å, which means it is a relative weak bond (Pellerito *et al.*, 2002). Therefore, the compound able to release the bioactive species, $RnSn^+$ and express biological activity and that pre-dissociation mechanism is important for the antitumour activity.

2.7: Comparison between ligands, Vitamin K_3-2-Hydrazinopyridine and Thiocarbohydrate

Comparisons among the chemical and physical properties of the two ligands are important (Table 1), because these characteristics able to contribute to the strength of antibacterial effect against the bacterial strains.

The principle of molar conductivity is based on the dissolved ability of a particular compound in a hyper polar solution such as DMF and DMSO to produce delocalized electrons which able to conduct electricity. Thus, if the value of molar conductivity, $\Omega^{-1}cm^2mol^{-1}$ of the compounds is high, it indicates the power of dissociation of the compounds in the hyper solution is strong. Hence, there are more free electrons be generated to conduct electricity. Nevertheless, molar conductance value of the compounds which in the range from 1 to 50 Ω^{-1} cm^2mol^{-1} can be considered as non-electrolytes.

Based on the table, the value of molar conductivity of Vitamin K_3-2-hydrazinopyridine is $9.71\Omega^{-1}cm^2mol^{-1}$, whereas the molar conductance value of thiocarbohydrazine is 8.15 $\Omega^{-1}cm^2mol^{-1}$. The value shows that thiocarbohydrazine is more stable than Vitamin K_3-2-Hydrazinopyridine, since its dissociation strength is weaker than Vitamin K_3-2-Hydrazinopyridine (Dr.Abu Affan, *pers com*). Metallation of vitamin K_3-2-hydrazinopyridine with Tin, Sn^{4+} atom able to exert hexacoordinated organtin (IV) complexes, while penta-coordinated organotin (IV) complexes are formed when the thiocarbohydrazine is cohered to the Tin, Sn^{4+}atom (Figure 1 and Figure 2). Furthermore, the coordinating behaviour of the ligands with the organotin (IV) complexes also leads to different geometry exhibition. Organotin (IV) derivatives of vitamin K_3-2-Hydrazinopyridine possesses octahedral geometry, but organotin (IV) complexes that are derived from thiocarbohydrazine have trigonal bypiramid geometry.

Table 1: Comparison of chemical and physical characteristics of **Vitamin K_3-2-Hydrazinopyridine and Thiocarbohydrate**

Characteristics	**Vitamin K_3-2-Hydrazinopyridine**	**Thiocarbohydrazine**
Electron donor to the Tin atom	N-donor	S-donor, O-donor and N-donor
Coordinated behavior with the tin atom	Hexa- coordinated	Penta-coordinated
Geometry exhibited with organotin (IV) complexes	Octahedral	Trigonal bypyramid
Available aromatic groups	three phenyl rings	one phenyl group
Available delocalized Hydrogen, H^+	More	Less
Colour	Dark red	Milk yellow
Melting point	196-198 ° C	194-195° C
Molar conductivity in DMF , Ω^{-1} cm^2mol^{-1}	9.71	8.15

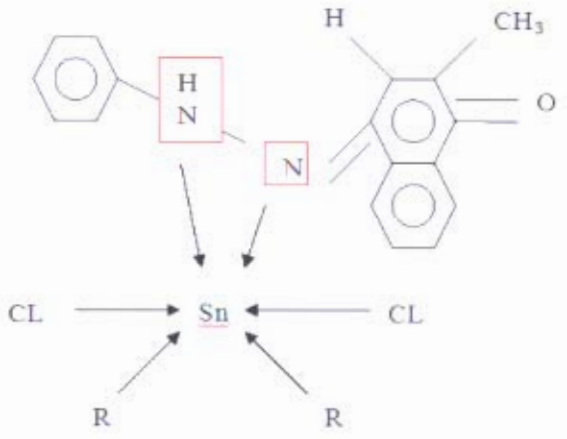

Figure 4: Structure of vitamin K_3-2-hydrazinopyridine. N-N-donor is hexa-coordinated to the organotin (IV) compound

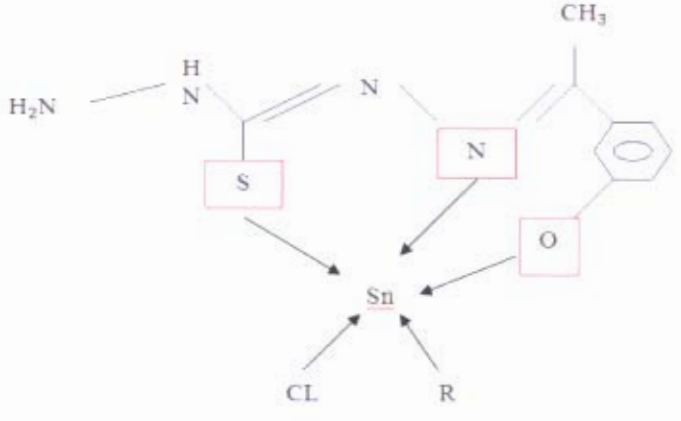

Figure 5: Structure of thiocarbohydrazine. N-donor, S-donor and O-donor are penta-coordinated to the organotin (IV) compound

3.0 Materials and Methods

3.1: Chemical compounds

There were twelve organotin (IV) compounds and two ligands, Vitamin K_3-2-hydrazinopyridine and thiocarbohydrate used in this project (Table 1). They were provided by Assoc. Prof. Dr. Md Abu Affan from Chemistry Department of FRST, UNIMAS.

Table 2: Codes that represent the organotin (IV) compounds

Organotin (IV) derivatives of Vitamin K_3-2-hydrazinopyridine		Organotin (IV) derivatives of Thiocarbohydrazine	
Vitamin K_3-2-hydrazinopyridine	L2	Thiocarbohydrazine	DNA-008
Dibutyltin (IV) complex	O1	Monomethyltin (IV) complex	DNA-009
Diphenyltin (IV) complex	O2	Monobutyltin (IV) complex	DNA-010
Monobutyltin (IV) complex	O4	Monophenyltin (IV) complex	DNA-011
Dimethyltin (IV) complex	O6	Dimethyltin (IV) complex	DNA-012
Monomethyltin (IV) complex	O7	Dibutyltin (IV) complex	DNA-013
Monophenyltin (IV) complex	O8	Diphenyltin (IV) complex	DNA-014

3.2: Tested Microorganisms

The pathogens that were used for assay were Gram-positive *Staphylococcus aureus* and Gram-negative pathogens were *Escherichia coli*, *Salmonella typhi* and *Enterococci aeruginosa*. The microorganisms were obtained from the culture collection of Faculty Science and Technology, UNIMAS.

3.3: Preparation of stock solution of organotin (IV) compounds

Organotin (IV) compounds were in powder form, thus it had to be weighted by electronic balancer to obtain four milligram. Subsequently, the powder was diluted with 10 µl of absolute DMSO and 990 µl of MHB to 1 ml of stock solution. Later, 0.5 ml of the stock solution was transferred into an eppendroft tube with 0.5 ml of MHB to form concentration at 200 µg/ml. Serial dilution was continued until concentration of 1.5625 µg/ml was obtained. Conversions of organotin (IV) compounds concentrations from µg/ml to mol/µl were required (Table 3 and Table 4).

3.4: Antibacterial test

Antibacterial test of pathogens was carried out by using disc diffusion method (Bauer *et al.*, 1966). The bacteria from stock culture were lightly inoculated into the MHB and let it grew overnight at 37 ° C in an ambient air incubator. The culture was diluted with a new MHB in order to achieve optical density value of 2.0×10^6 colony forming units (CFU/ml) or 0.168 at wavelength of 550 nm in the spectrophotometer. Later, dipped a sterile cotton swab into the broth culture and inoculated on the MHA.

Sterile filter paper discs (Whatmann No.1) with 6 mm diameter were placed on the agar in equal distance. Subsequently, 10 µl of aliquot organotin (IV) compounds at concentration of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 µg/ml that already diluted in 0.5% DMSO were dispensed individually to each of the discs. The agar plate was incubated at 37 ° C overnight. For each plate, antibiotic such as streptomycin and penicillin (10µg/disc) was plated at centre to act as positive control. DMSO mixture was used as negative control. The presence of inhibition zone on the plate was recorded and measured in diameter, mm. Thus, MIC of that compound could be determined based on the clear inhibition zone.

Table 3: Conversion of concentration from **µg/ml** to **mol/µl** of Organotin (IV) compounds with Vitamin K_3-2-Hydrazinopyridine

Compounds codes	**Conversion of concentration: µg/ml to mol/µl**							
	200	100	50	25	12.5	6.25	3.125	1.5625
L2	7.60x10^-10	3.80x10^-10	1.90x10^-10	9.51x10^-11	4.75x10^-11	2.38x10^-11	1.18x10^-11	5.94x10^-11
O1	3.53x10^-10	1.77x10^-10	8.83x10^-11	4.42x10^-11	2.21x10^-11	1.11x10^-11	5.53x10^-12	2.76x10^-12
O2	3.30x10^-10	1.65x10^-10	8.25x10^-11	4.13x10^-11	2.06x10^-11	1.03x10^-11	5.15x10^-12	2.58x10^-12
O4	3.69x10^-10	1.83x10^-10	9.17x10^-11	4.59x10^-11	2.29x10^-11	1.15x10^-11	5.72x10^-12	2.86x10^-12
O6	5.33x10^-10	2.67x10^-10	1.33x10^-10	6.67x10^-11	3.33x10^-11	1.67x10^-11	8.33x10^-12	4.16x10^-12
O7	4.15x10^-10	2.07x10^-10	1.04x10^-10	5.19x10^-11	2.59x10^-11	1.30x10^-11	6.48x10^-12	3.24x10^-12
O8	3.54x10^-10	1.77x10^-10	8.85x10^-11	4.42x10^-11	2.21x10^-11	1.11x10^-11	5.53x10^-12	2.76x10^-12

Table 4: Conversion of concentration from **µg/ml** to **mol/µl** of Organotin (IV) compounds with Thiocarbohydrazine

Compounds codes	**Conversion of concentration: µg/ml to mol/µl**							
	200	100	50	25	12.5	6.25	3.125	1.5625
DNA-008	6.06x10^-10	3.03x10^-10	1.52x10^-10	7.58x10^-11	3.79x10^-11	1.90x10^-11	9.47x10^-12	4.73x10^-12
DNA-009	4.02x10^-10	2.01x10^-10	1.01x10^-10	5.03x10^-11	2.52x10^-11	1.26x10^-11	6.29x10^-12	3.14x10^-12
DNA-010	3.70x10^-10	1.85x10^-10	9.23x10^-11	4.63x10^-11	2.31x10^-11	1.16x10^-11	5.79x10^-12	2.90x10^-12
DNA-011	3.57x10^-10	1.79x10^-10	8.93x10^-11	4.46x10^-11	2.23x10^-11	1.12x10^-11	5.58x10^-12	2.79x10^-12
DNA-012	4.19x10^-10	2.10x10^-10	1.05x10^-10	5.24x10^-11	2.62x10^-11	1.31x10^-11	6.55x10^-12	3.28x10^-12
DNA-013	3.57x10^-10	1.78x10^-10	8.91x10^-11	4.46x10^-11	2.23x10^-11	1.11x10^-11	5.57x10^-12	2.79x10^-12
DNA-014	3.33x10^-10	1.66x10^-10	8.32x10^-11	4.16x10^-11	2.08x10^-11	1.04x10^-11	5.20x10^-12	2.60x10^-12