SYNTHESIS AND CHARACTERIZATION OF NOVEL POLYAMINE SULPHUR ANALOGUES AND ITS EFFECTS ON CANCER CELL PROLIFERATION

BY

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ABSTRACT

Natural polyamines such as putrescine, spermidine, and spermine are crucial for cell growth and proliferation. However, over accumulation of polyamines can lead to the worst scenario which is the progression of cancer cells. The upregulation of polyamine transport system (PTS) activity leads to an opportunity to develop more specific and effective cancer treatment. Researches in compound containing sulphur and the polyamine transport system is beneficial in oncology as both affect cancer cells. Compounds containing sulphur such as organo sulphur are known for its anti-cancer properties. Recent studies revealed, several new compounds were successfully synthesized to combat cancer cells. Some of the compounds that were successfully synthesized were the polyamine-sulphur compounds. By adding sulphur to known natural polyamine, the analogues will be delivered specifically to cancer cells via polyamine transport system (PTS). For the synthesis of the compound, carbon disulphide and benzyl chloride were added to the polyamine (Putrescine or Spermidine) via a method called Room Temperature Mediated Synthesis. The addition of carbon disulphide was to add the sulphur element while the addition of benzyl chloride was to make the compound bulkier and stable. The analogues were named and categorized based on the starting material. The analogues named PSA-1 and SSA-1 are the analogues that were created by synthesizing the starting materials (putrescine and spermidine) with carbon disulphide only. For the addition of both carbon disulphide and benzyl chloride, the analogues were named PSA-2 and SSA-2. The resulting analogues were analyzed by using Fourier Transformed Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectroscopy (GC-MS) to determine the successful addition of sulphur and benzyl compound. In addition, the compounds were then tested with human lung adenocarcinoma cells (A549), human breast adenocarcinoma cell (MCF-7) and human colorectal adenocarcinoma cells (HCT-8). The cytotoxicity effects of these compounds were determined by using MTT assay technique against these in-vitro cells. The results show the cytotoxic effects were not potent against these cell lines as a higher concentration of compounds were needed to inhibit the cells growth.

خلاصة البحث

تعتبر عديدات الأمين الطبيعية مثل البوتريسين والسبيرميدين والسبيرمين ضروريةً لنمو الخلايا وتكاثرها، ولكن تراكم عديدات الأمين المفرط قد يؤدي إلى أسوأ السيناريوهات وهو تطور الخلايا السرطانية، ولتطوير علاج أكثر تحديدا وفعالية ضد السرطان فإن ذلك قد يكمن في تنظيم نشاط نظام نقل البوليامين (PTS). تعتبر الأبحاث في المركبات المحتوية على الكبريت ونظام نقل عديدات الأمين مفيدة في علم الأورام حيث يؤثر كلاهما على الخلايا السرطانية. المركبات المحتوية على الكبريت مثل الكبريت العضوي معروفةٌ بخصائصها المضادة للسرطان. كشفت الدراسات الحديثة أن العديد من المركبات الجديدة قد تم تصنيعها بنجاح لمكافحة الخلايا السرطانية، بعض تلك المركبات الناجحة هي مركبات عديدة الأمينات–الكبريت. إضافة الكبريت إلى عديدات الأمين الطبيعية المعروفة سيسلم النظائر مباشرة إلى الخلايا السرطانية عبر نظام نقل عديدة الأمينات. لتركيب المركب تمت إضافة ثاني كبريتيد الكربون وكلوريد البنزيل إلى عديدات الأمينات (البوتريسين أو السبيرميدين) عبر طريقة تسمى التركيب المحكم بدرجة حرارة الغرفة. إضافة ثاني كبريتيد الكربون كانت لإضافة عنصر الكبريت بينما إضافة كلوريد البنزيل كانت لجعل المركب أكبر حجمًا واستقرارا. تم تسمية النظائر وتصنيفها إلى فئات بناءً على المادة الأولوية. النظائر المسماة PSA-1 و SSA-1 هي النظائر التي تم إنشاؤها من خلال توليف المواد الأولية (بوتريسين وسبيرميدين) مع ثاني كبريتيد الكربون فقط، ومع إضافة كل من ثاني كبريتيد الكربون وكلوريد البنزيل تمت تسمية النظائر بـ PSA-2 و SSA-2. تم تحليل النظائر الناتجة باستخدام مطيافية الأشعة تحت الحمراء باستخدام تحويل فورييه (FTIR) والكروماتوغرافيا الغازية (GC-MS) لتحديد الإضافة الناجحة لمركب الكبريت والبنزيل. بالإضافة إلى ذلك، تم اختبار المركبات بعد ذلك باستخدام خلايا سرطان الغدة الرئوية البشرية (A549)، وخلايا سرطان الثدي البشري (MCF-7)، وخلايا سرطان القولون والمستقيم البشرية (HCT-8). تم تحديد تأثيرات السمية الخلوية لهذه المركبات باستخدام طريقة فحص MTT ضد هذه الخلايا في المختبر. أظهرت النتائج أن التأثير السمي لم تكن فعالة ضد خطوط الخلايا السرطانية حيث كانت هناك حاجة إلى تراكيز أعلى من المركبات لتثبيط نمو الخلايا.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Masters of Biomedical Science.

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Radiah Abdul Ghani
Supervisor

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Throughout history, numerous diseases and illnesses such as the smallpox and diabetes are a threat to human society. One of the major diseases is cancer. According to the World Health Organization (2017), in 2015, cancer had caused 8.8 million deaths globally. The most lethal which is lung cancer (along with trachea and bronchus cancers) had caused 1.7 million deaths which is the 5^{th} most caused death worldwide. Various new methods and drugs on ways to prevent as well as to cure, treat and reduce the effects of cancer have been discovered and researched. However, most of the drugs, artificial and natural, have been known to cause one or several side effects towards the patient while some may cause the cancer patient to undergo painful treatments (Komarov *et al.*, 1999).

Despite most anticancer drugs possess potent cell killing activity *in vitro* studies; the selective delivery of drugs to cancer cells is thus far becomes a major challenge to oncology studies. The non-specific actions of drugs on healthy cells cause many restrictions in clinical use due to their systemic toxicity. In response to this matter, specific targeting of anticancer drugs towards cancer cells can be achieved by attaching them to a molecule that is transported into cancer cells via a selective transport system. One strategy to achieve this selective delivery is by exploiting specific transport mechanisms such as the polyamine transport system (PTS). The PTS is an energy-dependent machinery frequently over activated in cancer cells with a high demand for

polyamines. Cancer cells differ from healthy cells by the amount of polyamine. In cancer cells, the amount of polyamine is higher as it needs more polyamine to grow (Thomas and Thomas, 2003). By using the PTS, it is hoped that the anti-cancer compound can be transferred to the targeted cells and minimize the harm to healthy cells.

1.2 PROBLEM STATEMENT

Although most recent anti-cancer drugs are known to minimize the growth of cancer cells, it still causes side effects towards the patients. Several cases have shown that the current anti-cancer drugs have cause one or more side effects such as nausea, mouth soreness, vomiting, loss of appetite, constipation and diarrhea. The side effects were due to the drug's reaction towards healthy cells. This shows that the current anti-cancer drugs are lacking specificity.

1.3 RESEARCH QUESTIONS

i. Can a polyamine-sulphur conjugate be successfully synthesized using putrescine, spermidine and spermine?

ii. What is/are the method used to optimize the synthesis of polyamine-sulphur conjugates?

iii. What are the physical and chemical characteristic of the newly synthesized polyamine-sulphur conjugates?

iv. Does the polyamine-sulphur conjugates induce cytotoxicity against various cancer cell lines?

1.4 AIM AND OBJECTIVE

The main aim of this study is to synthesize polyamine-sulphur conjugates and determine its cytotoxic effect on various cancer cell lines.

The specific objectives are:

i. To synthesize polyamine-sulphur conjugates using putrescine, spermidine and spermine as starting materials.

ii. To optimize the synthesis process based on two methods which are Cold Temperature Mediated Synthesis and Room Temperature mediated Synthesis for the synthesis of polyamine-sulphur conjugates.

iii. To ascertain the physical and chemical characteristics of the polyamine-sulphur conjugates using Solubility Test, Melting Point Determination, FT-IR, GC-MS, and TLC techniques.

iv. To determine the cytotoxicity effect of the polyamine-sulphur analogue against selected cancer cell lines.

1.5 RESEARCH HYPOTHESES

i. Successfully synthesized polyamine-sulphur analogues using natural polyamines with carbon disulphide.
ii. High yield of conjugates is produced using cold temperature and room temperature mediated synthesis.
iii. The chemical and physical characteristic of the polyamine-sulphur analogues are determined and recorded.
iv. The polyamine-sulphur compound shows cytotoxic effect against selected cancer cell line.

1.6 SIGNIFICANCE OF STUDY

The polyamine – sulphur conjugates produced by the synthesis process will be a potential anti-cancer drug that might have the ability to minimize the side effect by increasing the drug delivery specifically into cancer cells by using known delivery pathway which is the polyamine transport system.

CHAPTER TWO

LITERATURE REVIEW

2.1 CANCER AND THE EPIDEMIOLOGY

Cancer or malignant tumor is a term for addressing a group of illnesses involving abnormal growth of cells that has the potential to infect and affect other organs in the human body (Deng et. al., 2020). According to the American Cancer Society, cancer have been around for centuries. The oldest realized case of cancer dates back to about 300BC where evidence of cancer is found among fossilized bone tumors, human mummies in ancient Egypt (American Cancer Society, 2014). Cancer works by targeting healthy cell in a multistage process where it starts from pre- cancerous lesion to malignant tumors. The changes of the stages is the result of the interaction between a person's genetic factors with three categories of external agents including; physical carcinogens, such as ultraviolet and ionizing radiation; chemical carcinogens, such as asbestos, components of tobacco smoke, aflatoxin and arsenic; and biological carcinogens, such as infections from certain viruses, bacteria or parasites (World Health Organization, 2015). As of 2014, the World Health Organization's (WHO) International Agency for Research on Cancer (IARC) has identified more than 100 chemicals, physical, and biological carcinogens where most of it were recognized for causing cancer (American Cancer Society, 2015). A researched by Tomasetti et al., (2017) however said that cancers are caused by mutations that may be inherited, induced by environmental factors, or result from DNA replication errors (R). This was concluded in his research where he finds the relationship between the number of normal stem cell divisions and the risk of 17 cancer types in 69 countries throughout the world.

In recent years, cancers have been the leading cause of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related death in 2012 (WHO,2015). In Malaysia, an estimate of 90, 000 - 100,000 people in Malaysia living with cancer at any one time (National Cancer Society Malaysia, 2015). According to the National Cancer Registry of Malaysia (NCR) in 2008, there are about 21,773 recorded cancer patients in Malaysia and almost 10,000 more cases that are not recorded each year. The National Cancer Society Malaysia also stated that the top five type of cancer recorded in Malaysia in descending order are breast cancer, colorectal cancer, lung cancer, cervical cancer and nasopharyngeal cancer. Lung cancer, of which non-small-cell lung cancer (NSCLC) is the most common form, remains the leading cause of cancer-related mortality worldwide, with many patients presenting with advanced disease at initial diagnosis (Deng et al., 2020). In 2010, the National Institutes of Health estimates that more than 1.5 million new cases of cancer will be diagnosed in the United States, with an overall projected cost of US$263.8 billion (Kennedy et al., 2011)

2.2 CARCINOGENESIS

The process when normal cells are transformed into cancer cells are called carcinogenesis or tumorigenesis (Tanaka, 2009). The characterization of the process is due to several developments which are changes at the cellular, genetic, and epigenetic levels and abnormal cell division. The progression is a physiological process that transpires in almost all tissues. Carcinogenesis transforms normal cells to malignant cells in three fundamental stages including tumor initiation, tumor promotion and tumor progression (Wenston and Harris, 2003). Tumor initiation stage begins with permanent genetic changes by chemical carcinogens. Chemical carcinogens case alteration towards

DNA molecular structures and cause significant mutations during DNA synthesis. Proto-oncogenes and tumor suppressor genes are the earliest susceptible genes undergoing changes in response to these insults. In consequence, mutated proto-oncogenes effect normal cell growth and proliferation whereas mutated tumor suppressor genes are unable to suppress carcinogenesis, resulting in unceasing cell division.

2.3 CHALLENGES IN CURRENT CANCER TREATMENT

Although they have been advances in cancer treatment, there are still several challenges in the treatment of cancer. one of the main challenges is to reduce the toxicity of the treatment towards normal cells. Current cancer treatment such as chemotherapy, are also harming surrounding healthy cells as well as the cancer cell (Komarova and Gudkov 2000, Senapati et al., 2018).

Another type of treatment that is used to combat cancer cell nowadays is the use of human epidermal growth factor receptor 2 (HER2) targeted therapies. Treatment that uses HER2 targeted therapies, have shown improvement in the outlook of early stage breast cancer patients. However, a significant proportion of these patients still relapse and die of breast cancer (Arteaga et al., 2011). This treatment was applied to patients as a monotherapy after completion of chemotherapy. This shows that the treatment is not effective in treating the cancer cells as it is not fully cured.

The detection methods for the presence of cancer is also one of the challenges in combatting cancer. The late detection of cancer has reduced the chances of survival for almost nearly 50% of cancer patients. Improvement in the early detection of tumours

and cancer cells can increase the chances of survival and early treatment for cancer patients. Early tumor detection with non-invasive imaging is proving increasingly promising for discriminating between indolent and aggressive tumors.

2.4 NEW APPROACH OF CANCER TREATMENT

Reflecting back on our knowledge on cancer and the causes, many new researches have been done in finding the causes and ways to treat and prevent cancer. New methods and drugs have been identified to fight and prevent cancer cells from spreading. One new approach in cancer treatment is by implementing evidence-based strategies, early detection of cancer and management of patients with cancer (World Health Organization, 2002). According to WHO (2015), by detecting cancer in its early stages, the patient will have a higher chance of survival and cure than later detection. One of the findings shows there are several ways to treat cancer. Some of the preferable method is by radiation therapy, surgery, chemotherapy and targeted therapy.

The most efficient method used nowadays is by implementing several of these methods at once. For radiation therapy and chemotherapy, one of its drawbacks is it will cause harmful side effect for patients while surgery may cause death. Therefore, one possible approach is by harnessing already establishes activity of known anticancer drugs by attaching them to a molecule which is transported into cancer cells via a selective transport system (targeted therapy).

A review by Zhang (2009), kinases have become one of the most intensively pursued classes of drug target with approximately 30 distinct kinase targets being developed to the level of a Phase I clinical trial. A majority of these kinases are being investigated

for the treatment of cancer. With approximately 518 kinases encoded in the human genome, virtually every signal transduction process is wired through a phosphotransfer cascade, suggesting that inhibition of kinase activity can elicit a real physiological response.

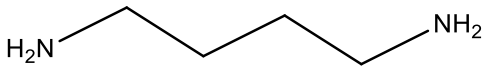
Another review paper by Arteaga *et al*. (2011), said that new approaches in battling HER2-positive early stage breast cancer are being developed some of these treatments are monoclonal antibodies and small-molecule tyrosine kinase inhibitors targeting HER2 or other HER family members, antibodies linked to cytotoxic moieties or modified to improve their immunological function, immunostimulatory peptides, and targeting the PI3K and IGF-1R pathways.

Nanotechnology-based cancer treatment approaches potentially provide localized, targeted therapies that aim to enhance efficacy, reduce side effects, and improve patient quality of life. Gold-nanoparticle-mediated hyperthermia shows particular promise in animal studies, and early clinical testing is currently underway (Kennedy et al., 2011).

2.5 WHAT IS POLYAMINES?

Polyamines are naturally occurring aliphatic compound which contain two or more anime groups. Some of the naturally produce polyamine are putrescine, spermidine and spermine (Figure 2.1). They are one of the oldest groups of substances known in biochemistry (Galston 1991). The study of polyamines was first intrigued by a researcher named Antoine van Leeuwenhoek when he discovered unknown crystals in semen in the year 1678 (Agrell, 1968). The observation was done using a primitive microscope and was reported to the newly formed Royal Society of London. It was not

until the 1920s that the chemical structure is determined by Rosenheim through synthesis. Subsequently a triamine (spermidine) and a diamine (putrescine; 1,4-diaminobutane) were isolated from prokaryotic and eukaryotic systems. It was not until 1958 however that the first published paper describing polyaminated compounds in cancer tissues. Many other publications and reviews on polyamine were done between the years 1960-1985. Within those years, more than 5000 papers and reviews were published, most of it about polyaminated small molecules and compounds. Based on those researches, it became clear that there were several natural biologically active polyaminated molecules in various form of life (Criss, 2003). One of the importance of polyamine is they are important for cellular growth. Natural polyamines such as spermidine are closely regulated by the cell according to the state of growth (Pegg and McCann 1982).



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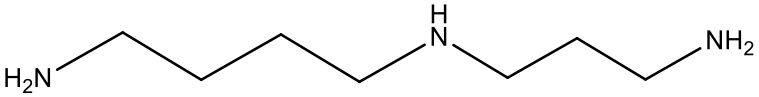

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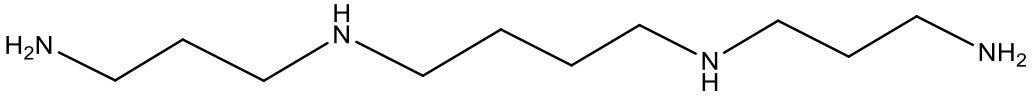

(C)

Figure 2.1: Structure of Putrescine (A), Spermidine (B), and Spermine (C)

2.6 POLYAMINES AND CANCER

Natural polyamines are low molecular weight aliphatic cations which are constituents of eukaryotic cells (Amendola et al., 2009). Polyamines are present naturally in all types of lifeform which include plants, animals, bacteria and humans. In mammals, natural polyamines exist in the form of putrescine, spermine and spermidine which are small naturally occurring aliphatic cations. These compounds are required in many physiological functions including stabilizing RNA and DNA (Rato et al., 2011), signal transduction and gene expression (Tsujinaka et al., 2011) cell proliferation and apoptosis (Igarashi et al., 1975; Marton & Pegg, 1995; Seiler & Raul, 2005), immunity (Seiler & Atanassov, 1994) and oxidative stress response (Cerrada-Gimenez, et al., 2011). Their intracellular levels are tightly controlled by machinery that allows rapid and efficient fluctuations in order to respond to specific needs and to avoid excessive accumulation (Phanstiel et al., 2007). This is vital since the increase in polyamine concentrations and deregulated polyamine metabolism is known to play an important role in the development of cancer at all stages (Martinez et al., 2003).

Thus far, because of its properties, polyamines have been a key interest in therapeutic application. These include cancer therapy and drug delivery. Based on earlier studies of polyamines, it is highly recognized that the concentration of polyamines affects the rate of cell growth in mammalian cells where in rapidly prolifering cells is the results of high concentration of polyamines (Wallace and Niiranen, 2007). Recent studies on these compounds have resulted in a variety of polyamine analogues and conjugates. Having similar structure to natural polyamines, these analogues efficiently enter the cells via the polyamine transport. At the same time, they have shown better specificity for cancer