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THE STUDY OF ANTICANCER PROPERTIES OF BENZYL-O-VANILLIN AND IT'S DERIVATIVES

By

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Thesis submitted in fulfillment of the requirement for the degree of Master of Pharmaceutical Science (MPharmSc.)

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Dedication

This dissertation is dedicated to my loving husband Dr Ammar Muallah for his friendship, enduring patience and support. He was my source of encouragement throughout this work and gave me the strength that kept me going. I also would like to dedicate this work to my beautiful daughter Maria who was born during my M.Sc.

This thesis is also especially dedicated to the many Iraqi cancer patients who suffered as a consequence of the use of depleted uranium weapons during the Iraqi war.

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Declaration

No portion of the work referred to in this thesis has been submitted in support for another degree or qualification of this or any other university, or institute of higher learning.

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List of abbreviation

2MP	2-(2-benzyloxy-3-methoxyphenyl)-1H-benzimidazole
2XP	N-1-(2-benzyloxy-3-methoxybenzyl)-2-(2-benzyloxy-3-methoxyphenyl)-1H-benzimidazole
3BS	(R) and (S)-1-(2-benzyloxy-3-methoxyphenyl)-2, 2, 2-trichloroethyl benzenesulfonate
Å	Angstrom
A.D.	Anno Domini
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
B.C.	Before Christ
Bn1	2-Benzyloxy-3-methoxybenzaldehyde
DMSO	Dimethyl Sulfoxide
DMEM	Dulbecco's Modification of Eagles Medium
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
G1	Gap 1 phase
G2	Gap 2 phase
M	Mitotic phase
MGB	Minor groove binder
Min.	minute
P35	tumor suppressor gene
PBS	Phosphate buffered saline
PMS	Phenazine methosulphate
RNA	Ribonucleic acid

S	Synthesis phase
UV	Ultraviolet
VB	Vogel-Bonner medium E
XTT	2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide

KAJIAN CIRI-CIRI ANTI-KANSER BENZYL-O-VANILLIN DAN TERBITANNYA

Benzyl-o-vanili dan nukleus benzimidazole adalah pharmacophore yang penting dalam penemuan ubatan kerana mempunyai kepentingan ubat yang signifikan. Oleh yang demikian, kita menerokai aktiviti anti-kanser daripada tiga sebatian iaitu 2 - (2-benzyloxy-3-metoksifenil)-1H-benzimidazole, 2MP, N-1-(2-benzyloxy-3-methoxybenzyl) -2 - (2-benzyloxy-3 -metoksifenil)-1H-benzimidazole, 2XP, dan (R) dan (S) -1 - (2-benzyloxy-3-metoksifenil) -2, 2, 2-trichloroethyl benzenesulfonate, 3B dan membandingkan aktiviti mereka dengan 2-benzyloxy- 3-methoxybenzaldehyde, (BN1) iaitu sebatian induk. 2XP dan 3B yang telah ditemui memberi sitotoksik yang selektif terhadap sel leukemia U937 menyebabkan kematian sel melalui apoptosis dengan mengaktifkan kegiatan caspase 9.

Kajian pengikatan DNA terutamanya dengan ujian titrasi keseimbangan pengikatan menunjukkan aktiviti pengikatan DNA dengan konstan pengikatan 7.39µM/bp dan 6.86µM/bp untuk 3B dan 2XP masing-masing. Kajian viskometri menunjukkan bahawa 3B dan 2XP mengikat terutamanya di kawasan-kawasan alur. Mutagenisiti 3B yang lebih tinggi menunjukkan bahawa sebatian ini menyasarkan N7 dalam guanina dalam alur utama dan GC di alur kecil yang lebih luas manakala kekurangan keputusan mutagen yang signifikan oleh 2XP menunjukkan bahawa sebatian ini mungkin menyasarkan urutan kaya dengan AT , yang ditemui dalam sebahagian besar di bahagian sempit alur kecil asid nukleik.

Keputusan kajian kami menunjukkan bahawa benzylvanillin sahaja tidak mempunyai aktiviti anti-kanser kuat walaupun digabungkan dengan benzimidazole, tetapi setelah disubstitusi dengan benzylvanillin lain, aktiviti yang lebih kuat diperhatikan. Kombinasi benzylvanillin dengan benzenesulfonate juga meningkatkan secara signifikan aktiviti 3B kerana kehadiran benzenesulfonate membolehkannya untuk memiliki aktiviti alkilasi yang baik. Penemuan kajian ini menyokong hasil pemodelan komputer yang juga menunjukkan bahawa 3B dan 2XP mempunyai pengikatan DNA yang baik. Kedua-dua sebatian (2XP dan 3B) gagal untuk membentuk ikatan langsung dengan DNA, tidak seperti BN1 dan 2MP. Namun begitu, kedua-dua sebatian (BN1 dan 2MP) gagal untuk menunjukkan aktiviti sitotoksik.

Oleh itu, kajian ini memberikan pendekatan baru bagi derivatif novel benzylvanillin sebagai agen terapeutik yang berpotensi terhadap leukemia.

THE STUDY OF ANTICANCER PROPERTIES OF BENZYL-O-VANILLIN AND IT'S DERIVATIVES

Benzyl-o-vanillin and benzimidazole nucleus serve as important pharmacophore in drug discovery as it has a significant medicinal importance. Thus we explored the anticancer activity of three compounds namely 2-(2-benzyloxy-3-methoxyphenyl)-1H-benzimidazole, **2MP**, N-1-(2-benzyloxy-3-methoxybenzyl)-2-(2-benzyloxy-3-methoxyphenyl)-1H-benzimidazole, **2XP**, and (R) and (S)-1-(2-benzyloxy-3-methoxyphenyl)-2, 2, 2-trichloroethyl benzenesulfonate, **3BS** and compared their activity to 2-benzyloxy-3-methoxybenzaldehyde, (**Bn1**) the parent compound. 2XP and 3BS were found to be selectively cytotoxic towards U937 leukemic cell causing cell death via apoptosis by activating caspase 9 activity.

DNA binding studies primarily by the equilibrium binding titration assay indicate DNA binding activity with binding constant of 7.39µM/bp and 6.86µM/bp for 3BS and 2XP respectively. Viscometry studies show that 3BS and 2XP bind mainly in the groove region. The higher mutagenicity of 3BS suggests that this compound targets the N7 of guanine in the major groove and the GC in the wider minor groove while the lack of significant mutagenic outcome by 2XP suggests that this compound may target mainly the AT rich sequences, found largely in the narrow minor groove region of the nucleic acid.

Our results show that the benzylvanillin alone have no strong anticancer activity even after it was combined with the benzimidazole, but after being

disubstituted with another benzylvanillin, stronger activity was observed. Also, the combination of benzylvanillin with benzenesulfonate significantly improved the activity of 3BS as the presence of benzenesulfonate enabled it to have good alkylating activity. The findings of this study support our computer modeling results which also shows that 3BS and 2XP have good DNA binding. Both compounds (2XP and 3BS) failed to form any direct linkage with the DNA unlike Bn1 and 2MP. However these two compounds (Bn1 and 2MP) failed to show any cytotoxic activity.

Hence, the present study provides a new insight of the novel benzylvanillin derivatives serving as potential therapeutic agents against leukemia.

CHAPTER ONE

INTRODUCTION

1.1 Cancer Overview:

1.1.1 Introduction:

Cancer is considered as the second most common cause of death after heart disease. From 58 million deaths worldwide in 2005, cancer accounts for 7.6 million (or 13%) of all deaths. Deaths due to cancers across the globe are expected to rise, with an estimate of 9 million people dying from cancer in 2015 (Selvan, 2007).

Cancer could be defined as a group of diseases at which abnormal cells divide without control (U.S. Department of health and human services, 2003) . In other words, cancer is a disease characterized by uncoordinated and undesirable cell division accompanied with the spread of abnormal cells to the remote area, metastasis (Caligiuri, 2007b; Evert, 2010; SEER Training Modules, 2011b).

It results from a breakdown of the regulatory mechanisms that govern normal cell behaviour, which are the proliferation, differentiation, and survival of individual cells in multicellular organisms (Bjornsti & Osheroff, 1999). Unlike normal cells, cancer cells are aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues), and/or metastatic (spread to other locations in the body) (Evert, 2010). These three malignant properties of cancers differentiate them from benign tumors that are self-limited in their growth and do not invade or metastasize (Bjornsti & Osheroff, 1999). Frequently, benign tumor is completely enclosed in a protective capsule of tissue and it typically does not pose danger to human life like malignant tumors do (Bjornsti & Osheroff, 1999; Evert, 2010; Fayed, 2009; SEER Training Modules, 2011b; U.S. Department of health and human services, 2003; Yu, Blanchard, Wang, & You, 2008). Over time, changes may take place in benign tumor cells that cause them to invade and interfere with the function of normal tissues (Fayed, 2009; U.S. Department of health and human services, 2003).

Although cancer is often referred to a single condition, it actually described as a group of more than 100 different diseases that have similar characteristics (SEER Training Modules, 2011b). It is usually classified according to the tissue from which the cancerous cells originate, as well as location (Harnden, Lorenzen, Pusztai, & McGee, 1998). The most common type is carcinoma that develops from epithelial tissue lining the surfaces of certain organs, such as the lung, liver, skin, breast or the lining of blood vessels. Another group of cancers is sarcoma that arises from cells in bone, cartilage, fat, connective tissue, supportive tissue, and muscle (Harnden, et al., 1998; U.S. Department of health and human services, 2003).

Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells (U.S. Department of health and human services, 2003). These genetic abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals and infectious agents (U.S. Department of health and human services, 2003). Other cancer-promoting genetic abnormalities may be randomly acquired through errors in DNA replication or inherited from birth. However, a complex interaction between carcinogens and the host genome exist that might explain a difference in the incidence of cancer after exposure to a known carcinogen. Thus, new aspects of cancer pathogenesis needs an extensive and further exploration to assess the other possible pathways (Harnden, et al., 1998).

1.1.2 Cancer History:

The signs of cancer in the bones of ancient Egyptian mummies dating back as far as 3000 B.C (Britannica Online, 2005). These had been described by Edwin Smith Papyrus in 1862 (Grant, 2001; Morton & Moore, 1997; The American Cancer Society Inc., 2002). This document is considered to be the oldest written description of cancer known to exist, described eight cases of breast tumours or ulcers in Egypt that were treated with cauterization. However, the document also states that there is no treatment for cancer at that time (Emory University, 2010; Hutchinson, 2006).

In 50 A.D., Romans found that some tumours could be removed by surgery followed by cauterization, but no medicine seemed to work. They found that surgery sometimes increased the spread of cancer or sometimes grew again. Later on caustic (burning) pastes, usually containing arsenic, were used for control of more extensive cancer. In addition, phlebotomy (blood-letting), diet, herbal medicines, powder of crab and other symbolic charms were also used (Cancer Council Victoria, 2010).

Though treatment remained the same, the development of microscopes in 1650 A.D. had led to a major step in improving the understanding of cancer. Since then different school of thought regarding the causes and treatment modalities had evolved (Cancer Request, 2010d).

In 19th century, asepsis improved surgical hygiene and as the survival statistics went up, surgical removal of the tumour became the primary treatment for cancer (Britannica Online, 2005). During the same period X-ray was discovered by German physicist Wilhelm Conrad Roentgen. This powerful ray was being used to detect and effectively treat cancer (Radiology Society of North America, 2008).

After World War II (WWII), Japanese medical community observed that the bone marrow of bomb victims in Hiroshima and Nagasaki was completely destroyed. They concluded that diseased bone marrow could also be destroyed with radiation, and this led to the discovery of bone marrow transplants for leukaemia (Hiroo, 1998).

In 1939, Charles Brendon Huggins discovered that hormones were necessary for the growth of certain cancers and this led the groundwork for hormone therapy for certain cancers(Machtens, Schultheiss, Kuczyk, Truss, & Jonas, 2000; Raju, 1998). Later on in 1946, Louis Goodman studied the chemical warfare agents during WWII. He discovered that nitrogen mustards could be used in the treatment of cancer. This is considered as the first chemotherapeutic agents against Hodgkin's Disease, lymphosarcoma, and leukemias (Britannica Online, 2005; Freireich, 1984; Goodman et al., 1984) .

First DNA microarray chip was constructed and used to measure gene expression levels in plants during 1995. This technology has been advanced and used to study cancer in humans. Currently 'gene chips' are being investigated as a tool in the development of individualized treatment plans (Emory University, 2010; Hutchinson, 2006).

1.1.3 Biology of Cancer:

Tumorigenesis is a multistep process where genetic changes such as point mutation, gene amplification, translocation, deletion, and viral infection accumulate over time **(Figure 1.1)**. These genetic changes, in turn, would cause downstream alterations in transcription, protein expression, and other cellular functions that eventually lead to the stepwise transformation of normal cells to malignant cells (Bjornsti & Osheroff, 1999). These malignant cells will soon acquire the ability to invade tissues, induce angiogenesis to support their own growth, and finally establish metastatic sites in remote organs (Yu, et al., 2008).

There are distinct biological properties that a cell must accumulate in order to develop cancer (Perspectives on Cancer, 2009). These hallmarks are:

- Self-sufficiency in growth signals, which means that cells grow even when it’s not getting a message to grow (Caligiuri, 2007a; Douglas Hanahan & Robert A. Weinberg, 2000a; Mitchell, 2007; Perspectives on Cancer, 2009).
- Insensitivity to anti-growth signals, which means that cells ignore messages to stop growing (Caligiuri, 2007a; Douglas Hanahan & Robert A. Weinberg, 2000a; Mitchell, 2007; Perspectives on Cancer, 2009).
- Evasion of apoptosis, which means that cells avoid all the messages to die (Caligiuri, 2007a; Douglas Hanahan & Robert A. Weinberg, 2000a; Mitchell, 2007; Perspectives on Cancer, 2009).
- Angiogenesis, which means the ability to develop new blood vessels to get the nutrients that fuel continued growth and division (Caligiuri, 2007a; Douglas Hanahan & Robert A. Weinberg, 2000a; Mitchell, 2007; Perspectives on Cancer, 2009).

- Unlimited replication potential, which means that cells keep replicating without control (Caligiuri, 2007a; Douglas Hanahan & Robert A. Weinberg, 2000a; Mitchell, 2007; Perspectives on Cancer, 2009).
- Invasion and metastasis, which means that cells spread into areas where they should not normally present. Tissue invasion and metastasis are what make many cancers lethal (Caligiuri, 2007a; Douglas Hanahan & Robert A. Weinberg, 2000a; Mitchell, 2007; Perspectives on Cancer, 2009).
- An inflammatory microenvironment, newly discover in 2008. The cancer-related inflammation contributes to proliferation and survived of malignant cells, angiogenesis, metastasis, subversion of adaptive immunity and reduced response to hormones and chemotherapeutic agents. It is induction of genetic instability by inflammatory mediators, leading to accumulation of random genetic alterations in cancer cells (Colotta, Allavena, Sica, Garlanda, & Mantovani, 2009). It was suspected (G. Smith & Missailidis, 2004) then represented as the seventh hallmark of cancer (Colotta, et al., 2009).

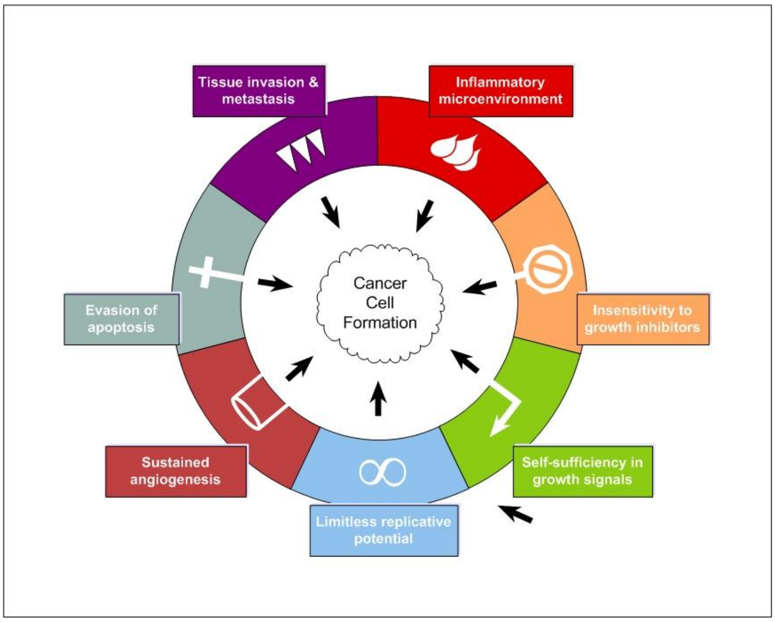
Tissue invasion & metastasis
Inflammatory microenvironment
Cancer Cell Formation
Evasion of apoptosis
Insensitivity to growth inhibitors
Sustained angiogenesis
Self-sufficiency in growth signals
Limitless replicative potential

Fig. 1.1 The hallmarks of cancer (Douglas Hanahan & Robert A. Weinberg, 2000b; Mantovani, 2009).

1.2 Cell Cycle:

The cell cycle, or cell-division cycle, is the series of events that takes place in a cell leading to its division and duplication (Malumbers, 2008; J. A. Smith & Martin, 1973).

The cell cycle consists of two parts or four distinct phases (**Figure 1.2**): the interphase: G1 phase, S phase (synthesis), G2 phase (collectively known as interphase) and M phase (mitosis): mitosis, in which the cell's chromosomes are divided between the two daughter cells, and cytokinesis, in which the cell's cytoplasm divides in half forming distinct cells (J. A. Smith & Martin, 1973). Activation of each phase is dependent on the proper progression and completion of the previous one. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G0 phase (Rubenstein & Wick, 2008). **Table 1.1** is shown a detailed description involved in each phase.

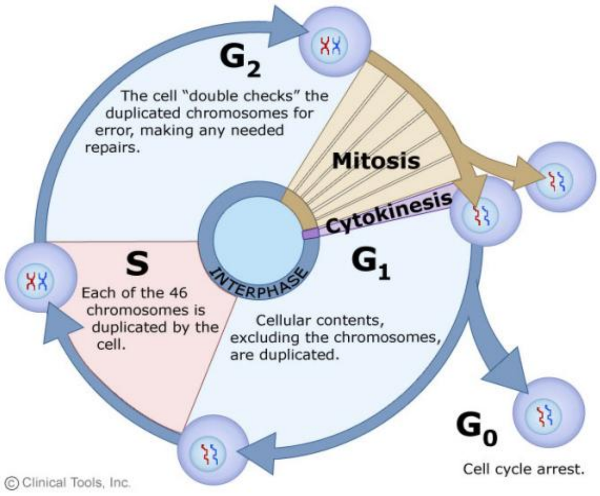
G_2
The cell "double checks" the duplicated chromosomes for error, making any needed repairs.
Mitosis
Cytokinesis
S
Each of the 46 chromosomes is duplicated by the cell.
INTERPHASE
G_1
Cellular contents, excluding the chromosomes, are duplicated.
G_0
Cell cycle arrest.
© Clinical Tools, Inc.

Figure 1.2 The cell cycle consists of two parts or four distinct phases: the interphase (G1 phase, S phase (synthesis), G2 phase) and M phase (mitosis) (mitosis and cytokinesis) (Virtual Genetics Education Centre, 2011).

Table 1.1 The cell cycle phases and their description (HighWire Press, 2003; Malumbers, 2008).

State	Phase	Abbreviation	Description
quiescent/ senescent	Gap 0	G0	A resting phase where the cell has left the cycle and has stopped dividing. occurs in response to DNA damage or degradation that would make a cell's progeny nonviable; it is often a biochemical alternative to the self-destruction of such a damaged cell by apoptosis
Interphase	Gap 1	G1	Cells increase in size in Gap 1. The G1 checkpoint control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	S	DNA replication occurs during this phase.
	Gap 2	G2	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The G2 checkpoint control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell division	Mitosis	M	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (Metaphase Checkpoint) ensures that the cell is ready to complete cell division.

Several cell cycle checkpoints are used by the cell to monitor and regulate the progress of the cell cycle. These checkpoints are designed to ensure that damaged or incomplete DNA is not passed on to daughter cells which may lead to tumor formation. The two main checkpoints exist are G1/S checkpoint also known as restriction point and G2/M checkpoint (Kumar, Abbas, & Fausto, 2004).

In cancer therapy, the cells which are actively undergoing cell cycle are targeted as the DNA is relatively exposed during cell division and hence susceptible to damage by drugs or radiation. But in general, cells are most radiosensitive in late M and G2 phases and most resistant in late S because the resistance and sensitivity correlates with the level of sulfhydryl compounds in the cell which are natural radioprotectors and tend to be at their highest levels in S and at their lowest near mitosis (Rubenstein & Wick, 2008).

1.3 Cell Death:

1.3.1 Introduction:

Cell death is a major endpoint in toxicological assessment both *in vivo* and *in vitro* and numerous methods have become available for the characterization and quantitation of cell death. Recent developments in cell biology have made great strides in articulating two generally distinct modes of cell death (Poot, Pierce, & Kavanagh, 2002).

In general, the cell death divided into apoptosis and necrosis, and each involves different biochemical features and, possibly, kinetics(Raffray & Cohen, 1997). Necrosis appears to be a rapid response (often to exogenous agents) involving cellular injury, cell swelling, loss of cytoplasmic ATP, release of sequestered calcium, and uncontrolled activation of calcium-dependent enzymes (proteases, lipases and DNAases) leading to early loss of cytoplasmic membrane integrity(Cotran, Kumar, Collins, & Robbins, 1999). DNA single-strand breaks are formed, but the morphology of the cell nucleus usually remains intact (Mellor et al., 2002; Nguyen, 2006). Cells undergoing rapid necrosis *in vitro* do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers (Nguyen, 2006).

Apoptosis, in contrast, is a more controlled process involving a decrease in cell size, the loss of mitochondrial membrane potential, alterations in mitochondrial structure and function, altered cellular redox status (D. Green & Kroemer, 1998; D. R. Green & Reed, 1998), as well as activation of caspases, exposure of cell-surface phosphatidylserine residues, and ordered double-strand DNA fragmentation (Poot, et al., 2002). Cultured cells that are undergoing apoptosis *in vitro* eventually undergo secondary necrosis. Markers of apoptosis such as caspase activity may be present only transiently (Nguyen, 2006).

Apoptosis has been associated with ischemic injury (like myocardial infarction, and reperfusion injury) (MacManus, Buchan, Hill, Rasquinha, & Preston, 1993; Walton et al., 1997), immunoreactive and immunodegenerative states and a variety of neurodegenerative disorders (including Alzheimer's disease, ALS and motor neuron degeneration, Parkinson's disease, etc.), and retinal ganglion cell death in experimental glaucoma (Garcia-Valenzuela, Shareef, Walsh, & Sharma, 1995; Laquis, Chaudhary, & Sharma, 1998; Nickells, 1996).

The ability of tumor cell populations to expand in number is determined not only by the rate of cell proliferation but also by the rate of cell attrition. Programmed cell death—apoptosis—represents a major source of this attrition (Douglas Hanahan & Robert A. Weinberg, 2000a). If this equilibrium is disturbed, one of two potentially fatal disorders occurs: (Potten & Wilson, 2004) the cells are dividing faster than they die, effectively developing a tumor (Bevec, Cavalli, Cavalli, & Bacher, 2010; Potten & Wilson, 2004), which is the hallmark of most and perhaps all types of cancer (Douglas Hanahan & Robert A. Weinberg, 2000a), and the cells are dividing slower than they die, which results in a disorder of cell loss (Bevec, et al., 2010; Potten & Wilson, 2004).

The life of most cells is in part maintained by cell–matrix and cell–cell adherence-based survival signals whose abrogation elicits apoptosis (Giancotti & Ruoslahti, 1999; Ishizaki, Cheng, Mudge, & Raff, 1995). Both soluble and immobilized apoptotic regulatory signals likely reflect the needs of tissues to maintain their constituent cells in appropriate architectural configurations (D. Hanahan & R. A. Weinberg, 2000).

Resistance to apoptosis can be acquired by cancer cells through a variety of strategies. Surely, the most commonly occurring loss of a proapoptotic regulator through mutation involves the *p53* tumor suppressor gene (Douglas Hanahan & Robert A. Weinberg, 2000a) that arrests the cell cycle in G_1 in response to damaged DNA and is required for apoptosis induced by a variety of stimuli (Bjornsti & Osheroff, 1999; Camden, 2001b; Harris, 1996).

1.3.2 Historically:

The cell death is a completely normal process in living organisms and was already discovered by scientists more than 100 years ago. The German scientist Carl Vogt was first to describe the principle of apoptosis in 1842. In 1885, anatomist Walther Flemming delivered a more precise description of the process of programmed cell death. However, it was not, until 1965, resurrected. Apoptosis (Greek: *apo* - from, *ptosis* - falling) was distinguished from traumatic cell death by John Foxton Ross Kerr at the University of Queensland in 1965 (Potten & Wilson, 2004). The possibility that apoptosis serves as a barrier to cancer was first raised in 1972, when Kerr, Wyllie, and Currie described massive apoptosis in the cells populating rapidly growing, hormone-dependent tumors following hormone withdrawal (Kerr, Wyllie, & Currie, 1972).

1.4 DNA Structure:

1.4.1 DNA Overview:

a. Introduction:

Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms and some viruses. The main role of DNA molecules is the long-term storage of information. DNA segments that carry the genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information (Calladine, Drew, Luisi, & Travers, 2004).

DNA can be damaged by many sorts of mutagens, which change the DNA sequence. Mutagens include oxidizing agents, alkylating agents and also high-energy electromagnetic radiation such as ultraviolet light and X-rays (Jeffrey, 1985).

DNA chain is 22 to 26 Angstroms (Å) wide (2.2 to 2.6 nanometres), and one nucleotide unit is 3.3 Å (0.33 nm) long (Watson & Crick, 1953). Although each individual repeating unit is very small, DNA polymers can be very large molecules containing millions of nucleotides. For instance, the largest human chromosome, chromosome number 1, is approximately 220 million base pairs long (Mandelkern, Elias, Eden, & Crothers, 1981).

b. DNA's Discovery:

DNA was first isolated by the Swiss physician Friedrich Miescher in 1869 that he called it at that time by "nuclein" but two years earlier, the Czech monk

Gregor Mendel, had closely connected to the finding of nuclein by studying the traits in the peas and their inherit. In 1919, the molecular structure of DNA had been discovered by Phoebus Levene. He identified the base, sugar and phosphate nucleotide unit (Levene, 1919) and he thought that the chain was short and the bases repeated in a fixed order. In 1928, Frederick Griffith discovered that the DNA carried genetic information. Later on, William Astbury, in 1937, produced the first X-ray diffraction patterns that showed that DNA had a regular structure (Maddox, 2003). Oswald Avery, along with coworkers Colin MacLeod and Maclyn McCarty, identified DNA as the transforming principle in 1943 (Meselson & Stahl, 1958).

In the late 1940's, the scientific were skeptical that DNA was most likely the molecule of life, they also knew that DNA included different amounts of the four bases adenine, thymine, guanine and cytosine (usually abbreviated A, T, G and C), but nobody had the slightest idea of what the molecule might look like (Fredholm, 2003).

In order to solve this puzzle, a couple of distinct pieces of information needed to be put together. One was that the phosphate backbone was on the outside with bases on the inside; another that the molecule was a double helix. It was also important to Figure out that the two strands run in opposite directions and that the molecule had a specific base pairing (Fredholm, 2003).

In 1953 James D. Watson and Francis Crick suggested the first correct double-helix model of DNA structure (Watson & Crick, 1953), **Figure 1.3**. Their double-helix, molecular model of DNA was then based on a single X-ray diffraction image (F. Crick & Watson, 1953) taken by Rosalind Franklin and Raymond Gosling in May 1952, as well as the information that the DNA bases were paired—also obtained through private communications from Erwin Chargaff in the previous years. Chargaff's rules played a very important role in establishing double-helix configurations for B-DNA as well as A-DNA.

In 1957, Crick laid out the "Central Dogma" of molecular biology, which foretold the relationship between DNA, RNA, and proteins (F. H. C. Crick, 1957, 1958; Strasser, 2006), and articulated the "adaptor hypothesis" (F.H.C. Crick, 1955). Final confirmation of the replication mechanism in 1958, the discovery of the codons by Crick and coworkers and the decipher of the genetic code by Har Gobind Khorana, Robert W. Holley and Marshall Warren Nirenberg (Nobelprize.org, 1968) lead to the birth of molecular biology (Friedberg, 2002; Hayes, 1985; Ingram, 2002).

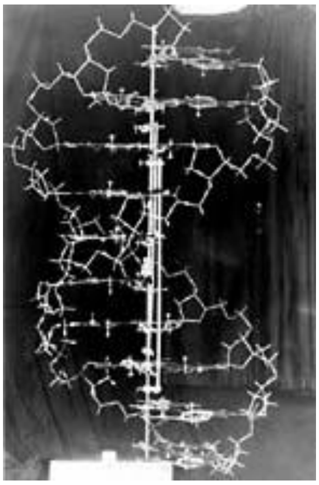

Figure 1.3 The original DNA model by Watson and Crick Photo: Cold Spring Harbor Laboratory Archives (F. Crick & Watson, 1953).

1.4.2 Properties:

DNA consists of two long polymers (Alberts, 2002; Butler, 2000; Saenger, 1984) that are held tightly together (Berg, Tymoczko, & Stryer, 2002; Gregory et al., 2006) by simple units, nucleotides, in the shape of a double helix (Moss, 1970). This double helix contains both the segment of the backbone of the molecule, which holds the chain together, and a base, which interacts with the other DNA strand in the helix. A base linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. If multiple nucleotides are linked together, as in DNA, this polymer is called a polynucleotide (Moss, 1970).

The backbone of the DNA strand is made from alternating phosphate and sugar residues (Ghosh & Bansal, 2003). The sugar in DNA is pentose 2-deoxyribose that differs from RNA which has pentose sugar ribose (Berg, et al., 2002). The sugars are joined together by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings. These asymmetric bonds mean a strand of DNA has a direction. In a double helix the direction of the nucleotides in one strand is opposite to their direction in the other strand therefore are antiparallel. The asymmetric ends of DNA strands are called the 5′ (five prime) and 3′ (three prime) ends, with the 5’ end having a terminal phosphate group and the 3’ end a terminal hydroxyl group.

The DNA double helix is stabilized by hydrogen bonds between the bases attached to the two strands. The four bases found in DNA are adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T). These four bases are attached to the sugar/phosphate to form the complete nucleotide (Keller, 2007), see **Figure 1.4**.

The sequence of these four bases along the backbone encodes information. This information is read using the genetic code, which specifies the sequence of the amino acids within proteins. The code is read by copying stretches of DNA into the related nucleic acid RNA, in a process called transcription.

The four bases are classified into two types; adenine and guanine are fused five- and six-membered heterocyclic compounds called purines, while cytosine and thymine are six-membered rings called pyrimidines (Berg, et al., 2002; Clausen-Schaumann, Rief, Tolksdorf, & Gaub, 2000). A fifth pyrimidine base, called uracil (U), usually takes the place of thymine in RNA and differs from thymine by lacking a methyl group on its ring. Uracil is not usually found in DNA, occurring only as a breakdown product of cytosine. In addition to RNA and DNA, a large number of artificial nucleic acid analogues have also been created to study the proprieties of nucleic acids, or for use in biotechnology (Verma & Eckstein, 1998).

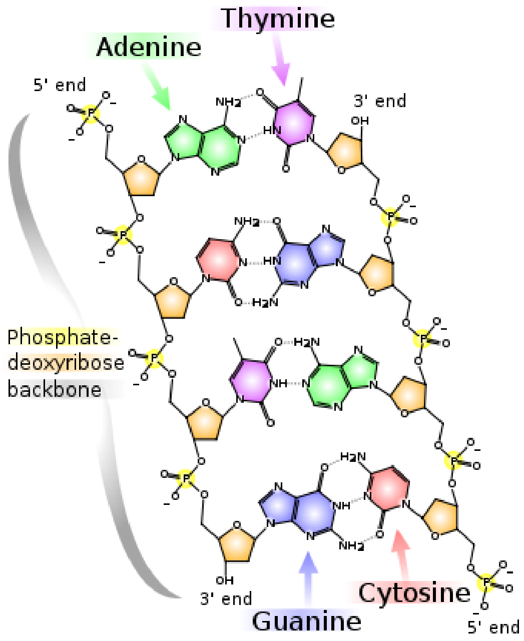

Figure 1.4 The Chemical structure of DNA. Hydrogen bonds shown as dotted lines (Keller, 2007; Madeleine Price Ball, 2007).

a. Grooves:

Double helix may be found by tracing the spaces, or grooves, between the strands. These voids are adjacent to the base pairs and may provide a binding site. As the strands are not directly opposite each other, the grooves are unequally sized. One groove, the major groove, is 22 Å wide and the other, the minor groove, is 12 Å wide (Calladine, et al., 2004; Wing et al., 1980), **Figure 1.5**. The narrowness of the minor groove means that the edges of the bases are more accessible in the major groove. This situation varies in unusual conformations of DNA within the cell, but the major and minor grooves are always named to reflect the differences in size that would be seen if the DNA is twisted back into the ordinary B form.

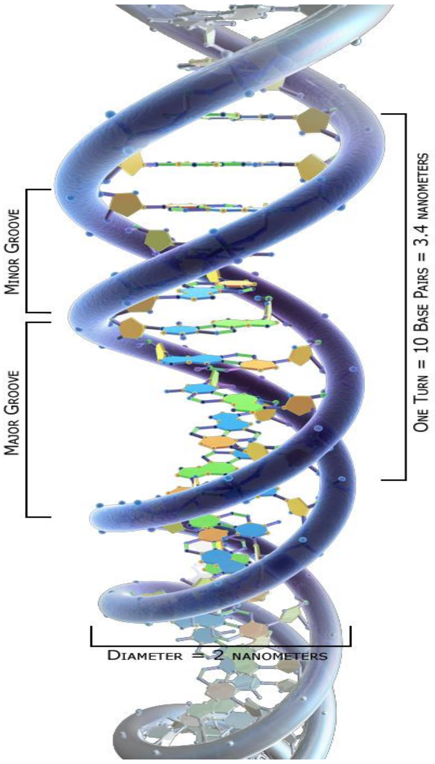
Minor Groove
Major Groove
One Turn = 10 Base Pairs = 3.4 nanometers
Diameter = 2 nanometers

Figure 1.5 The groove of DNA structure (Zygote Media Group, 2007).

b. Base pairing:

Each type of base on one strand forms a bond with just one type of base on the other strand. This is called complementary base pairing. Here, purines form hydrogen bonds to pyrimidines, with A bonding only to T, and C bonding only to G. This arrangement of two nucleotides binding together across the double helix is called a base pair (Clausen-Schaumann, et al., 2000). The two types of base pairs form different numbers of hydrogen bonds, AT forming two hydrogen bonds, and GC forming three hydrogen bonds, as shown in **figure 1.4**.

c. Supercoiling:

DNA can be twisted like a rope in a process called DNA supercoiling. With DNA in its "relaxed" state, a strand usually circles the axis of the double helix once every 10.4 base pairs, but if the DNA is twisted the strands become more tightly or more loosely wound (Benham & Mielke, 2005). If the DNA is twisted in the direction of the helix, this is positive supercoiling, and the bases are held more tightly together. If they are twisted in the opposite direction, this is negative supercoiling, and the bases come apart more easily. In nature, most DNA has slight negative supercoiling that is introduced by topoisomerases enzymes (Champoux, 2001) that also included to relieve the twisting stresses during transcription and DNA replication (Wang, 2002).

d. Alternate DNA structures:

DNA exists in many possible conformations that include A-DNA, B-DNA, and Z-DNA forms, although, only B-DNA and Z-DNA have been directly observed in functional organisms (Ghosh & Bansal, 2003) as in **Figure 1.6**. The conformation that DNA adopts depends on the hydration level, DNA sequence, the amount and direction of supercoiling, chemical modifications of the bases, the type and concentration of metal ions, as well as the presence of polyamines in solution (Basu, Feuerstein, Zarling, Shafer, & . 1988).

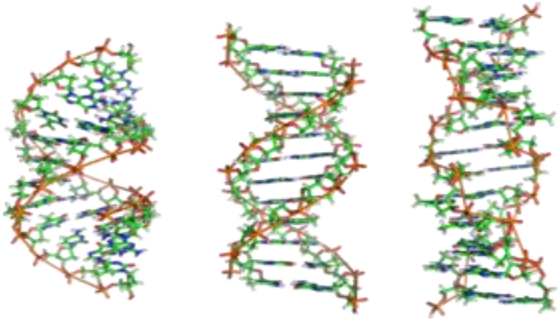

Figure 1.6 From left to right, the structures of A, B and Z DNA (Arnott, Chandrasekaran, Birdsall, Leslie, & Ratliff, 1980; R. Chandrasekaran & Arnott, 1996; R Chandrasekaran et al., 1989).

The `B-DNA form' is most common under the conditions found in cells (I. C. Baianu, 1980; Leslie, Arnott, Chandrasekaran, & Ratliff, 1980) that occur at the high hydration levels present in living cells (I. Baianu, 1978; Gaylord & Kaufman, 1963).

Compared to B-DNA, the A-DNA form is a wider right-handed spiral, with a shallow, wide minor groove and a narrower, deeper major groove. The A form occurs in the cell due to the hybrid pairings of DNA and RNA strands, as well as in enzyme-DNA complexes (Lu, Shakked, & Olson, 2000; Wahl & Sundaralingam, 1997). Segments of DNA where the bases have been chemically modified by methylation may undergo a larger change in conformation and adopt the Z form. Here, the strands turn about the helical axis in a left-handed spiral, the opposite of the more common B form (Rothenburg, Koch-Nolte, & Haag, 2001). These unusual structures can be recognized by specific Z-DNA binding proteins and may be involved in the regulation of transcription (Oh, Kim, & Rich, 2002).

1.5 Cancer Treatment:

1.5.1 Introduction:

The treatment given for cancer is highly variable and dependent on a number of factors including the type, location and amount of disease and the health status of the patient. The treatments are designed to directly kill and/or remove the cancer cells or lead to their eventual death by stopping the cell division. Other treatments work by stimulating the body defence mechanisms (Cancer Request, 2010d).

The standard methods of treating cancer include surgery, radiation therapy, chemotherapy, hormonal therapy (Camden, 2001b; Fayed, 2009; Lam, 2001; Maranga, 2009; Mishra, 2009), immunotherapy and monoclonal antibody therapy (Camden, 2001a, 2001b; Fayed, 2009; Maranga, 2009). In medical science, different cancer treatments are used in combination, either simultaneously or sequentially (Cancer Request, 2010d)depending on the type, size, location, and stage of the tumour in additional to the person's general health (Mishra, 2009).

a. Surgical Intervention

Surgery is the oldest form of treatment for many solid tumours (Cancer Request, 2010d; Mishra, 2009) It is used to prevent, treat and diagnose cancer (Fayed, 2009), which involves the complete removal of tumour or as much of the cancerous tissue as possible (Mishra, 2009). This is usually the first preference by the physicians; however, it is not helpful if the cancer has already metastasise to a remote area (Cancer Request, 2010d; Fayed, 2009; Selvan, 2007). Surgery is often performed in conjunction with chemotherapy or radiation therapy (Fayed, 2009; Maranga, 2009; Park & Posner, 2003; Selvan, 2007).

b. Radiation Therapy

This type of treatment uses certain types of ionized energy beams or waves such as gamma rays or x-ray to shrink tumours and/or eliminate cancer cells (Cancer Request, 2010d; Fayed, 2009; Maranga, 2009; Mishra, 2009; Selvan, 2007). It works by damaging their genetic constitutes and making it unable to multiply (Fayed, 2009; Maranga, 2009). This type of cancer treatment is given in fractions in order to allow healthy tissue to recover between remission periods (Maranga, 2009). Radiotherapy may be used in conjunction with surgery and/or drug treatments (Cancer Request, 2010d; Mundt & Roeske, 2003).

c. Chemotherapy

It is a term used for a wide variety of medicines that are used to kill cancer cells by damaging the dividing cancer cells and preventing their further reproduction (Cancer Request, 2010d; Maranga, 2009; Mishra, 2009). Systematic chemotherapy generally uses anticancer drugs that enter the bloodstream and reach all areas of the body, making this treatment potentially useful for cancer that has spread (Ewesuedo & Ratain, 2003; Mishra, 2009). This type of treatment will be discussed in details in the following part **1.5.2**.

d. Hormone Therapy

Hormone therapy is used against certain type of cancers which are dependent on hormones as signals for their growth. This treatment includes the use of drugs that stop the production of certain hormones (Cancer Request, 2010d; Maranga, 2009; Mishra, 2009). The hormonal therapy also used in conjugation with surgery and/or radiation therapy.

e. Immunotherapy/Biological Response Modifiers

It involves several strategies that promote naturally occurring proteins to stimulate our body's own immune system defence to fight cancer (Cancer Request, 2010d; Maranga, 2009; Mishra, 2009; Selvan, 2007). These are considered as vaccines that usually contain proteins found on or produced by cancer cells. Thus treatment aims to increase the response of the body against the cancer cells (Cancer Request, 2010d; Gajewski, 2003).

f. Targeted Therapy

This class of drugs is relatively new in the treatment of cancer since 1990. They work by targeting specific proteins and processes that are limited primarily to cancer cells or that are much more prevalent in cancer cells. Some types work by blocking the biological processes that allow the tumour to thrive and grow, which are antibodies. Others cut off the blood supply to the tumour, angiogenesis, causing it to basically starve and die. This type of treatment is usually given in conjunction with other cancer treatments (Cancer Request, 2010d; Fayed, 2009).

g. Complementary and Alternative Medicines

These treatment methods are not practiced by conventional western medicine, usually used in the South East Asia. They can include herbal, animal derived, and mind-body approaches to treating cancer (Cancer Request, 2010d).

1.5.2 Chemotherapy:

The term chemotherapy, currently used cytotoxic drugs, refers to a wide range of drugs used to treat cancer which affect rapidly dividing cells (Cancer Request, 2010b; Ingwersen, 2001). Unlike surgery, chemotherapy affects the entire body by targeting rapidly multiplying cells (Camden, 2001b; Fayed, 2009; Ingwersen, 2001; Maranga, 2009). Ideally cytotoxic agents that have specificity for cancer cells while not affecting normal cells, would be extremely desirable (Camden, 2001a, 2001b; Ingwersen, 2001). Nevertheless, the treatment of cancer by cytotoxic drugs produces some of the common side-effects, like hair loss and vomiting, because of the death of normal cells (Baquiran & Gallagher, 2001; Camden, 2001b; Cancer Request, 2010b; Fayed, 2009; Maranga, 2009).

There are many universal cytotoxic drugs that are used to treat cancer; based on their chemical structures and the way they act on cancer cells (SEER Training Modules, 2011a). These chemotherapeutic agents can be divided as follows, (Camden, 2001a; Fayed, 2009; Skeel, 2007):

a. Antimetabolites:

Are drugs that interfere with the formation of key bio-molecules within the cell including nucleotides, the building blocks of DNA. These will interfere with DNA replication and therefore cell division (Baquiran & Gallagher, 2001; Cancer Request, 2010b; Ingwersen, 2001).

b. Genotoxic Drugs:

Drugs that damage DNA that eventually interfere with DNA replication and cell division (Baquiran & Gallagher, 2001; Cancer Request, 2010b).

c. Spindle Inhibitors:

These agents prevent proper cell division by interfering with the cytoskeletal components that enable one cell to divide into two (Baquiran & Gallagher, 2001; Cancer Request, 2010b).

d. Other Chemotherapy Agents:

These agents inhibit cell division by mechanisms that are not covered in the three categories listed above (Baquiran & Gallagher, 2001; Cancer Request, 2010b).

1.6 Genotoxic Drugs:

1.6.1 Introduction:

These drugs affect nucleic acids and alter their function either directly through binding to DNA or indirectly through enzymes involved in DNA replication lead to DNA damage. Cancer cells are particularly sensitive to genotoxic agents because they are actively synthesizing new DNA and if enough damage occurs, will often undergo apoptosis (Cancer Request, 2010c; Ewesuedo & Ratain, 2003). These agents in addition to being cytotoxic (cell poisoning and having the ability to kill the cell), are also mutagenic and carcinogenic (Cancer Request, 2010c).