CHAPTER I

INTRODUCTION

Cancer is one of the leading causes of premature death and morbidity worldwide. It is in a class of diseases involving unregulated cell growth and is able to cause severe damage when invading other tissue.

Depending on the type of cancer, there are several types of treatments such as surgery, radiation therapy, biological therapy, immunotheraphy, and chemotheraphy. Chemotherapy, elimination of cancer cells through medicine such as doxorubicin, taxol, psorospermin and vinblastine, is currently the most effective cancer treatment for several types of cancers. These agents act by killing cells that divide rapidly. This result also detrimentally affects normal cells. Although there are many chemical treatments for this disease, searching for alternative agents is available in clinics.

For example, some cancer cell types have high expressions of drug efflux transporters, such as P-glycoprotein, and are resistant to doxorubicin and many of its analogues (Fojo and Coley, 2007). Although increasing the drug dosage is an effective strategy to overcome drug resistance, it typically leads to increased side effects including hypotension and cardiac arrhythmia (Octavia et al., 2012).

Human papillomavirus (HPV) is present in over 90% of cervical cancers, the second most common form of cancer in women (DiPaolo JA, 2004) and (Parkin et al., 2005). High-risk HPV infection is the major risk factor for the development of this disease worldwide (Kaur et al., 1989). In general, after high-risk infection, tumor suppressor p53 will be inactivated by E6 protein. Thus, nonfunctional or absent p53 allows an accelerated cell division rate and promotes genetic instability, facilitating malignant transformation (Scheffner, 1998). Activation of p53 can further induce downstream target genes involved in cell cycle arrest and apoptosis (Vousden, 2002).

The cytotoxic effect of p53 is mediated by transcriptional activation of the cyclin-dependent kinase (cdk) inhibitor by deactivation of a antiapoptotic gene product, anti-apoptotic B-cell lymphoma-2 or Bcl-2 (Alnemri et al., 1992) and (Fisher et al., 1993).

Although p53 has an important role in the function of many cells, some experiments have recently demonstrated that the elevated levels of p53 protein did not result in an increased transcriptional activity of p53-regulated gene (Lutzker and Levine, 1996). Although p53-dependent apoptosis suggest how a DNA damaging agent such as cisplain can kill neoplastic cells, the relationship between cisplatin sensitivity and p53 function remains unclear. In addition, recent work has indicated that p53 is not a determinant of cisplatin cytotoixicity in some cancer cells including ovarian and non-small cell lung cancer cells (Fujiwara et al., 1994) and (Lutzker and Levine, 1996). These cell lines were sensitized to cisplatin by the inactivation of p53 and do not exhibit a predominant apoptotic response to chemotherapy (Fan et al., 1995) and (Hawkins et al., 1996).

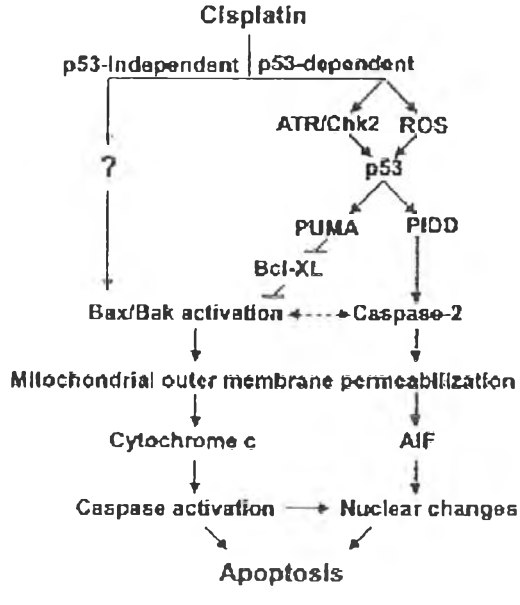


Figure 1. Schematic diagram showing the effects of cisplatin independent and dependent apoptosis

Cisplatin (Figure 2) has been used to treat a wide variety of cancers in addition to testicular tumors (Loehrer and Einhorn, 1984) and (McEvoy et al., 1994). However, its effectiveness is often limited by its inherently poor activity against many tumor types and by the development of resistance (Hawkins et al., 1996). The molecular anti-cancer mechanism of cisplatin is not clearly understood. It is generally accepted that it acts through the formation of DNA adducts (Zamble and Lippard, 1995). The fact that some cells can be killed by DNA damage while others are resistant to such damage is most likely a consequence of many cellular pathways (Scanlon et al., 1991) and (Chu et al., 1994). This must be explored further to provide more comprehensive treatment strategies

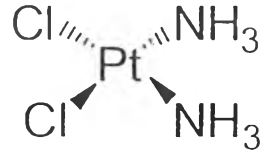


Figure 2. Chemical structure of cisplatin

Doxorubicin or hydroxyl daunorubicin (Figure 3) is commonly known as adriamycin. This is classified as an antibiotic drug suitable for treated diverse human carcinomas. The chemical structure contains four fused rings and positively charged amino sugars. It is a broad-spectrum chemotherapeutic agent that has been widely used in clinics to treat various cancers (2004; Muggia and Green, 1991). The cytotoxicity of this drug has been reported to inhibit DNA synthesis by intercalation DNA, including p53 and oxidative stress by increasing the production of reactive oxygen species (ROS) as described in previous literature (Momparler et al., 1976), (Agbandje et al., 1992) and (McKnight et al., 2004).

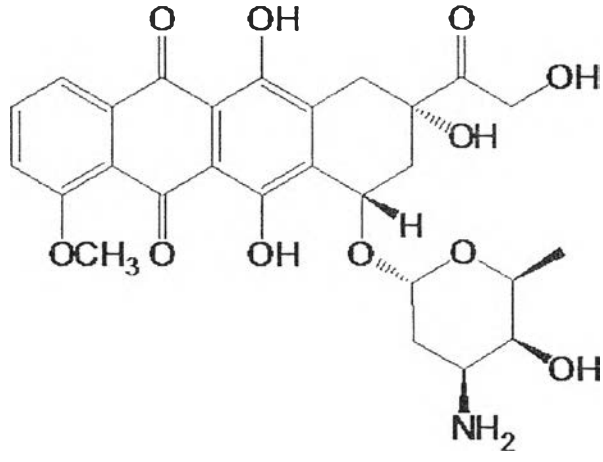
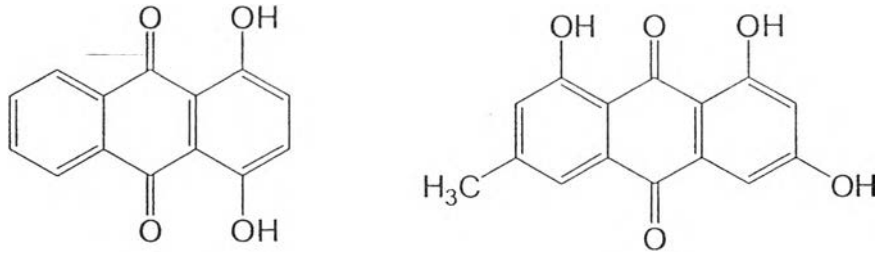


Figure 3. Chemical structure of doxorubicin

One of the failures of these chemotherapy agents is that some transporters require glutatione for substrate transport including multi-drug resistant proteins (MRPs) (Lu et al., 1993). Glutatione (L-glutamyl-cysteinyl-glycine, GSH) is involved with the biotransformation and detoxification of cells developing resistance to chemotherapeutic drugs (Kuo, 2009)). For example, glutatione can conjugate with active agents such as cisplatin (Figure 2) by glutathione transferase protecting cells against cytotoxic stress (Siddik, 2003). This phenomenon produces less reactive chemical species, which correlate with resistance to anti-cancer drugs through accelerated detoxification of the drug's substrates (Mannervik and Danielson, 1988). Reduced glutathione becomes the most abundant intracellular thiol which acts as a major antioxidant by protecting the cells against the damaging effects of free radicals and reactive oxygen species (ROS) (2004; Cervantes et al., 1988).

Anthraquinones or anthracene, 9-10-dione to quinone-based chemotherapies was reported to overcome some drug resistant cancer (Figure 4a) and have been commonly used as a pharmacophore for the treatment of various diseases including cancer [Sangthong *at al.*, 2013]. The anthraquinone derivative, emodin (Figure 4b), has been reported to suppress the proliferation of various cancer cells. Co-treatment of

emodin with a number of anti-cancer drugs was shown to be effective against a multi-drug resistance cell line, working as a reactive oxygen species (ROS) generator. In addition to ROS generation, emodin targets a number of cancer pathways and molecular targets such as tyrosine kinases, phosphoinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and NF-kappaB (Hunger et al., 2003). Moreover, novel anthraquinone platinum derivatives have shown improved *in vitro* cytotoxic activity in tumor cell lines representative of human ovarian (A2780 and A2780cisR) and breast cancers (T47D) (Ruiz et al., 2008).



(a) (b)

Figure 4. Chemical structures of (a) anthraquinone and (b) emodin

Since the development of inhibiting drug resistance and specific activity on cancer cells has been a major impediment toward successful cancer treatments, it has encouraged the development of novel chemotherapeutics that can overcome drug resistance and specifically inhibit cancer cells.

The objective of this research is to synthesize new anthraquinone derivatives to develop the effectiveness of cytotoxicity based on overcoming drug resistance and the inhibition of cancer cells using MTT and Colony Formation Assay for cytotoxic activity. This report also studies molecular anti-cancer mechanisms based on cell cycle, protein expression and apoptosis.