CHAPTER I
INTRODUCTION

1. Introduction

1.1 Structure-Activity Relationship

The relationship studying between a chemical structure and its biological activity enables the identification and determination of the chemical groups that are specific with the target biological effect in the organism. The basic hypothesis is that similar molecules have similar activities. This principle is also called Structure-Activity Relationship (**SAR**). However, created hypotheses usually depend on the chemical structure and biological data. The widely used anti-cancer drugs such as doxorubicin and its derivatives were referred to the created hypothesis in mechanisms and intermolecular interactions study in rotenoid compounds. That leads to study and prove the important hypothesis of molecular structures and functional groups of rotenoid compounds that are necessary on cytotoxicity.

Doxorubicin (Fig. 1), also known as adriamycin, is an antibiotic used as a treatment for a wide range of solid tumors. This compound possesses an anthracycline chromophore containing four fused rings and a positively charged amino sugar. It interacts with DNA by intercalation and inhibits both DNA replication and transcription [1]. When the drug intercalates with DNA, the cyclohexane 'A' ring resides in the minor groove acting as an anchor, hydrogen bonding to base-pairs above and below. The planar 'D' ring resides in the major grove. The drug is held in place by the formation of favorable hydrogen bonds to the bases within DNA; for example, the hydroxyl group in the 9 position forms two hydrogen bonds to N2 and N3 of an adjacent guanine [2]. However, this drug does not specific to the base-pair sequence and shows a high level of toxicity as they will also interact with the DNA in many other tissues.

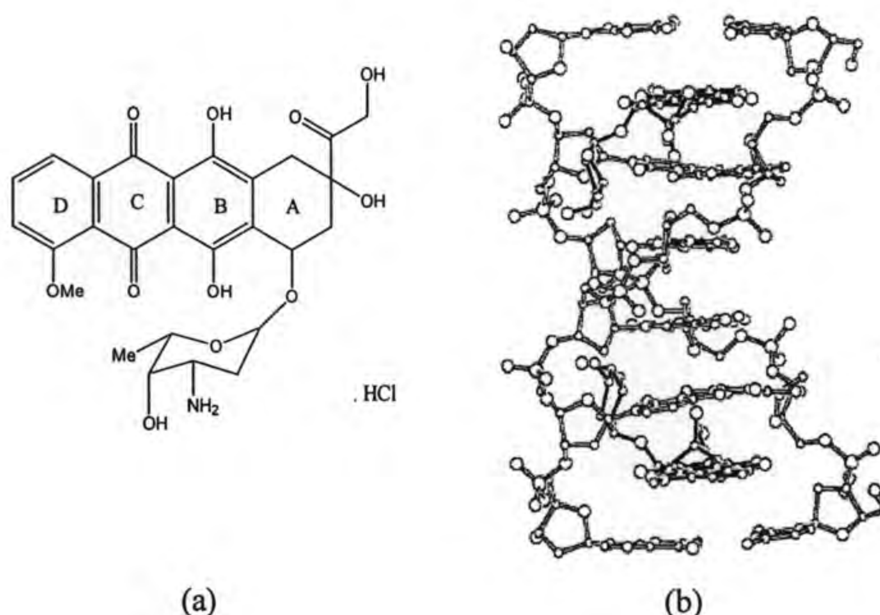

Fig. 1 Chemical structure of (a) doxorubicin HCl (b) Intercalation binding of doxorubicin- d(CGATCG)$_2$ [3]

1.2 Rotenoid compounds

Rotenoid, a four fused ring A, B, C and D [Fig. 2a], possess various bioactivities such as antimicrobial, antiviral action, insecticide and antifeedant properties. One of the interesting activities of rotenoid compounds is anticancer activity. For example, 6-deoxyclitoriacetal from the roots of *Clitoria macrophylla* [4] is known to show strong cytotoxic activity against culture P388 lymphocytic leukemia cell [5]. Rotenoids from *Amorpha fruticosa* are found to be inhibitors of human cancer cell line [6, 7, 8]. In Thailand, the rotenoid compound, 6-deoxyclitoriacetal [Fig. 2b], was isolated from the dried roots of of *Stemona collinsae* Craib. It has been known to have cytotoxic activity against various types of human carcinoma [9]. 6-Deoxyclitoriacetal presents both planar structures, heteroaromatic ring systems corresponding with the important characteristic of intercalation drug molecules. Not only the planar molecule, 6-deoxyclitoacetal present the bent shape as well. This is similar to the structure of known anticancer drug, doxorubicin. Although, 6-deoxyclitoacetal is not a derivative of doxorubicin, it shares structural similarities with doxorubicin.

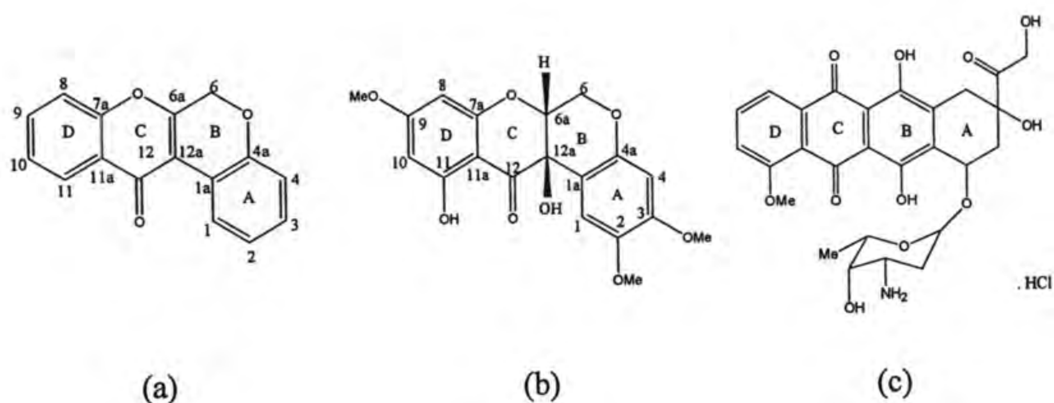

Fig. 2 Chemical structure of (a) rotenoid structure, (b) 6-deoxyclitoriacetal (c) doxorubicin HCl

Even though DNA-intercalating molecules are mostly planar, being planar is not enough to make a molecule be an anti-cancer drug. The structural similarities of Doxorubicin derivatives and 6-deoxyclitoacetal shed light to other necessary characteristics. Therefore, this research proposes a hypothesis that a substance has a good chance to be a DNA-intercalating anti-cancer drug if it possesses three characteristics: (i) molecule has a bent shape, (ii) a part of the molecule is planar and (iii) it has functional groups that have intermolecular interactions, such as π-π interactions of hydrogen bonding, with DNA. If this hypothesis is proven, these characteristics can be used to find novel anti-cancer drug candidates. Known cytotoxic substances such as 6-deoxyclitoacetal may be modified to enhance its effectiveness as an anti-cancer drug candidate. Also, substances that lack some characteristics can be modified to satisfy the requirements.

1.3 Drug-DNA interaction

In general, the major target for many anticancer drugs is deoxyribonucleic acid (DNA). DNA is the genetic material that stores genetic information. It is a long polymer made from repeating units called nucleotides. Chemically, DNA consists of two long polymers of simple units called nucleotides, with a backbone made of sugars and phosphate groups joined by phosphodiester bonds. DNA is double helix stabilized by hydrogen bonds between the bases attached to the two strands (Fig. 3).

The four bases found in DNA are adenine (abbreviated A), cytosine (C), guanine (G) and thymine (T) [10].

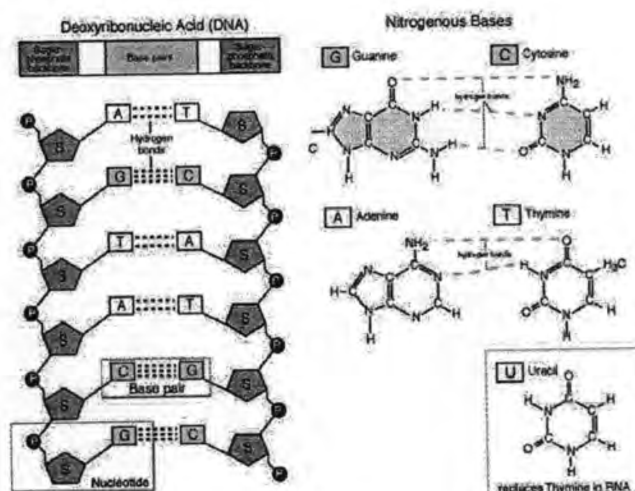

Fig. 3 Deoxyribonucleic acid (DNA) and hydrogen bonding of nitrogenous bases

The binding of small organic and inorganic molecules to DNA can interfere with the numerous processes, including transcription and replication, a major step in cell growth and division. Such interference by the anticancer drug can retard or prevent cell growth. Many of the anticancer agents in clinical used are known to interact strongly with DNA (e.g. adriamycin, cis-platin, mitoxantrone). The exact mechanism of action remains unresolved in most cases. There is a wide range of studies of the drug-DNA interactions. The major objective of such studies has been to determine the structure of the drug-DNA complex, with the objective being to use this information in the design of new derivatives [11].

The interaction of the anticancer drugs with DNA occurs principally by three different ways [12]. The first one is through control of transcription factors and polymerases; in which drug interacts with proteins that bind to DNA. The second is through RNA binding either to the DNA double helix to form nucleic acid triple helix structures or to exposed DNA single strand forming DNA-RNA hybrids that may interfere with transcriptional activity. The third type of interaction involves the binding of small aromatic ligand molecules to DNA double helical structures.

The binding of small molecules to DNA involves electrostatic interaction with the negatively charged nucleic acid sugar-phosphate structure, intercalation of planar

aromatic ring systems between base pairs and minor and major grooves binding interaction.

DNA helix is shown associated and interacted with proteins, such as Topoisomerase I and II. Blocking proteins from binding to DNA is the way of interfering with protein-DNA complexes. Topoisomerase are nuclear enzymes regulating the topoisomeraselogical state of DNA by breaking and rejoining of DNA strands [13]. Topoisomerase I generates single-strands breaks [14], and topoisomerase II introduces double-stranded breaks [15]. The cytotoxicity of topoisomerase I and II is due to stabilization of the enzyme-DNA covalent cleavage intermediates, which are referred to as cleavage complexes. Compounds that block access of topoisomerase to DNA do not show clinical efficacy; however, compounds that trap an intermediate in which the protein is covalently bound to DNA result in potent cytotoxicities [14, 15]. Clinically effective drugs that are believed to show their cytotoxicities through this mechanism include SN-38 (topoisomerase I), and doxorubicin and mitoxanthone (topoisomerase II). In addition, two principal modes for binding of small molecules to DNA are intercalation binding and minor groove binding [16].

The first mode is DNA-intercalation. Intercalating drugs should have planar, heteroaromatic ring systems which insert themselves between two adjacent base pairs in a DNA helix. The drug-DNA complex is stabilized by π-π and van der Waals interactions between the DNA base and the drug molecule. Intercalating drugs also cause structural perturbations in the DNA to accommodate the binding, such as the unwinding of the helix and a lengthening of the DNA, thereby, inhibits the ability of enzymes such as topoisomerase II to interact with DNA (Fig. 4). This enzyme cleaves double stranded DNA to reduce the strain that comes from local unwinding. The formation of drug-DNA-enzyme complex leads to breakage of the DNA backbone and inhibits DNA replication and transcription of the target DNA [17]. Intercalators include ethidium bromide, doxorubicin and its derivatives (Fig. 5).

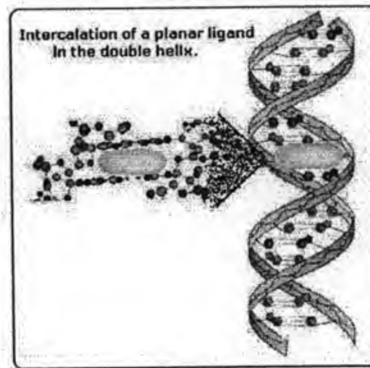

Fig. 4 Intercalation of a planar ligand in the double helix [18]

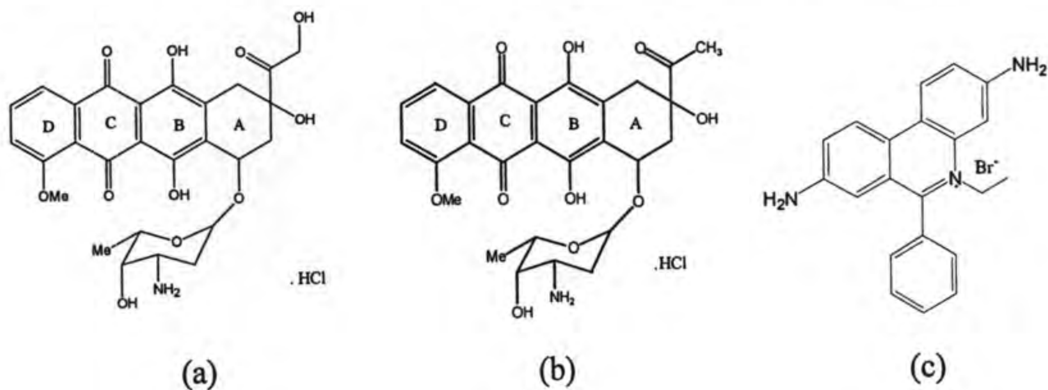

Fig. 5 Chemical structures of (a) doxorubicin HCl (b) daunorubicin HCl and (c) ethidium bromide

The second mode is a minor groove binding. These drugs consist of several aromatic rings (e.g. benzene or pyrrole). The drug-DNA binding is stabilized by hydrophobic interactions, as well as van der Waals interactions and hydrogen bonding. The drug-DNA binding preference is to the A-T base pairs. Minor groove binders do not induce significant structural changes to the DNA. Drugs in this category include Hoechst 33258 (Fig. 6).

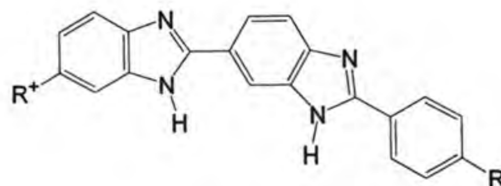

Fig. 6 Chemical structure of Hoechst 33258 [18]

Many anticancer drugs have the mechanism relationships of ligand binding to DNA. Many intercalators have cytotoxic activity by stabilizing the ternary DNA intercalator-Topoisomerase complex [19]. Many anticancer compounds have not yet fully understood but were suggested including DNA intercalation and inhibition of topoisomerase II acitivity. Also, the antitumor activity of anthracyclines, such as doxorubicin, mainly resulted from an inhibition of mammalian topoisomerase II. [20, 21]. The topoisomerase is involved in the control of the shape of DNA. The DNA intercalators have cytotoxocity by inhibiting topoisomerase. The representative DNA intercalators are anthracyclines, acridines, alkaloids, anthracenediones, cumarins, indoles, quinolines and quinoxalines [20].

1.4 DNA binding studies

1.4.1 Spectrophotometric methods

The interaction of DNA with nucleic acid binding molecules has been studied by a variety of techniques [22-26] such as UV-vis spectroscopy, NMR spectroscopy, X-ray crystallography, cyclic voltammetry, circular dichrorism. All procedures relied on a variety of physico-chemical methods to monitor changing the overall properties of either the drug (e.g. absorbance, fluorescence, CD) or the DNA (e.g. viscosity and sedimentation). These techniques are discussed as follows.

1.4.1.1 UV-vis spectroscopy

The binding of a drug to DNA produces hypochromism and red shift in the drug absorption band. This effect is especially pronounced for intercalators [27, 28]. Red shift and hypochromism in the absorption band of DNA intercalating drugs are usually assigned to the π-π interaction between DNA bases and intercalated drugs. Changes in the absorption spectrum of the drug after mixing with DNA indicated that the drug directly forms a complex with double-helical DNA and evaluates the structural changes in the DNA helix.

1.4.1.2 Melting temperature (*Tm*)

Classic interactions of drugs with DNA base pairs result in changes in the DNA melting temperatures (*Tm*). It is well known that when the temperature in the solution increases, the double-stranded DNA gradually dissociates to single strands, and generates a hyperchromic effect on the absorption spectra of DNA bases and increasing in melting temperature (*Tm*). It is occasionally used as an indication of drug-DNA interaction [27, 28].

1.4.1.3 Circular dichroism (CD) spectroscopy

Circular dichroism (CD) spectroscopy can be used for studying the interactions of DNA with the molecule that possesses the chiral center. A CD signal is induced upon binding to DNA. It produced from the interactions between the electronic transition moments of drugs and chirally arranged electric transition moments of DNA bases. For the intercalators, the induced CD signal is generally weak, while a strong positive CD signal in the drug absorption region is apparent for the minor groove binders [29].

1.4.1.4 Nuclear magnetic resonance (NMR)

NMR can provide the detail of localized information on molecules and materials. NMR can be used to observe static as well as dynamic interactions between molecules. 1D-2D NMR spectroscopy indicate that the binding of drug to the DNA through H-bonding formation. All resonances and chemical shifts have been assigned. The changes in chemical shift between the free and the bound forms are associated with the hydrogen bonding between drug molecule and base of DNA [30].

1.5 Inhibition of topoisomerase II

DNA topoisomerase are enzymes that catalyze the passage of individual DNA strands (type I) or double helices (type II). These enzymes have important roles in replication, recombination, transcription, chromosome condensation, and the maintenance of genome stability, and hence are good targets for antineoplastic drugs. Topoisomerase II catalyzes DNA topoisomerases logical reactions via a DNA breakage/reunion mechanism. The breakage/reunion reaction of topoisomerase II can

be interrupted by many anticancer drugs, resulting in the accumulation of a topoisomerase II- DNA covalent intermediate, the cleavable complex. Accumulation of topoisomerase II-DNA cleavable complexes causes tumor cell death. [14, 15]. The antitumor drugs doxorubicin and etopoisomeraseside are representative topoisomerase II inhibitors. Therefore, in this study, to investigate one possible mechanism of action of the cytotoxic activity of rotenoid, we evaluated their ability to inhibit topoisomerase II in activities with DNA relaxation.

1.6 Objectives of this research

1. To isolate and derivatize 6-deoxyclitoriacetal extracted from the dried roots of *Stemona collinsae* Craib.
2. To characterize 6-deoxyclitoriacetal and its derivatives.
3. To test the cytotoxicity of 6-deoxyclitoriacetal and its derivatives.
4. To study the structure-activity relationship on cytotoxicity of 6-deoxyclitoriacetal and its derivatives base on the hypothesis of DNA-intercalating anti-cancer substance.
5. To study the % inhibition topoisomerase II 6-deoxyclitoriacetal and its derivatives.