CHAPTER IV

CONCLUSION

4. CONCLUSION

6-Deoxyclitoriacetal (**1**) and stemonal (**2**) are rotenoid compounds extracted from the dried root of *Stemona collinse* Craib. 6-deoxyclitoriacetal has good cytotoxic activity against various types of human carcinoma. Therefore, in order to enhance its cytotoxic activity, the structure-activity relationship on cytotoxicity was investigated. The hypothesis that might attribute to cytotoxic activity of a compound was proposed and proved. That is, the molecule should possess three characteristics: (i) the molecule has a bent shape, (ii) the molecule has a planar part to intercalate with DNA and (iii) the molecule has functional groups that have intermolecular interactions to stabilize the intercalation.

The crystal structures of **1**, **2** and their cytotoxic activities revealed that not only the planar structure is necessary in the intercalation process, but also the bent shape and functional groups can help to stabilize the intercalation.

The interactions between **1**, **2** with ct-DNA and $d(CGTACG)_2$ have been studied by spectroscopic methods. The results from UV, T_m and CD showed that **1**, **2** can bind with DNA and deform the DNA strand. From the NMR titration, the 1:2 stable complex of $d(CGTACG)_2$ with **1** and **2** were investigated. From 1D and 2D NMR spectroscopy, it can be concluded that **1** and **2** can intercalate between C1pG2 and C5pG6 base pairs. However, the complex of **1** with $d(CGTACG)_2$ are strong binding than that of **2**, changing the spectra of the complex. These results correspond with the good anticancer activities of **1**. It can be concluded that this is probably because **1** can bind with DNA better than **2**. Owing to **1** has a planar moiety that can intercalate with DNA. Its bent shape can promote locking the molecule in DNA grooves. Whereas, **2** is a planar molecule, it lacks of a molecular part to lock the molecule within the DNA double strand.

In order to study the effect of the functional groups on the DNA-binding ability, the 6-deoxyclitoriacetal analogues were prepared and then tested for their cytotoxicities. All compounds were tested the cytotoxicity against KB (Human mouth

carcinoma), MCF7 (Breast cancer) and NCL-H187 (Human small lung cancer) cell lines. There are three groups of 6-deoxyclitoriacetal analogues.

Among the 6-deoxyclitoriacetal – amino acid analogues **(A, A1** to **A5),** The compound **A** showed strong cytotoxicity against KB, MCF7 and NCI-H187 with the IC_{50} value of 2.87, 7.33 and 3.21 μg/ml, respectively. Because the amine groups of the amino acid can open the epoxide ring and then form stable DNA complex. Among the 6-deoxyclitoriacetal – pyrimidine base analogues **(B** and **B1** to **B3),** uracil derivative showed the strongest cytoxicity. This is consistent with the commercial anticancer drug containing uracil pharmacophore. Among the 6-deoxyclitoriacetal – aromatic carboxylic analogues **(C** to **G),** 4-aminobenzoic acid derivative showed the strongest cytotoxic activities against KB and NCI-H187 with IC_{50} of 2.64 and 8.28 μg/ml, respectively. This is probably because the amine group can participate strong hydrogen bonding, hence leading to the stable DNA-compound complex.

To further elucidate the mechanism of action of 6-deoxyclitoriacetal, stemonal and 6-deoxyclitoriacetal analogues (**A1** to **A5**) for cytotoxicity, the relaxation of supercoiled plasmid pBR322 DNA was evaluated. Etoposide are selective topoisomerases II inhibitors being used as positive control. The compound A1 to A5 showed the moderate inhibitory topoisomerase II, giving 50.10, 35.60, 31.50, 30.20, 39.50 % inhibition, respectively. The results of topoisomerase II inhibition of **A1** to **A5** are consistent with the cytotoxicity activity.

All of the cytotoxic activities and % inhibition topoisomerases II were tabulated in Table 23. In conclusion, the results have confirmed the hypothesis.

Table 23 The summary of the characteristics, cytotoxic activities and % inhibition topoisomerases II of all compounds

Compound	Characteristics				IC_{50} (μg/ml)			Relaxation activity for Topoisomerase II % Inhibition
	Planar	Bent shape	Functional group	Steric	KB	MCF7	NCl-H187	
Doxorubicin	√	√	√		0.325	0.822	0.041	-
Ellipticine	√				0.147	Inactive	0.441	-
Etoposide	√	√			-	-	-	68.94
6-deoxyclitoriacetal **(1)**	√	√	√		0.08	0.26	0.04	73.51
Stemonal **(2)**	√		√		Inactive	Inactive	Inactive	37.25
A	√	√			2.87	7.33	3.21	-
A1	√	√		√	23.22	Inactive	20.74	50.10
A2	√	√		√	35.54	25.61	3.31	35.60
A3	√	√		√	5.05	18.57	6.01	31.50
A4	√		√	√	Inactive	Inactive	Inactive	30.20
A5	√		√	√	1.45	38.45	Inactive	39.50
B	√	√	√		0.017	Inactive	0.018	-
B1	√		√	√	18.65	Inactive	11.61	-
B2	√		√	√	25.39	Inactive	11.61	-
B3	√		√	√	1.45	Inactive	0.255	-
C	√	√		√	Inactive	Inactive	Inactive	-
D	√	√		√	40.21	Inactive	22.50	-
E	√			√	32.06	Inactive	19.13	-
F	√		√	√	47.83	Inactive	27.29	-
G	√		√	√	2.64	Inactive	8.28	-

(-) = not determined