CHAPTER IV

GENERAL DISCUSSION AND CONCLUSION

Flavonoids are found ubiquitously in higher plants and constitute an important component of the majority of peoples' daily diets. Our research group has focused for more than ten years on their anticancer and antioxidant activity. We have considered the polydroxylated and polymethoxylated flavonoids as a potential new generation of anticancer candidates. They are the mediated antiproliferation of various cancer cell types, such as MDA-MB 435, erythomyelogeneous leukemic K562, and small cell lung carcinoma GLC4. The particular finding is that these molecules exhibited more cytotoxicity against multidrug resistant cells than their corresponding parent cells such as K562/*adr* cell with overexpression of P-gp and GLC4/*adr* with overexpression MRP1. In addition, they did not inhibit cell growth nor cell differentiation of blast cells compared with doxorubicin (the anticancer drug use in clinic).

This study emphasized on the physicochemical properties of molecules, especially their behavior in aqueous physiological solutions, the predominant active form and the active sites responsible for anticancer activity. Indeed, the efficacy and the specific activity of pharmaceutical molecules depend on their intracellular target concentration. With regard to this subject, the transport of molecules across the membrane is an important parameter determinant of the intracellular target concentration and cellular distribution of the molecules. The micromultilamellar

vesicle labeled DPH is a suitable model for determining the interaction and the mean rate influx coefficient of flavonoids. The study provides a new method for determining the mean rate influx coefficient of non-intrinsic fluorescent molecules such as flavonoids.

On the basis of our results, all flavonoids: catechin, eriodictyol, apigenin, kaempferol, quercetin, 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (WP279), 5,5′-dihydroxy-6,7,3′,4′-tetramethoxyflavone (WP280) and 5,3′-dihydroxy-3,6,7,8,4′-penta methoxyflavone (WP283), were studied (except catechin and WP280) and can determine the rates of deprotonation by means of spectrophotometry. The deprotonated anionic form of flavonoids is highly soluble in an aqueous physiological buffer and is not prone to aggregation. The protonated form of these flavonoids is much less soluble and tends to aggregate following precipitation. The methoxyl group substitutes in place of hydrogen atoms and/or hydroxyl groups at various positions of carbon atoms in ring A, B and C resulting in an increase in solubility and lipophilicity.

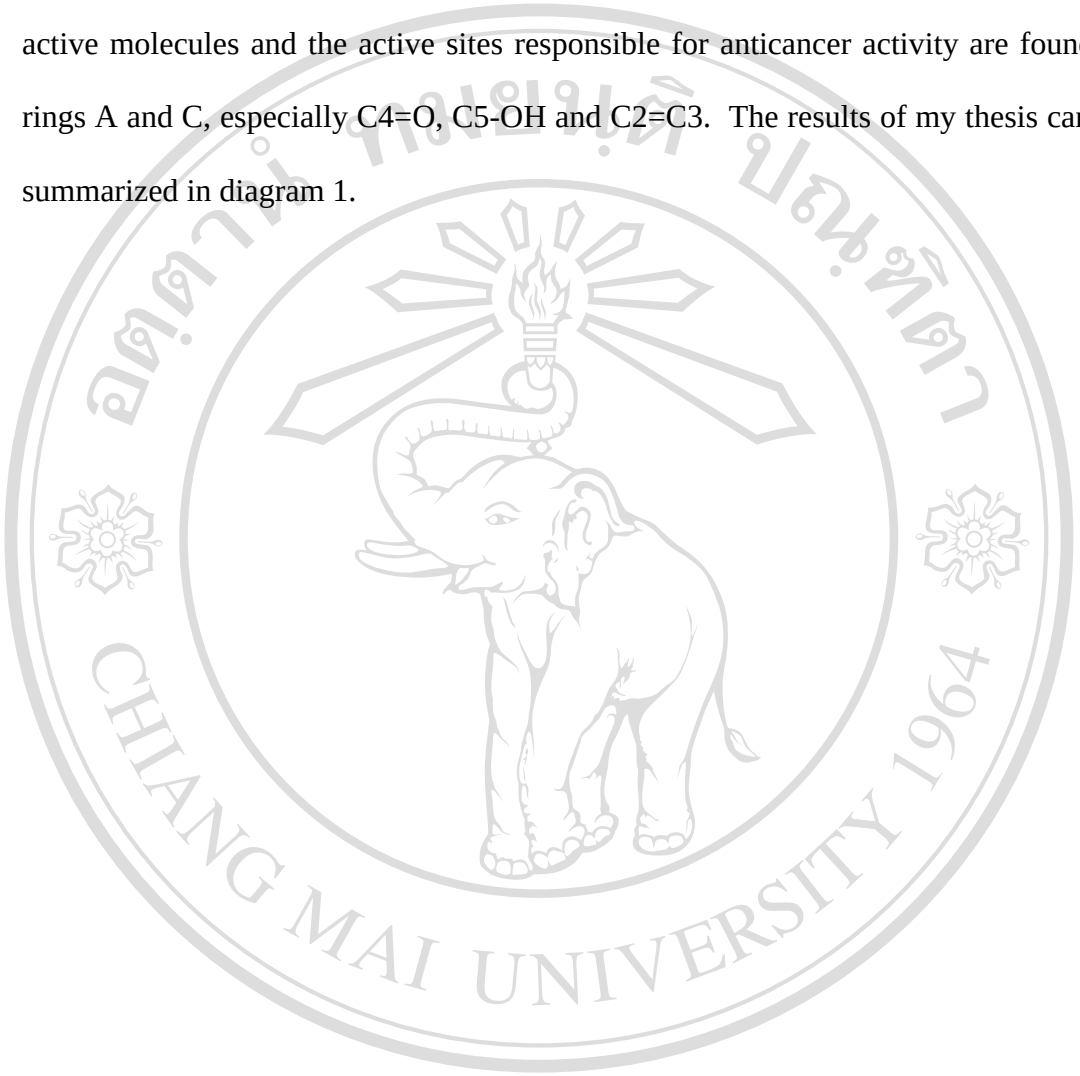
The interaction of flavonoids with myristyl myristate-Tween20 bilayers in terms of the permeability and distribution on those lipid bilayers membranes was determined. The neutral form of flavonoids was passively diffused and it accumulates in hydrophobic zone where DPH molecule is located following DPH fluorescence which is quenched by flavonoid collision. Then, flavonoids are translocated across the lipid bilayer membrane to the inside of liposome. Let us consider chemical structure; eriodictyol that is lacking in a double bond at C2=C3 and hydroxyl group at C3 have k_+ value slower than apigenin which contains a double bond at C2=C3 and is lacking in hydroxyl at C3 by factor 4. The particular observation was that eriodictyol has

k_+ value slower than quercetin that contains double bond at C2=C3 and hydroxyl at C3 by factor 8. The results clearly show that both C2=C3 and C3-OH are essential in the facilitation of the flavonoids to translocation across the lipid bilayer membrane. The methoxyl group substitutions are increase at the rate of diffusion. The methoxyl group substitution occurred at C6, C7, C3′, C4′ and hydroxyl group at C5′ but was lacking in the C3-methoxyl group (WP 280) which caused an increased in k_+ value by factor 6 compared to eriodictyol. The substitution of methoxyl groups at various positions of carbon atoms on ring A, B and C (WP 279 and WP283) caused an increase in k_+ value by factor 8.

In addition, the translocation across the lipid bilayer membrane of the flavonoid effects the conformation of bilayers. In physiological pH, the phase transition temperature of the liposomal bilayer was shifted to lower temperatures. The lower phase transition temperature implies a loose packing state of the liposomal bilayer. It is suggested that (1) flavonoids underwent ketalisation in which the head of myristly myristate (ketalization, see in scheme 2) yielded hemiketal and ketal group resulting in an increase in the distance between lipid-lipid molecules and (2) flavonoids located in the hydrophobic zone of bilayers.

Flavonoids used in this study exhibited anticancer activity in micro-molar concentration range, slightly more efficacy in MDR cells than their corresponding sensitive cell lines. The anticancer efficacy of molecules did not change when the molecules contained C2=C3, C4=O and C3-OH (kaempferol and quercetin). But the molecules lacking in C2=C3 (eriodictyol) decreased in activity against the multidrug resistant K562/*adr* and dramatically decreased against K562 when the molecule was lacking in both C2=C3 and C4=O (catechin). The methoxyl substitutions are

increased in cytotoxicity against all cell lines with similar efficacy in both drug-sensitive and drug-resistant cells. The neutral forms of flavonoids are predominantly active molecules and the active sites responsible for anticancer activity are found in rings A and C, especially C4=O, C5-OH and C2=C3. The results of my thesis can be summarized in diagram 1.



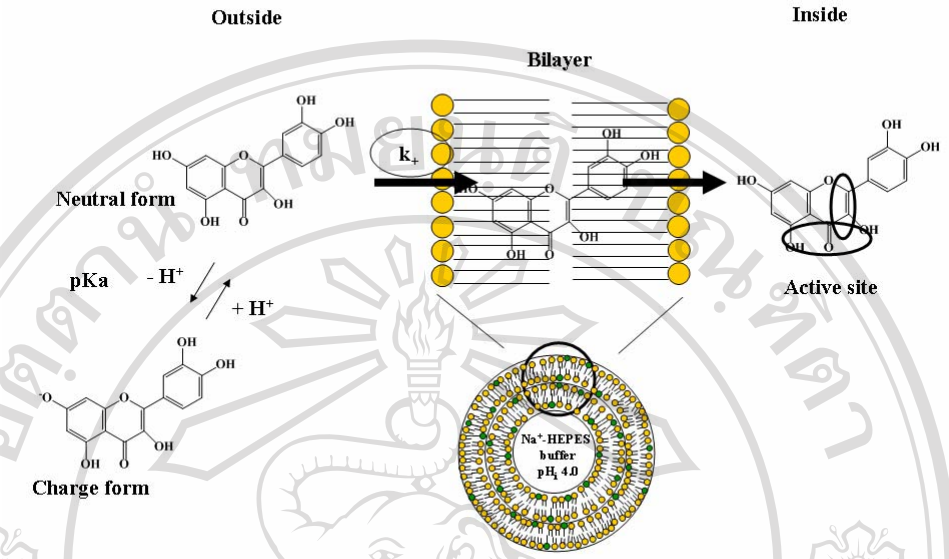


Diagram 1 The behavior of flavonoids in aqueous physiological condition are crucially important in determining the potential active form. For example, flavonoids can be found in neutral state and charge form when soluble in physiological solution and their neutral form tends to be aggregate following precipitation. This can be prevented by methoxyl substitution at C3. The neutral form can passively be diffused across and a molar fraction can reside in the hydrophobic zone of the membrane. The reversible ketolisation of flavonoids within keto group of biological molecules seem to play a predominant role in the cellular distribution pattern. Once the flavonoids find at intracellular target, we propose it shall be the neutral form that contains C2=C3, C4=O, C3-OH and C5-OH as its active site