CHAPTER I

INTRODUCTION

Cytotoxic compounds have become an effective cure for some previously lethal forms of malignancies. Currently, it is the treatment of choice in many rapidly progressing tumorous diseases. Selected combinations of cytotoxic agents with different intracellular targets are especially useful in the treatment of cancer patients (Sarkadi and Muller, 1997; Sauna *et. al.*, 2001).

Most of the antineoplastic drugs have narrow therapeutic index because they target proteins and nucleic acids commonly found in both cancerous and normal cells. The signs and symptoms of toxicity of these compounds include mucositis, diarrhea, myelosuppression, alopecia, and infertility. Generally, the cells with relatively high proliferative rate are more susceptible to the cytotoxic effects of these chemotherapeutics (Rubin and Hait, 2006). The therapeutic failure occurs occasionally because of patients' intolerability and resistance to treatment.

Chemotherapy has a limited potential for cancer cure due to acquired or intrinsic resistance of cancer cells to anticancer drugs. Resistance to one drug often implies resistance to a series of different drugs. This phenomenon is known as multidrug resistance (MDR), which leaves the clinician with few therapeutic options and the patient with a sinister prognosis. Gastrointestinal, hepatobiliary and renal cancers are largely unresponsive to chemotherapy because of a high degree of intrinsic MDR. Some cancers such as leukemia, lymphomas, ovarian and breast cancers respond to treatment initially, but they acquire resistance during the course of the disease. Several mechanisms have been suggested in MDR development. For example, growing tumors often develop physical obstacles rendering the decrease in tumor blood flow and drug delivery. In addition, increases in metabolic inactivation as well as active removal of anticancer drugs have been demonstrated in MDR cancer cells (Sikic *et al.*, 1997; Lehne, 2005).

P-glycoprotein (P-gp) is one of the important multidrug transporters in MDR phenomenon (Germann and Chambers, 1998). Despite the diversity in chemical structures and pharmacological actions of the cytotoxic drugs, P-gp can effectively pump them out of the cancer cells resulting in reduction of their therapeutic efficacy

(Ozben, 2006). The inverse correlation between P-gp expression and chemosensitivity has been established in leukemia, lymphomas, osteogenic sarcoma, small-cell lung cancer, breast cancer, and pediatric solid tumors (Sikic *et al.*, 1997; Thomas and Coley, 2003).

A large number of chemicals with heterogeneous molecular structure are able to bind with P-gp and influence its activity. Generally, P-gp substrates share a common structure of a basic nitrogen atom and two aromatic planar domains in hydrophobic molecule. They are chemically diverse and may carry a positive charge at physiological pH (Ambudkar *et al.*, 1999). Examples of P-gp substrates include anticancer drug such as anthracyclines (*e.g.*, doxorubicin), *Vinca* alkaloids (*e.g.*, vincristine, vinblastine), podophyllotoxins (*e.g.*, etoposide), and taxanes (*e.g.*, taxol) (Sauna *et al.*, 2001; Sawicka *et al.*, 2004).

The structure activity relationship of P-gp inhibitors is also poorly established. Similarly to P-gp substrate, the inhibitors share a basic molecular structure comprising a cationic protonable site linked to an aromatic lipophilic part (Wang *et al.*, 2003). Examples of these compounds are arylalkylamine (verapamil), aryloxypropanolamines (*e.g.*, propafenone), indole alkaloids (*e.g.*, vindoline, reserpine), quinoline (*e.g.*, chloroquine, quinine), and isoquinoline alkaloids (*e.g.*, cepharantine) (Hirai *et al.*, 1995; Avendano and Menendez, 2002).

The clinical application has been adopted to suppress MDR by using the non-cytotoxic P-gp modulators to inhibit P-gp-mediated efflux of cytotoxic drugs. Several classes of modulators have been identified among drugs that were originally developed for other therapeutic indications such as calcium channel blockers, calmodulin antagonists, steroid hormone, and immunosuppressive agents. Verapamil, a calcium channel blocker, was the first agent that was shown to modify MDR *in vivo* and *in vitro* (Ford and Hai, 1990). Unfortunately, its MDR modulating activity requires concentrations that are associated with severe cardiac toxicity in patients. Cyclosporine A, an immunosuppressive agent, is a highly potent inhibitor of P-gp in a number of cell lines and animal models, but its immunosuppression restricts its clinical use (Liscovitch and Lavie, 2002; Thomas and Coley, 2003; Mahadevan and List, 2004). The MDR reversing agent may expose the patient to unacceptable side effects or toxicity at effective dose and affect the pharmacokinetics of anticancer

drugs (Sikic, 1997; Krishna and Mayer, 2000). These limitations have supported the need to search for new more effective compounds.

Recently, marine natural products have yielded a considerable number of drug candidates. These natural products exhibit a broad spectrum of biological activities such as anticancer, antibacterial, antifungal, anti-inflammatory and antiviral activities (Haefner, 2003). For example, Ecteinascidin-743 (ET-743) is a marine tetrahydroisoquinoline alkaloid isolated from tunicate *Ecteinascidia turbinate*, which is currently under clinical investigation in Europe and the United States as a promising new class of anticancer drug (D'Incalci *et al.*, 2002; Minuzzo *et al.*, 2005). ET-743 exhibits a potent cytotoxic activity against a variety of tumor cell lines *in vitro* and against several rodent and human tumors and human tumor xenografts *in vivo* (Fricker, 2001). Furthermore, this compound is classified as the fourth generation of MDR1 inhibitors with ability to suppress P-gp expression in cell culture by down-regulating P-gp/*MDR1* gene (Kanzaki *et al.*, 2002; Liscovitch and Lavie, 2002; Mahadevan and List, 2004).

Renieramycin M (RM) is a new tetrahydroisoquinoline compound which can be isolated from a blue sponge, *Xestospongia sp.*, growing around Sichang Island in the Gulf of Thailand. RM has been reported its anti-tumor activity in several cell culture models including human colon carcinoma (HCT116), human lung carcinoma (QG56), human lung carcinoma (NCI-H460), and human colon carcinoma (DLD1) (Suwanborirux *et al.*, 2003; Saito *et al.*, 2004). However, being a novel natural compound, the characteristic property and selectivity of RM toward different cell types is very limited, especially the normal cells and the cells with high MDR activity. Furthermore, the effects of RM on P-gp modulation have not been reported.

Besides its cytotoxic activity, it is possible that RM is able to modulate P-gp function. Consequently, the cancerous cells with MDR activity should be more sensitive to RM treatment.

Hence, the objectives of this thesis are to

1. Determine the cytotoxic effects of Renieramycin M in different cell lines including dermal fibroblast (CC2511), renal epithelial cells (LLC-PK_1), buccal carcinoma (KB), lung carcinoma (H460) and *MDR1* gene-transfected epithelial cells (LLC-MDR_1).
2. Determine the interaction of Renieramycin M with P-gp and elucidate the potential action of Renieramycin M as P-gp modulators.