CHAPTER I

INTRODUCTION

Geldanamycin, the 17-substituted benzoquinoid ansamycin antibiotic, was reported as the secondary metabolite produced by the terrestrial *Streptomyces hygroscopicus* var. *geldanus* (DeBoer *et al.*, 1970; Heisey and Putnam, 1986). Geldanamycin binds to the ATP binding site of the chaperone heat shock protein 90 (Hsp90), resulting in prevention of several signaling proteins from reaching their mature form, inhibiting their activity and affecting their stability (Roe *et al.*, 1999). This compound possesses antitumor activity against cancer cell lines and in animal models (Schulte and Neckers, 1998).

The antitumor activity of geldanamycin has been attributed to destabilization of key client proteins, including receptor and nonreceptor kinases (Erb-B2, epidermal growth factor receptor, and Src family kinases), serine/threonine kinases (c-Raf-1 and Cdk4), steroid hormone receptors (androgen and estrogen), and cell cycle and apoptosis regulators such as mutant p53 (Hostein *et al.*, 2001). However, the development of geldanamycin as a clinical agent has so far been limited by its toxicity, especially liver toxicity. Due to the promising antitumor property and the toxicity of geldanamycin, the development of other biologically active geldanamycin derivatives has become an interesting and important endeavor (Schulte and Neckers, 1998).

The crystal structure of geldanamycin-Hsp90 complex revealed that geldanamycin binding to Hsp90 is highly compact with more than 85% surface area of geldanamycin buried in the complex. The benzoquinone group of geldanamycin binds near the entrance of Hsp90 binding pocket, whereas the ansa ring inserts into the binding pocket (Cheng *et al.*, 2005; Stebbins *et al.*, 1997). SAR studies showed that C-7 position and C-11 position of geldanamycin are critical for its anticancer activity. Modification of these two positions resulted in inactive compounds. However, modification of C-17 position on the quinone ring maintained the anticancer activity within nanomolar range (Cheng *et al.*, 2005; Schnur *et al.*, 1995 (a); Schnur *et al.*, 1995(b)).

Among the modified geldanamycin derivatives, 17-allylamino-17-demethoxygeldanamycin showed the highest activity against $p185^{erb-B2}$ (Schnur *et al.*, 1995 (a)). It compared favorably with geldanamycin in terms of animal toxicity and caused fewer hepatic side effects. The compound is currently in phase II clinical trials at the National Cancer Institute, USA (Cheng *et al.*, 2005).

Importantly, geldanamycin treatment induced different response in different cell lines. It triggered apoptosis of PC12 cells, whereas it induced differentiation with neurite outgrowth in the murine neuroblastoma N2A (López-Maderuelo *et al.*, 2001). Interestingly, geldanamycin prevented neurotoxic effects of anticancer drugs on cultured dorsal root ganglion neurons from chick embryo at low doses (Sano, 2001) and protected rat brain from focal ischemia (Lu *et al.*, 2002). The effect of geldanamycin on neuronal cells is not easily explained. Nevertheless, geldanamycin binds specifically to Hsp90 and may protect neuronal cells from apoptosis by suppressing the signal transduction pathway(s) involving Hsp90. However, interaction of limited doses of geldanamycin with other specific target molecule(s) to suppress apoptosis cannot be ruled out (Sano, 2001). According to the effect of geldanamycin on neuronal cells, our interest is focused on the neuroprotective property of geldanamycin and its derivatives on other neuron cultures.

Cell lines used in the assay on neuronal cells are SH-SY5Y human neuroblastoma cells, LA-N-5 cells (human neuroblastoma cells less differentiated than SH-SY5Y), PC12 cells (rat pheochromocytoma), N2A cells and NS20Y cells (mouse neuroblastoma) (Gold, 2004). However, in this study, we are interested in the differentiated P19 cells as an *in vitro* model for study the effect of geldanamycins on neuronal cells culture. P19 cells represent an early stage of neuronal development, while other cell lines such as pheochromocytoma (PC12) or neuroblastoma represent late stages of neuronal differentiation (Parnas and Linial, 1995). In addition, there was no previous study in the effect of geldanamycin on P19 cells and P19-derived neurons. The information from this study will be remarkably useful for the further development of geldanamycin as a novel drug for neuroprotection which might also give us more knowledge in the mechanism of action of geldanamycin as a neuroprotective agent for the treatment of Alzheimer's disease.

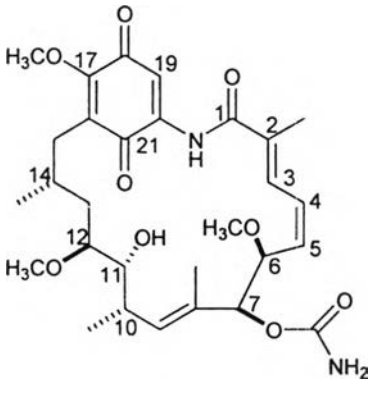
The P19 embryonal carcinoma cell is a pluripotent stem cell line which is differentiated into neurons by retinoic acid (McBurney and Rogers, 1982; MacPherson and McBurney, 1995; Jones-Villeneuve *et al.*, 1982). Cultures of P19-derived neurons contain various neurotransmitters such as γ-aminobutyric acid or GABA, and contain glutamic acid decarboxylase, the enzyme responsible for GABA synthesis. In addition, GABA-transaminase, the first enzyme in the GABA degradative pathway, was detected, which represented the GABAergic neurons (MacPherson and McBurney, 1995). The neurotransmitter acetylcholine was also detected, suggesting the presence of cholinergic neurons (Staines, 1994; Jones-Villeneuve *et al.*, 1983). Less than 1% of the neurons differentiated from P19 cells are catecholaminergic, containing tyrosine hydroxylase. The derived neurons are irreversibly postmitotic and exhibit many characteristics of mature CNS neurons (MacPherson and McBurney, 1995).

As a part of our interest in exploring biologically active secondary metabolites of marine microorganisms obtained from Thai marine environment, several *Streptomyces* isolates from mangrove forests along the Andaman sea coast have been investigated. The notably antifungal activity of *Streptomyces* sp. TRA9875-2, isolated from a rotten bark collected from mangrove forest in Trang province, was discovered. The EtOAc extract prepared from the fermentation broth of the strain exhibited the promising antifungal activity against *Candida albicans* ATCC 10231. Antifungal-assay guided fractionation led to the isolation of a new ansamycin analog, 17-*O*-demethylgeldanamycin hydroquinone, from the antifungal EtOAc extract prepared from the fermentation broth of the strain, together with two known metabolites, geldanamycin as a major metabolite and 17-*O*-demethylgeldanamycin (Tadtong, 2000). Including the advantages of P19 cell line as described above, therefore, the objectives of this research are to

1. Modify the chemical structures of geldanamycin isolated from *Streptomyces* sp. TRA9875-2.
2. Identify and elucidate the chemical structures of the modified geldanamycin derivatives.
3. Evaluate cytotoxicity and biological effect of the geldanamycin

derivatives on P19 cells and P19 neuron like cells and compare with those of geldanamycin.

The information mentioned above leads us to further investigate the modification of geldanamycin in several positions as follow



Geldanamycin

1. Modification of 17-OCH_3 into other alkoxy derivatives.
2. Modification of 17-OCH_3 into alkylamino derivatives.
3. Modification at C-19 position.
4. Modification of 11-OH into other alkoxy derivatives.
5. Reduction of double bond at C-2 to C-4 positions.