CHAPTER I

INTRODUCTION

Nowadays, cancer and infectious diseases are serious public health problems in worldwide because of drug resistance (Levy and Marshall, 2004; Dean, Fojo, and Bates, 2005; Roberts *et al.*, 2005). Although, there are a lot of therapeutic agents that were used extensively in clinical status, they are insufficient to get rid of these problems. The researchers attempt to discover new therapeutic agents by several strategies (Shoemaker *et al.*, 2002). Those agents could be derived from natural products, semi-synthesis or even total synthesis. The natural products seem to be the best source to derive excellent therapeutic agents (Mann, 2002). There is a biodiversity in Thailand to obtain new compounds and evaluate for their biological activities.

Topoisomerase I play a key role in cellular processes affecting the topology and organization of intracellular DNA. It has been isolated from prokaryotes and eukaryotes. Thus, it is an interesting target for anticancer or antimicrobial agents. Camptothecin is a first prototype anticancer affecting this enzyme (Bailly, 2000). Because of its severe side effect, camptothecin was not used in clinical level. At present, only two topoisomerase I inhibitors, topotecan and irinotencan, are approved by FDA to treat cancer (Takimoto *et al.*, 1998). In addition, drug resistance of these inhibitors had been previously reported (Parchment and Pessina, 1999).

Conventional topoisomerase I assays for screening inhibitors are typically based on gel electrophoresis (Champoux, 2001; Stewart and Champoux, 2001). There are two assays for clarifying the mechanism of inhibitors. The First one was called DNA relaxation assay which indicated only general topoisomerase inhibition of unknown compounds and the second one was called DNA cleavage assay which showed specific inhibition of unknown compounds, called topoisomerase poisons. The Disadvantages of these assays are time consuming and low throughput. They are unsuitable to manage a large number of unknown compounds. Many researchers attempt to develop the fast, uncomplicated assays (Hwang *et al.* 2000; Kwon *et al.*, 2004). However, these assays are expensive because of its modified substrate.

Andrea *et al.* (1991) had introduced a quantitative fluorescence assay using ethidium bromide as a probe. Enzyme reactions of this method were similar to that of conventional assay. Nevertheless, the assay was not performed in 96-well microplate format. Due to the genotoxic of ethidium bromide, this method was not interested to use for screening. There was an evidence of fluorescent dye that demonstrated the difference fluorescence intensity on topoisomer state of plasmid which used as topoisomerase I substrate (Sandhu, Warters, and Dethlefsen, 1985). Therefore, it is possible to use other dyes instead of ethidium bromide.

Rock *et al.* (2003) had developed method for determining nicked plasmid in solution in 96-well microplate format. This method is possible to detect cleavage complex induced by the enzyme or topoisomerase poisons.

In the present study, DNA relaxation assay and DNA cleavage assay for topoisomerase I inhibitory activity were combined and were developed to microplate format and using some sensitive fluorescent dyes.

Objective

To develop a rapid fluorescence microplate assay for topoisomerase I compared with gel electrophoresis, a conventional method.

Conceptual framework

The conceptual framework was showed in Figure 1. The fluorescence dyes are used to measure the difference intensity on topological state of plasmid. The Z′ value is taken to investigate assay performance of each dye. If the proposed assay has an acceptable Z′ value, time-course study of enzyme activity was performed by proposed assay compared with conventional assay using various DNA concentrations. Known topoisomerase inhibitors are included to test the detection ability of both assays. The IC_{50} values of these topoisomerase inhibitors are compared between two assays. Unknown compounds are carried out to screen for topoisomerase I inhibitors by both assays. For DNA cleavage assay, fluorescence ratio assay is used to determine the cleavage complex in enzyme reactions which are prepared like in DNA relaxation assay. The IC_{50} values of some topoisomerase inhibitors are compared between two assays.

Contribution of the study

This study will establish a rapid fluorescence microplate assay for screening topoisomerase I inhibitors.

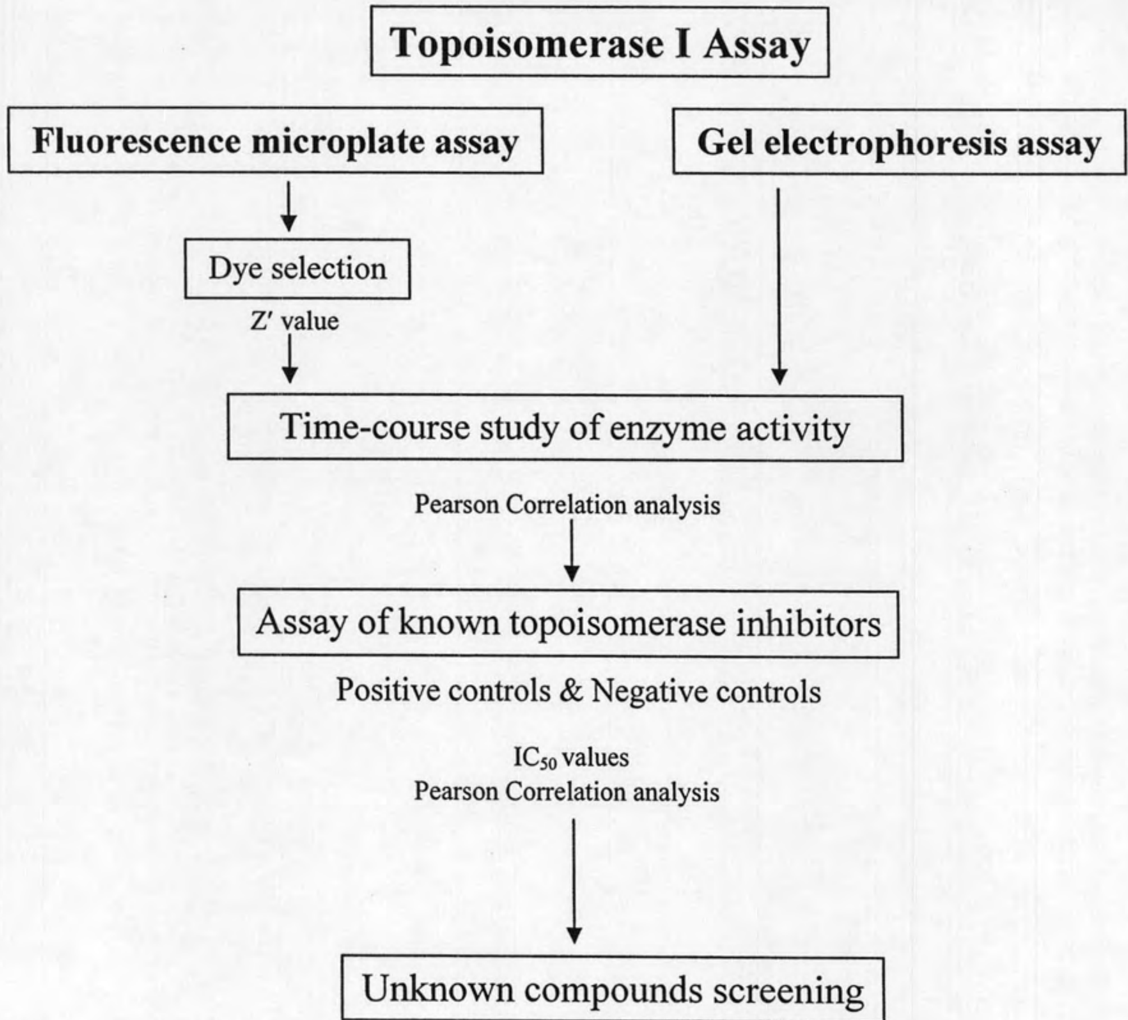

Figure 1 Conceptual framework of this study