DOCTORAL THESIS

Isolation, identification, bioactivity evaluation and structure-activity relationship studies of tricothecenes and the miliusa constituents

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ABSTRACT

Background:

Natural products have attracted high attention due to their great contribution in drug discovery. Many natural products have shown to be effectively against different disease targets including cancer, malaria and HIV. And their structural diversity is a rich resource for the discovery of novel bioactive compounds. This thesis is to explore plant natural products for their potential in treatment of malaria and cancer diseases.

Malaria is still considered as a major global health problem, affecting a large population of the world, especially in the developing countries. Effective drug discovery is still one of the main efforts to control malaria, and plant-derived compounds have played the most important role for treatment of malarial disease.

In our previous work, we have evaluated more than 2,000 plant extracts against the malaria parasite *Plasmodium falciparum*. As a result, we discovered dozens of antiplasmodium plant leads. Bioassay guided separation of these active plant extracts led to isolation of some potent antimalarial compounds. Among them, trichothecenes, the sesquiterpenes identified from the plant *Ficus fistulosa* and *Rhaphidophora decursiva*, were found to have potent inhibitory activity against *P. falciparum* with IC_{50} values in the sub-nano molar range in our previous study (Zhang *et al.* 2002). However, these compounds are significant cytotoxic. In order to improve the antimalarial activity of the trichothecenes, we evaluated the antimalarial activity of dozens of trichothecenes, and based on the structure-activity relationships (SAR) analysis, we synthesized trichothecene derivatives with low cytotoxicity.

On the other hand, cancer has stricken one-third of the world's population.

Through our anticancer drug program to discover bioactive leads from thousands of the plant extracts, the extract of *Miliusa sinensis* Finet *et* Gagnep. (Annonaceae) was found to exhibit cytotoxic activity against a panel of cancer cell lines. Our previous bioassay-directed fractionation of the leaf, twig and flower extracts of *M. sinensis* has led to the discovery of a novel class of anticancer lead molecules, which we designated as miliusanes (Zhang *et al,* 2006). *M. balansae*, in the same family with *M. sinensis,* also contains bioactive miliusanes. We thus collected the plant materials of *M. balansae* to isolated additional new anticancer miliusanes.

Objective:

The objective of this study is to discover novel antimalarial and anticancer natural compounds from plants using different techniques in combination of extensive literature review, phytochemical separation, SAR analysis, semi-synthesis and biological activity study. Trichothecenes and miliusanes are the two major classes of the compounds, which have been extensively explored in the current thesis for their antimalarial and anticancer potential.

Methodology:

There was a prior comprehensive review article entitled *"Antimalarial activity of plant metabolites"* by Schwikkard and Van Heerden (2002), which reported structures of those antiplasmodial active compounds and covered literatures up to the year 2000. As a continuation of their work, antimalarial compounds isolated from plants, including marine plants, which reported in the literatures from 2001 to the end of 2017 have been reviewed and organized according to their plant families.

Dozens of trichothecenes have been obtained by us and explored for their SARs.

Based on the SAR analysis, we designed and carried out the structure modifications of some trichothecenes. These compounds were evaluated for their antimalarial and cytotoxic activities.

M. balansae, in the same genus with *M. sinensis*, was selected to isolate bioactive miliusanes, as well as the other active components through bioassay-guided fractionation study. Columns chromatography was used in fractionation and separation of the dichloromethane extract of *M. balansae*. Preparative HPLC separation and LC-MS analysis were used to speed up the isolation process. All isolates were determined for their chemical structures by spectroscopic means such as NMR and MS, and then evaluated for their anticancer potential. The isolated compounds with abundant amounts were further explored to modify their structures in order to improve their biological activities.

Result:

During the span of the last 17 years (2001-2017), 175 antiplasmodial compounds were discovered from plants. These active compounds were organized in our review article according to their plant families. In addition, we also included ethnobotanical information of the antimalarial plants.

In order to decrease toxicity while retaining antimalarial activity of the trichothecenes, we analyzed SAR of 28 trichothecene analogues. Based on the SAR analysis, we were able to conclude that the diacetylation of C-4 and C-15, and the hydroxylation at C-3 or C-4 could significantly improve the therapeutic indices of trichothecenes. Subsequently, our synthesis of a trichothecene derivative demonstrated potent inhibitory activity against *P. falciparum* with an IC_{50} value of

10.4 nM and low toxicity against KB cells with an IC_{50} value of 556 nM.

The separation of the dichloromethane extract of *M. balansae* led to the isolation of 16 new miliusanes, along with 12 known ones, and 6 flavonoids. Seven of them exhibited significant cytotoxicity against human colorectal cancer cell lines HCT116 with IC_{50} values in the range of 1.24 - 4.2 µM. The SAR study of the miliusanes indicated that the α,β-unsaturated ketone was the active group of but may not be the essential group responsible for the bioactivity of miliusanes. Two flavonoids that showed moderate cytotoxic activity were carried out their structure activities modification. A total of 19 derivatives have been synthesized based on the two flavonoid structures.

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