DOCTORAL THESIS

Arylnaphthalene lignans from justicia plants as potent broad-spectrum antiviral agents

Ku, Chuen Fai

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ABSTRACT

Background:

The emergence of viral diseases has been the major threat to public health and social stability. A hundred years ago, 1918 Spanish flu (H1N1) pandemic spread worldwide, and about 3% ~ 5% of the world's population died from the flu-related illnesses. It is known as the deadliest catastrophic pandemics in human history. There have been five Public Health Emergency of International Concern (PHEIC) declarations over the past decade, including the 2014 Ebola outbreak in west Africa, the 2016 Zika outbreak and the ongoing COVID-19 pandemic. There is always a new strain of virus emerging on the horizon. We have urgent need to develop more broad-spectrum antivirals, which work effective against multiple viruses, for thwarting outbreaks in the future.

Objective:

Based on our previous experience in search of anti-HIV compounds from topical plants, we aimed to discover novel antiviral lead compounds from *Justicia* plants collected in Hong Kong. Further, structure modification of the natural compounds can lead to optimization of their drug properties for further development as drug candidates. To determine the antiviral targets of the lead compounds will further provide insights to elucidate the mechanism of actions. The present studies are to discover the antiviral lead compounds from *Justicia* plants, to analyze the structure-activity relationship of the modified structures, to identify the molecular targets of the lead compounds as antiviral agents against the multiple viruses.

Methodology:

Four common *Justicia* plants were collected in Hong Kong. The plant extracts and compounds isolated from the plants were explored for their antiviral activities via our established "One-Stone-Two-Birds" antiviral assay. Time-of-addition experiments were performed to determine the target stages of the antiviral compounds on the viral replication. Computational techniques (3D-QSAR and *in silico* pharmacokinetics evaluation) were employed to elucidate the structure-activity relationship of the compounds and thereby optimize their structures to enhance the antiviral activity. Comprehensive activity-based protein profiling (ABPP) of biotin-linked compounds using SWATH-MS technique was performed to identify the protein target(s) of the lead compounds in an unbiased manner. The role of the molecular target in viral replication was further verified by mRNA knockdown using siRNA.

Result:

The extracts of *Justicia procumbens* and *Justicia championii* showed potent antiviral effects with low cytotoxicity among the collected *Justicia* plants. By correlating the antiviral activity with their HPLC-UV profiles, arylnaphthalene lignans (ANLs) were determined as the principle active components. Among the isolated compounds from *J. procumbens*, diphyllin exhibited strong antiviral activities against VSV/HIV, H5N1/HIV and EBOV/HIV pseudoviruses with EC_{50} values ranging from 30-100nM. In time-of-addition experiments, diphyllin mainly acts on the entry stage of the viral infection. Considering the broad-spectrum antiviral properties and antiviral mechanism together, diphyllin is probably a host-targeting antiviral agent. In a subsequent lead optimization, a reliable and predictive 3D-QSAR was established from 25 synthesized ANLs. Compound **31** was found as the most potent antiviral agent based on the 3D-QSAR model. It showed 70 times more potent antiviral activity than the parent diphyllin, with retained broad-spectrum antiviral properties and improved predicted ADMET properties. In addition, comprehensive ABPP analysis of the biotin-linked diphyllin was employed for the target identification of the ANL compounds. Total 2343 proteins were captured by the ABPP probes. By quantitative analysis, the protein TFAM showed significant affinity to the diphyllin-based ABPP probes. The viral susceptibility of TFAM-deficient cells was shown to be reduced in the subsequent validation. We thus determined TFAM as the potential antiviral drug target of the ANL compounds against a broad spectrum of viruses.

Keywords: broad-spectrum antiviral activity, *Justicia*, *Justicia procumbens*, arylnaphthalene lignan, diphyllin, entry inhibitor, 3D-QSAR, *in silico* ADMET, ABPP, photoaffinity, SWATH-MS, TFAM

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