DOCTORAL THESIS

# Chemical synthesis of anti-HIV compounds based on the aryl naphthalene lignans identified from justicia plants

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# ABSTRACT

**Background**: Natural products have been a rich source for the discovery of lead compounds in modern drug discovery. 6,7'-Cyclolignans are a class of secondary metabolites which are widely distributed in more than 20 families. This important class of lignans continue to attract the interest of the pharmaceutical industry owing to their remarkable biological benefits, particularly for their anti-tumor and antiviral properties. Arylnaphthalene lignans (ANLs) belong to 6,7'-cyclolignans which contain a 2,3-dimethyl-1-phenyl-naphthalene core structure. ANLs are widely distributed in plants. *Justicia* cf. *patentiflora* was identified as an anti-HIV (human immunodeficiency virus) plant lead through the screening of more than 3,500 plant extracts. Bioassay-directed fractionation of the methanol extract of the stems and barks of this plant has led to the isolation of three ANL glycoside compounds, which displayed potent inhibitory activity against broad HIV clinical strains with $EC_{50}$ values in the range of 14-37 nM [Zidovudine (AZT): 77-95 nM]. They also showed significant inhibitory effects against drug-resistance HIV strains. Thus, the ANL glycosides have high potential as lead molecules for the development of new therapeutic drugs for HIV/AIDS.

**Objectives**: 1) To establish an efficient route for the total synthesis of ANL compounds and to synthesize a library of ANL compounds through the established total synthetic approach; 2) To evaluate the cytotoxicities and anti-HIV activities of the synthesized ANLs; 3) To elucidate the structure activity relationship (SAR) of ANLs as a basis for the optimization of drug efficacy, improvement of pharmacokinetic properties as well as minimization of the toxicity of ANLs; 4) To synthesize potent anti-HIV ANL molecules with high selectivity.

**Methods**: To achieve these objectives, a synthetic route for ANL was designed and a broad series of ANL derivatives were synthesized *via* modifications of rings A and B, as well as the functionalities at C-7. The synthesized compounds had been evaluated

for their toxicity and antiviral activities. Cytotoxicity was determined using the SRB (Sulforhodamine B) assay, while anti-HIV activity was evaluated by utilizing the “One-Stone-Two-Birds” protocol.

**Results**: We have accomplished the total synthesis of the key intermediate diphyllin with more than 20 g. Our modification of ANL derivatives focused on substitutions, additions and different configurations of the C-7 position, ring A and ring B. Specifically, the different structural components of the ANLs were systematically modified, resulting in the formation of six groups of compounds. A total of 72 ANL compounds with various functional groups were synthesized. Their structures have been confirmed by the MS and NMR spectral data. All the synthesized ANL compounds were purified to have purity ≥ 95%. The SAR of ANL compounds was revealed based on the analysis of the antiviral and cytotoxicity data of these synthetic analogues. After structural modification, all the modified derivatives based on rings A and B (**groups 1** and **2**) showed activity reduction in terms of both cytotoxic and anti-HIV activities. However, the modification of C-7 yielded divergent results, which included the groups of **3**-**6**. Most compounds in **groups 3**-**5** displayed comparable inhibitory effect with diphyllin (**5**). **Group 6** represents the largest number of analogues among the six groups. In this group, the stereochemical properties and functionalization of the hydroxy groups on the sugar units are essential for their activities. Among these series of derivatives, compound **17b** showed significant high potency of anti-HIV activity with an $EC_{50}$ value of 2.6 nM and SI of 815.

**Conclusion**: Using the synthesized diphyllin as the key intermediate, a compound library of 72 ANL derivatives was obtained. These compounds have been evaluated for their cytotoxicity and anti-HIV activity. Our bioactivity data revealed that the functionalization of the C-7 hydroxy group could significantly reduce the cytotoxicity and increase anti-HIV activity, while the modification on rings A and B would rather result in the reduction of both cytotoxicity and anti-HIV activity. Subsequently, novel

diphyllin glycosides containing various sugar moieties were further synthesized. Several of these ANL analogues showed high anti-HIV activity with $EC_{50}$ values in the nM range and low cytotoxicity (selective indices > 500).

**Future Perspective**: This study clearly suggests ANLs as anti-HIV lead compounds and they have high potential to be developed as therapeutic drugs for the treatment of HIV. To further confirm the antiviral potential of ANLs, live HIV strains including some drug resistant strains should be further investigated. Although our data have shown that the ANL compounds are targeting the viral post-entry stages, their antiviral molecular targets are still unknown. However, since our SAR information has clearly revealed that the substitution of rings A and B are not involve in the antiviral activity for enhancing ANL compounds, the carbon positions in these rings may be explored to link a biotin unit, which can be used as a viable approach to pull down the antiviral target proteins of ANLs. Once the target proteins are identified, molecular docking is then made possible for a rational synthetic design to fine tune the chemical structures of ANLs in order to improve their antiviral properties such as high antiviral activity, low toxicity and enhanced water solubility. A further step to advance ANLs as anti-HIV drugs is the investigation of their drug properties in *in vivo* studies including the assessment of their anti-HIV efficacy in the rhesus model as well as obtaining their pharmacokinetic and safety parameters. These studies will help to provide more evidence about the anti-viral properties of ANLs

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