DOCTORAL THESIS

Total Synthesis and Structural Modification of Plant Natural Products Ardisiphenol D and Napabucasin as Anticancer Lead Compounds

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ABSTRACT

Nature is a vast treasure for humans. Numerous cytotoxic phytochemicals have been derived as lead compounds for the discovery of novel chemotherapeutics over the years. However, chemotherapy resistance is common, particularly in cancer stem cells (CSCs) due to their self-renewal nature, and thus the search for novel anticancer agents must be continued.

In our continuing drug discovery programme, napabucasin has been isolated from the roots of *Radermachera boniana* Dop. (Bignoniaceae). Napabucasin was found to bypass the resistance problem against ordinary chemo- and radio-cancer therapies by selectively targeting cancer stem cells and metastasis, and therefore considered a new generation lead compound for preventing cancer relapse and metastasis. Structural modifications of napabucasin have been attempted but the study on its *N*-heterocyclic analogues is limited despite the fact that nitrogen-based heterocyclic compounds comprise the largest proportion among all the other heterocycles in the U.S. Food and Drug Administration (FDA) approved anticancer small molecules. More potent candidates have been suggested in several studies, but their research have not been extended to cancer stem cells.

In the present study, total synthetic routes for obtaining napabucasin (**Scheme 1**) and a series of nitrogen-based analogues (**Scheme 2**) mimicking the chemical structure of napabucasin have been established. The synthesized compounds have been subjected to tumorsphere formation assays in HCT116 human colon cancer derived CSCs. Napabucasin significantly inhibited sphere formation at 0.5 μM and even induced cell death of HCT116 cancer stem cells at higher dosage i.e. 1 μM. Interestingly, the nitrogen counterpart demonstrated no obvious effect in the assay even at high concentration of 5 μM. However, the inhibitory effect on cancer stem cell's sphere formation ability has been rescued in the synthesized analogue **A120**, which has a simpler structure and improved solubility. Sphere formation of cancer stem cells has been retarded upon treatment of 0.6 μM of **A120**, while 1.2 μM of the compound could induce cell death. The differential inhibitory effects of the chemically diversified compounds on cancer stem cells in our studies revealed that the oxygen atom in the heterocyclic ring system may act as a hydrogen bond acceptor in the interaction with its binding target (**Figure 7 – 8**).

On the other hand, bioactivity-guided fractionation and isolation of the active extracts of the roots of *Ardisia lindleynana* also led to the identification of ardisiphenol D in our drug discovery programme. The research on this type of compounds has been limited. Synthetic studies have barely been touched and the functional and structural requirements of the compound have not been reported so far. Extensive studies on structural modification, structure-activity relationship elucidation, target binding and lead optimization are crucial for the development of this type of compounds into clinically potent drug candidates.

In the present study, the first total synthesis of ardisiphenol D has been accomplished for a more economical and environmentally friendly approach of obtaining the natural compound (**Scheme 8**). The total synthesis route has also been optimized with fewer steps and can be applied in obtaining some of its analogues (**Scheme 13**). By several synthetic approaches as well as functional group interconversions (**Figure 17**), a total of 135 derivatives with rich chemical and structural diversity have been synthesized. Structure-activity relationship analysis of the derivatives (**Figure 18**), categorized into different classes according to the characteristic functional group constituents, has been conducted following *in vitro* SRB antiproliferation assay against a human colorectal cancer cell line. The acetyl group at C-4 or C-1 is determined to be crucial to the anticancer potency of this type of phenolic compounds, while the formation of an additional acyl group could enhance the activity. Compound **ad4** has demonstrated improved *in vitro* and *in vivo* antineoplastic effects, with reduced toxicity. Upon treatment of HCT116 human colorectal cancer cell induced tumour in nude mice by intraperitoneal administration of **ad4** at 10 mg/kg, the tumour suppression rate reached 56 %, which outperformed the mainstay colorectal cancer remedy 5-FU at 25 mg/kg.

More synthetic and mechanistic studies of napaubasin and ardisiphenol D, and their related derivatives have to be done in the future to transform the compounds into drug candidates to overcome chemotherapy resistance and metastasis.

Keywords: Phytochemicals, anticancer, cancer stem cells, napabucasin, ardisiphenol D, total synthesis, structural modification, SAR analysis.

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