Development of Anticancer Agents Derived from Natural Phenanthroindolizidine Alkaloids

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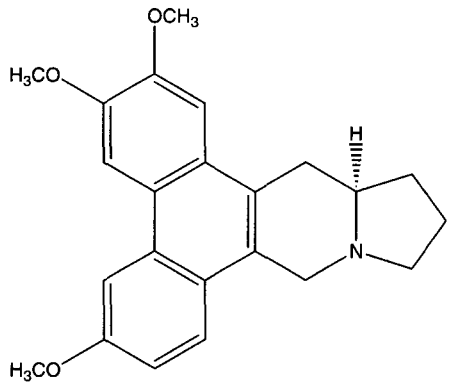
It is well known that natural products have played an important role in the discovery of useful antitumor agents. Especially, clinically relevant anticancer drugs such as taxol, camptothecin, vinblastine and vincristine have been uncovered from higher plants. Nonetheless, as exemplified by the frequent morbidity and mortality associated with metastatic conditions, there is a still clearly need for the discovery of new agents with higher clinical efficacy. During the course of searching for natural product-derived antitumor agents, we isolated a phenanthroindolizine alkaloid antofine (**1**) by bioassay-guided fractionation from the root of *Cynanchum paniculatum* Kitagawa (Asclepiadaceae), and antofine was found to exhibit a significant growth inhibitory activity with the IC_{50} values of approximately 10 nM against cultured several human cancer cell lines. The inhibitory activity of antofine was shown to be similar with those of paclitaxel and vinblastine. Based on the potential growth inhibitory activity of antofine against human cancer cells, we designed and synthesized antofine for further *in vivo* antitumor study in animal model. Two different asymmetric total syntheses of a potential antitumor phenanthroindolizidine alkaloid, (-)-antofine, were employed. An important feature of one of the syntheses was the creation of a stereogenic center by using enantioselective catalytic phase transfer alkylation, affording an unnatural α-amino acid derivative. Another route employed a chiral building block, (*R*)-(*E*)-4-(tributylstannyl)but-3-en-2-ol, and the Overman rearrangement with a total transfer of chirality. The problem of constructing the pyrrolidine ring was successfully addressed, primarily by using a ring-closing metathesis reaction. Using the synthesized (-)-antofine, the *in vivo* study for the evaluation of antitimor activity was conducted in nude mouse model. Treatment of (-)-antofine in nude mice bearing HCT 116 xenograft by i.p. injection resulted in a significant tumor growth suppression in a dose-dependent manner. Especially, treatment of (-)-antofine (8 mg/kg body weight) markedly inhibited the growth of tumor compared to vehicle-treated control groups ($p < 0.001$), suggesting that, along with the growth-inhibitory effect on several human cancer cells, (-)-antofine also possesses antitumor activity. Prompted to the high potency of antofine on the inhibition of cancer cell proliferation, further studies were performed to investigate the mechanism of the growth-inhibitory effect on human cancer cells. Utilizing cultured HCT 116 human colon carcinoma cells as a model, (-)-antofine

interestingly exhibited the growth arrest of the cell without apoptotic cell death. Treatment of (-)-antofine for 24 h down-regulated the expression of c-myc and cdk4 in a dose-dependent manner. Interestingly, p53 and p21 levels were also down-regulated, indicating that this compound might not induce DNA damage. However, cyclin D1, cyclin A, cyclin B1, cdk2, and PCNA expression were not affected by the treatment of (-)-antofine. These data suggest the potential of antofine to serve as a cancer chemotherapeutic agent. Additional mechanisms of action studies are underway to confirm the growth control of cancer cells by antofine.



Antofine (**1**)