DOCTORAL THESIS

Elucidation of anticancer efficacy of ent-kaurane diterpenes through structure-activity relationship and mechanism of action studies

Sarwar, Md Shahid

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ABSTRACT

Colorectal cancer (CRC) is the third most prevalent and second leading causes of cancer-associated deaths globally. Over the last few decades, *ent*-kaurane diterpenes have been widely investigated for their anticancer potentials. Flexicaulin A (**9**) is a naturally occurring *ent*-kaurane. Previously, our lab modified the structure of **9** by replacing the C-11 hydroxyl group with carbonyl group and obtained a novel compound oxoflexicaulin A (**11**). However, anticancer activities and mechanistic pathway of these two compounds are yet to be explored.

In the current thesis, we evaluated the cytotoxicity of compounds **9** and **11** in A549 lung, A375 melanoma, PANC1 pancreatic, HCT-116 and HT-29 colon cancer and 293T human embryonic kidney cells as well as compared the activity with a number of known natural *ent*-kauranes to describe their structure-activity relationship. Our study found that the presence of α, β-unsaturated ketone groups in *ent*-kaurane structure acted as the pharmacophore. The replacement of C-11 hydroxyl group by carbonyl in **9** (IC_{50}: 3.68 µM) gives a novel potent anticancer compound **11** (IC_{50}: 0.77 µM).

Considering the novelty and superior activity of **11**, its mechanistic pathway was studied in HCT-116 cells and compared with the natural scaffold **9**. Flow cytometry analysis by Annexin V/PI staining along with fluorescent staining by DAPI showed that both compounds induced apoptosis in HCT-116 cells. Induction of apoptosis is mediated through up-regulation of tumor suppressor protein p53 and pro-apoptotic protein Bax, Bak and puma as well as promoting the cleavage of PARP while down-regulation of anti-apoptotic protein Bcl-2 and PARP. Apart from their effect on apoptosis, compounds **9** and **11** stimulated the

event of senescence, a process of cellular aging, as confirmed by *β*-galactosidase assay. Induction of senescence is related with up-modulation of p21 and p27 while down-modulation of p16, Rb and its transcription factor E2F1. Moreover, immunofluorescence staining showed translocation of p21 and p27 from the cytoplasm to nucleus after treatment with **9** and **11**. Further study found that the two *ent*-kauranes inhibited the protein level of two NF-κB sub-units p65 and p50 in the nucleus as well suppressed the cytoplasmic level of NF-κB inhibitor IkB-α. Both compounds also inhibited the expression and phosphorylation of STAT3 in the cytoplasm and nucleus, so as for the expression and phosphorylation of Src, an up-stream kinase of STAT3.

In xenograft nude mice model, compound **11** remarkably inhibited tumor growths (volume and size) but the body weights of the mice were also reduced ($p < 0.05$). Therefore, we designed to synthesis a series of prodrug analogs from **11** by adding acetal protecting group to reduce the toxicity. One of the prodrug (**37**) significantly attenuated the tumor volume and size ($p < 0.05$) at 50 mg/kg without any toxicity ($p > 0.05$). The prodrug **37** is actually released as compound **11** in the mice due to its cleavage in the acidic microenvironment of tumor. Taken together, antitumor effect of compound **11** in CRC model is supposed to be mediated through induction of apoptosis and senescence via modulation of NF-κB and STAT3 signaling pathway.

Keywords: Colorectal cancer, *ent*-kaurane, flexicaulin A, oxoflexicaulin A, cytotoxicity, anticancer, apoptosis, pathway, NF-κB, STAT3

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