

## MASTER'S THESIS

### Anti-tumour and anti-angiogenic effects of euxanthone

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Anti-tumour and Anti-angiogenic Effects of Euxanthone

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## Abstract

Plants of *Polygala sp.* have long been used as folk medicines to improve the learning and memory process. The roots of *Polygala* have also been used as sedative agent and anti-tumor drug in some Chinese medicines. Both naturally occurring and synthetic xanthone derivatives have been reported to mediate anti-tumor activities on human cells *in vitro*. In the current study, the potential anti-tumor and anti-angiogenic effects of euxanthone isolated from the medicinal herb *Polygala caudata* were investigated. Murine neuroblastoma (Neuro 2A, subclone BU-1) was used as a model to study the anti-tumor activities, and the human umbilical vein endothelial cells (HUVECs) was employed as a model to evaluate the anti-angiogenic activities of euxanthone.

In the studies of anti-tumor activity, euxanthone (50 - 100  $\mu$ M) was found not only to inhibit the growth and arrest BU-1 at G2/M phase, but also to induce the morphological differentiation of the cells. The morphological differentiation of BU-1 cell was associated with the outgrowth of neurite and the enlargement of cell bodies. Previous studies suggested that protein kinase C (PKC) signaling pathway may be involved in the neuronal differentiation of Neuro-2A cells. Thus, the effects of PKC inhibitor on the growth and neuronal differentiation of euxanthone-treated BU-1 cells were also examined. Significant reduction of euxanthone-induced neuritogenic effect was observed when the conventional PKC isoform specific inhibitor Gö6976 was included in the culture. The results suggest that the conventional PKC isoforms may be involved in euxanthone-induced neuritogenesis in the BU-1 cells. Axonal (NF-H and tau) and dendritic (MAP-2) markers were employed to characterize the nature of

induced neuritic outgrowth in euxanthone-treated BU-1. Laser scanning confocal microscopy was used to study the distribution of these neuronal markers. A mixed phenotype of axon- and dendrite-like processes was observed 5 days after euxanthone treatment. These data suggested that euxanthone might be one of the anti-tumor compounds in the medicinal plant *Polygala caudata*.

In the second part of the study, the anti-angiogenic effect of euxanthone on HUVECs was examined. Euxanthone was found to inhibit the *in vitro* migration and proliferation of HUVECs. The growth inhibition of HUVECs was also supported by the results from the flow cytometric analysis. The proportion of HUVECs in S-phase was reduced after euxanthone treatment. The induction of morphogenesis of HUVECs, namely changing from the typical cobblestone appearance into elongated spindle shape, was observed after euxanthone treatment. Different *in vitro* and *in vivo* assays (*in vitro* tube formation assay and *in vivo* chick chorioallantoic membrane study) were evaluated on the effect of euxanthone on the vessel formation. The *in vitro* 3-D tube formation assay was finally selected in this study. The anti-angiogenic effect was also observed in the *in vitro* tube formation on Matrigel. It has been previously reported that cell adhesion molecules including platelet endothelial cell adhesion molecules-1 (PECAM-1) and cadherin-5 are critical molecules expressed on HUVECs for tube formation. In this study, PECAM-1 and cadherin-5 were found to express mainly at the cell junction of HUVECs. Euxanthone did not alter the expression level of these two adhesion molecules as revealed by the flow cytometric analysis. Inhibition of HUVEC migration might be responsible for the anti-angiogenic effect of euxanthone. The data generated in the study allow us to further

investigate the potential anti-angiogenic effects of euxanthone in other *in vivo* angiogenic models.

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