# SUPPLEMENTARY MATERIAL

*Calophylluminophyllum* and *Calophyllumsoulattri* Source of Anti-proliferative xanthones and their Structure-Activity Relationships

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Extensive chromatographic isolation and purification on the extracts of the stem bark of *Calophylluminophyllum* and *Calophyllumsoulattri*have resulted in eleven xanthones. *C. inophyllum*gave inophinnin (**1**), inophinone (**2**), pyranojacareubin (**5**), rheediaxanthone A (**6**), macluraxanthone (**7**) and 4-hydroxyxanthone (**8**) while *C. soulattri*afforded soulattrin (**3**), phylattrin (**4**), caloxanthone C (**9**), brasixanthone B (**10**) and trapezifolixanthone (**11**). The structures of these compounds were determined based on spectroscopic analyses such as 1D and 2D-NMR, GCMS, IR and UV. Cytotoxicity screening (MTT Assay) carried out *in vitro* on all the xanthones using five human cancer cell lines indicated good activities for some of these xanthones. The structure-activity relationship (SAR) study revealed the inhibitory activities exhibited by these xanthone derivatives to be closely related to the existence and nature of the pyrano and the prenylsubstituent groups on their skeleton.

Keywords: *Calophyllum*; cytotoxicity;structure-activity relationship;xanthone

# Experimental

# *Plant Material*

Stem bark samples of *Calophyllum inophyllum* (RG 5016) were collected from campus grounds in Universiti Putra Malaysia in 2010 while *Calophyllum soulattrii* (RG 5017) was collected from Sri Aman in Sarawak, Malaysia in 2011. The plant materials were identified by Dr Rusea Go from the Biology Department, Universiti Putra Malaysia where the herbarium samples were kept.

## Extraction and Isolation

Detailed purification of the dichloromethane extract of *Calophylluminophyllum*afforded two xanthones, inophinnin (**1**) (9 mg) and inophinone (**2**), along with pyranojacareubin (**5**) (6 mg), rheediaxanthone A (**6**) (8 mg) and macluraxanthone (**7**) (52 mg). The simple xanthone 4-hydroxyxanthone (**8**) (8 mg), was isolated from the ethyl acetate extract of this species.

Extensive chromatography carried out on the dichloromethane extract of *Calophyllumsoulattri*afforded another two xanthones, soulattrin (**3**) (7 mg) and phylattrin (**4**) (67 mg). Other compounds, macluraxanthone (**7**) (6 mg), caloxanthone C (**9**) (14 mg), brasixanthone B (**10**) (21 mg) and trapezifolixanthone (**11**) (10 mg) were also successfully isolated from the dichloromethane extract.

## Cell Culture

All the apparatus used for cell culture were sterilized and decontaminated using Hirayama HICLAVE HVE-50. Cell culture handling was carried out in an ESCO Class II BSC Biosafety Cabinet. The healthy cells were spun down, adherent together and separated from unhealthy and dead cells by using Thermo Scientific Sorvall ST 16R centrifuge machine. All cultures were incubated in 5% CO2 humidified incubator at 37 °C (ESCO Celculture CO2 Incubator with model number CCL-170B-8). Cell stocks were placed in a -86 °C ultra low freezer (ScancoolSCL 50 P) and preserved in a liquid nitrogen tank (Taylor-Wharton LS300).

***MTT Assay***

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed according to a previously described method ([Mosmann, 1983](#_ENREF_3_1)). The tests were performed in a sterile 96-well flat bottom plate. Stock solutions of each pure compound were prepared by dissolving them in DMSO to a concentration of 20 mg/mL. A six-point serial dilution was developed to obtain six different sub-stocks with different concentrations. For suspension cells, concentrations needed were 50.00, 25.00, 12.50, 6.25, 3.13 and 1.56 μg/mL. Meanwhile, 100.00, 50.00, 25.00, 12.50, 6.25 and 3.13 μg/mL were the essential concentrations for anchorage-dependant cells. Each pure compound was tested in triplicate together with the controls.

After 72 h incubation at 37 °C and 5% of CO2, MTT solution (20 μL) was added into all the filled wells and incubated again for 3 h. The plate was spun at 1500 rpm for 10 minutes followed by discarding approximately 80% of the supernatant carefully. The volume of supernatant discarded was the same as the volume of DMSO added into the wells. The absorbance of each well was determined by a microplate reader at 550 nm after the purple crystal formazan fully dissolved in DMSO. Three independent experiments for both suspension and anchorage-dependant cell lines were conducted. The average absorbance values were used in the calculation of percentage cell viability. The cytotoxicity index used was IC50, which is the concentration that yields 50% inhibition of the cell compared with the untreated control.