Antiproliferative xanthone derivatives from *Calophyllum inophyllum* and *Calophyllum soulattri*

Siau Hui Mah[1,2], Gwendoline Cheng Lian Ee[1,*], Soek Sin Teh[1] and Mohd Aspollah Sukari[1]
[1]Department of Chemistry, Faculty of Science, University Putra Malaysia, UPM Serdang, Selangor, Malaysia
[2]School of Bioscience, Taylor's University, Lakeside Campus, Subang Jaya, Selangor, Malaysia

Abstract: Structure-activity relationships of eleven xanthones were comparatively predicted for four cancer cell lines after the compounds were subjected to antiproliferative assay against B-lymphocyte cells (Raji), colon carcinoma cells (LS174T), human neuroblastoma cells (IMR-32) and skin carcinoma cells (SK-MEL-28). The eleven chemical constituents were obtained naturally from the stem bark of *Calophyllum inophyllum* and *Calophyllum soulattri.* Inophinnin (1) and inophinone (2) were isolated from *Calophyllum inophyllum while* soulattrin (3) and phylattrin (4) were found from *Calophyllum soulattri*. The other xanthones were from both *Calophyllum* sp. and they are pyranojacareubin (5), rheediaxanthone A (6), macluraxanthone (7), 4-hydroxyxanthone (8), caloxanthone C (9), brasixanthone B (10) and trapezifolixanthone (11). Compound 3 was found to be the most cytotoxic towards all the cancer cell lines with an IC_{50} value of 1.25μg/mL while the simplest xanthone, compound **8** was inactive.

Keywords: *Calophyllum inophyllum*, *Calophyllums oulattri*, Clusiaceae, cytotoxicity, xanthone, structure-activity relationship.

INTRODUCTION

Calophyllum species are well known for its medicinal uses and have been used in folk medicine. The habitats for these species are ridges in mountain forests, coastal swamps, lowland forest and coralcays. They are large lightweight hardwoods, which can attain 30 metres in height and their hardwoods are used to make boats, luxury furniture and flooring. The presence of secondary metabolites such as xanthones (Blanco-Ayala *et al.*, 2013; Dharmaratne *et al.*, 1999; Ee *et al.*, 2011a), coumarins (Ee *et al.*, 2011b; Ee *et al.*, 2004; Joshi *et al.*, 2013), triterpenoids (Dharmaratne *et al.*, 1985; Li *et al.*, 2010) and flavonoids (Ito *et al.*, 1999; Ravelonjato *et al.*, 1987) contributed to their various bioactivities such as antioxidant (Blanco-Ayala *et al.*, 2013), anti-inflammatory (Tsai *et al.*, 2012), anti-microbial (Alkhamaiseh, *et al.*, 2012) and cytotoxicity (Mah *et al.*, 2013; Mah *et al.*, 2012).This paper reports the structure activity relationships for eleven xanthones which were isolated from *Calophyllum inophyllum* and *Calophyllum soulattri*.

MATERIALS AND METHODS

Plant Material

The stem bark of *Calophyllum inophyllum* and *Calophyllum soulattri* were collected from the campus ground of University Putra Malaysia and Sri Aman District, Sarawak, Malaysia, respectively.

Extraction and Isolation

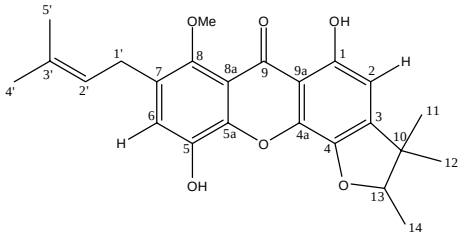
Repeated purification by column chromatography of the dichloromethane extract of *Calophyllum inophyllum* resulted in two xanthones, inophinnin (1) (Ee *et al.*, 2011a) (9mg) and inophinone (2) (Mah *et al.*, 2011a) (6 mg) and pyranojacareubin (5) (Iinuma *et al.*, 1994b) (6 mg), rheediaxanthone A (6) (Iinuma *et al.*, 1994b) (8 mg) and macluraxanthone (7) (Iinuma *et al.*, 1994b) (52 mg). The simple xanthone 4-hydroxyxanthone (8) (Iinuma *et al.*, 1994b) (8 mg), was obtained from the ethyl acetate extract of this same species.

Extensive chromatographic separation on the dichloromethane extract of *Calophyllum soulattri* gave another two xanthones, soulattrin (3) (Mah *et al.*, 2011b) (7 mg) and phylattrin (4) (Mah *et al.*, 2012) (67 mg). Other compounds, macluraxanthone (7) (Iinuma *et al.*, 1994b)(6 mg), caloxanthone C (9) (Iinuma *et al.*, 1994a) (14 mg), brasixanthone B (10) (Ito *et al.*, 2002) (21 mg) and trapezifolixanthone (11) (Somanathan *et al.*, 1974) (10 mg) were also successfully isolated from the dichloromethane extract.

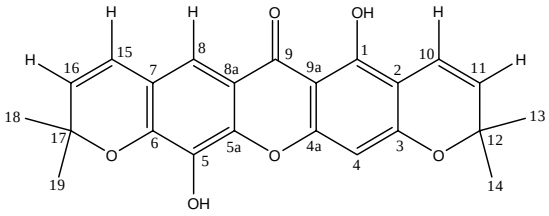
Cytotoxicity (MTT Assay)

Protocol by Mossman (Mosmann, 1983) was followed for the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The experiment was carried out in a sterile 96-well flat bottom plate. Pure compounds were dissolved in DMSO to prepare 20 mg/ml stock solutions. Serial dilutions were then prepared to obtain six different sub-stock solutions. Concentrations for suspension cells were 50.00, 25.00, 12.50, 6.25, 3.13 and 1.56μg/mL. Essential concentrations for anchorage-dependant cells were 100.00, 50.00, 25.00, 12.50, 6.25 and 3.13μg/mL. Each pure compound was tested in triplicate together with the controls.

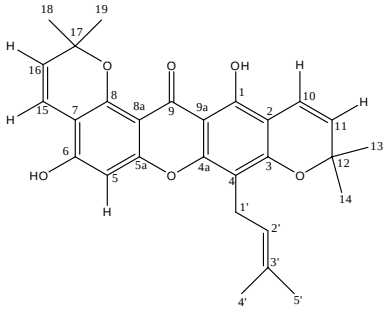
*Corresponding author: e-mail: gwen@upm.edu.my



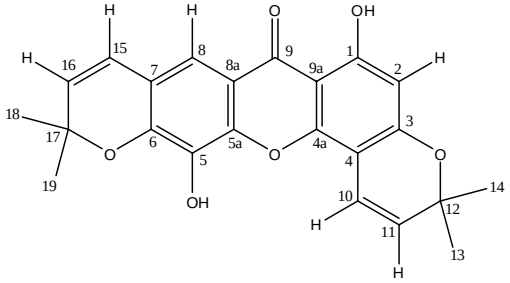
1



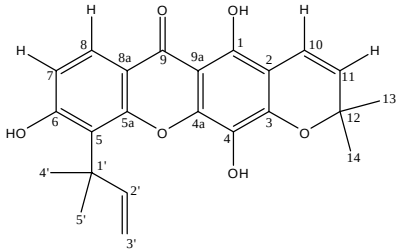
2



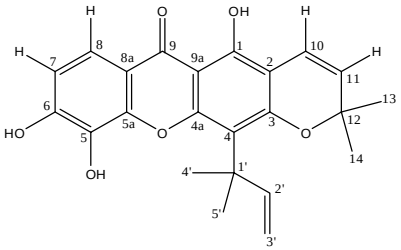
3



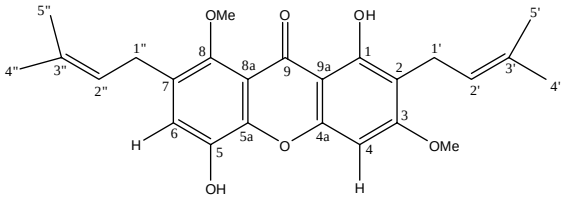
4



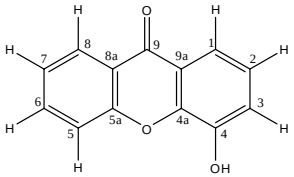
5



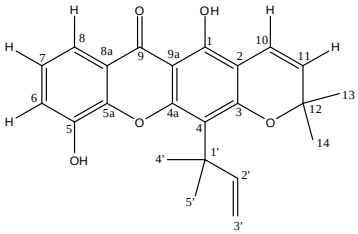
6



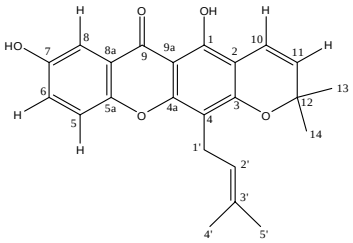
7



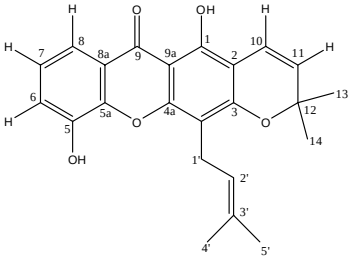
8



9



10



11

Table 1: IC_{50} values of inophinnin (1), inophinone (2), soulattrin (3), phylattrin (4), pyranojacareubin (5), rheediaxanthone A (6), macluraxanthone (7), 4-hydroxyxanthone (8), caloxanthone C (9), brasixanthone B (10) and trapezifolixanthone (11) against five human cancer cell lines

Compound	IC_{50} (μg/mL)			
	Raji	LS174T	IMR-32	SK-MEL-28
1	8.33±0.38	13.75±0.84	13.75±1.25	13.75±1.08
2	7.29±0.87	>400.0	>40.00	>40.00
3	1.01±0.80	1.25±0.55	0.27±0.91	0.57±0.65
4	4.95±0.93	4.68±1.09	7.81±1.07	4.68±0.78
5	7.03±0.82	15.62±0.07	>40.00	>40.00
6	7.29±0.84	14.58±0.95	>40.00	7.80±0.50
7	1.75±0.50	5.94±0.94	1.95±1.21	15.62±0.75
8	>40.00	>40.00	>40.00	>40.00
9	5.86±1.15	10.94±0.57	7.03±0.59	>40.00
10	9.89±0.47	13.75±0.37	35.00±1.00	10.93±1.08
11	7.29±0.84	14.58±0.95	>40.00	7.80±0.50
1	8.33±0.38	13.75±0.84	13.75±1.25	13.75±1.08
2	7.29±0.87	>400.0	>40.00	>40.00
3	1.01±0.80	1.25±0.55	0.27±0.91	0.57±0.65
4	4.95±0.93	4.68±1.09	7.81±1.07	4.68±0.78
5	7.03±0.82	15.62±0.07	>40.00	>40.00

Note: Each value of IC_{50} represents mean ± S.E.M.

After incubation at 37°C for 72 hours and with 5% of CO_2, MTT solution (20μL) was added into all the filled wells. This was followed by further incubation for 3 hours. The absorbance of each well was determined by a microplate reader at 550 nm. Both suspension and anchorage-dependant cell lines experiments were carried out in 3 independent triplicates. Percentage cell viability calculations were carried out using the average absorbance values. The cytotoxicity index used was IC_{50}.

RESULTS

The MTT Assay (Mosmann, 1983) cytotoxicity screening was carried out *in vitro* on all the eleven xanthones using four human cancer cell lines. These are B-lymphocyte cells (Raji), colon carcinoma cells (LS174T), human neuroblastoma cells (IMR-32) and skin carcinoma cells (SK-MEL-28). The IC_{50} values, which are expressed in μg/mL for the compounds are summarized from the graphs of percentage of cell viability versus concentration (See Table 1). The cytotoxic effect of these xanthones can be predicted to be closely related to the nature of the substituent groups on their skeleton. Therefore, structure-activity relationships for the xanthones towards the four cell lines are discussed. Kaempferol and quercetin were used as standard drugs in the assay.

DISCUSSION

Four previously reported as new xanthones namely soulattrin (3), phylattrin (4), inophinnin (1) and inophinone (2) exhibited potent cytotoxicity towards the proliferation of B-lymphocyte cell line, Raji cells. Their IC_{50} values were 1.0, 4.9, 7.0 and 8.3μg/mL, respectively. The known xanthones, pyranojacareubin (5), rheediaxanthone A (6), macluraxanthone (7), caloxanthone C (9), brasixanthone B (10) and trapezifolixanthone (11) also showed strong activity. For Raji cells, the xanthones with pyrano rings possess higher cytotoxic effects compared to those with furano rings. Therefore, inophinnin (1) has a higher IC_{50} value. Besides, the prenyl moiety in xanthones also contributed to the anti-proliferation of Raji cells. This was proven by phylattrin (4), which carries a di-prenylated moiety. The simplest xanthone without any substituent groups, 4-hydroxyxanthone (8) was inactive.

The LS174T cell line is the colon carcinoma cells. The pure compounds, soulattrin (3), phylattrin (4) and inophinnin (1) possess potent cytotoxicities with IC_{50} values of 1.2, 4.7 and 13.7μg/mL, respectively. Meanwhile, six other xanthones showed strong activity too which are macluraxanthone (7), caloxanthone C (9), brasixanthone B (10), rheediaxanthone A (6), pyranojacareubin (5) and trapezifolixanthone (11). The concentrations of these compounds needed to kill 50% of the LS175T cells are within 19.0 μg/mL. All these xanthones carry a prenyl moiety or a pyrano ring except for inophinnin (1), which carries a five membered furano ring. These substituents may contribute cytotoxic effects. By comparing the cytotoxic effects, the pyrano ring substituent possesses stronger inhibition rather than the prenyl moiety. The evidences are pyranojacareubin (5) and rheediaxanthone A (6) which have higher IC_{50} values.

Having a pyrano ring attached at C-2 and C-3 or C-6 and C-7 giving a higher antiproliferative rate towards the LS174T cells. Trapezifolixanthone (11) has the weakest activity among the active xanthones.

The human neuroblastoma cell line, IMR-32 was vulnerable to five xanthones including the three new xanthones, soulattrin (3), phylattrin (4) and inophinnin (1). Their IC_{50} values were observed at 0.3, 7.8 and 13.7 μg/mL, respectively. The other two xanthones are macluraxanthone (7) (IC_{50} value=1.9μg/mL) and caloxanthone C (9) (IC_{50} value=7.0 μg/mL). Brasixanthone B (10) and trapezifolixanthone (11) were less susceptible as these compounds required at least 35.0 and 43.7 μg/mL to kill half of the IMR-32 cells. The cytotoxic effects of the xanthones were probably contributed by the pyrano or furano ring attached to their skeleton. This was proven by pyranojacareubin (5), rheediaxanthoneA (6) and 4-hydroxyxanthone (8) which are inactive as they do not have pyrano or furano ring as substituent but only a prenyl moiety for 5 and 6. Inophinone (2) showed no cytotoxicity towards the IMR-32 cell and this may be due to the position of the pyrano ring at C-7 and C-8 and not at C-6 and C-7.

The cytotoxic effects of the pure compounds towards skin carcinoma cell line, SK-MEL-28 cells have a close similarity to that of IMR-32 cells. The three new compounds also possess strong cytotoxicity with low IC_{50} values. The SK-MEL-28 cells were the most vulnerable towards soulattrin (3) with an IC_{50} value of 0.6 μg/mL. This is followed by phylattrin (4) and inophinnin (1) with IC_{50} values of 4.7 and 13.7μg/mL, respectively. On the other hand, rheediaxanthone A (6), trapezifolixanthone (11), brasixanthone B (10) and macluraxanthone (7) were the known xanthones, which gave high cytotoxic effects. The inhibition effect against the SK-MEL-28 cells could be contributed by the prenyl moieties and pyrano or furano rings attached to the xanthone nucleus. This explains the cytotoxic effect of the above xanthones. Hence, the simplest xanthone, 4-hydroxyxanthone (8) is inactive. Pyranojacareubin (5) was found to exhibit mild activity towards the proliferation of the SK-MEL-28 cells and this may be due to the position of di-pyrano rings.

CONCLUSION

The structure-activity relationships of a series of xanthone derivatives were predicted to be closely related to the nature of the substituent groups on their skeleton. Future work on screening against the respective non-cancerous cell lines will be conducted in due course.

ACKNOWLEDGEMENTS

The authors acknowledge financial support from UPM and MOSTI under the Agri Science Fund.

REFERENCES

Alkhamaiseh SI, Taher M, Ahmad F, Qaralleh H, Althunibat OY, Susanti D and Ichwan SJA (2012). The phytochemical content and anti-microbial activities of Malaysian *Calophyllum canum* (stem bark). *Pak. J. Pharm. Sci.*, **25**(3): 555-563.

Blanco-Ayala T, Lugo-Huitrón R, Serrano-López EM, Reyes-Chilpa R, Rangel-López E, Pineda B, Medina-Campos ON, Sánchez-Chapul L, Pinzón E, Cristina T-S, Silva-Adaya D, Pedraza-Chaverrí J, Ríos C, Cruz VPdl and Torres-Ramos M (2013). Antioxidant properties of xanthones from *Calophyllum brasiliense*: Pprevention of oxidative damage induced by $FeSO_4$. *BMC Compl. Alternative. Med.*, **13**: 262.

Dharmaratne HR, Sotheeswaran S, Balasubramaniam S and Waight ES (1985). Triterpenoids and coumarins from the leaves of *Calophyllum cordato-oblongum*. *Phytochem.*, **24**(7): 1553-1556.

Ee GCL, Mah SH, Rahmani M, Taufiq-Yap YH, Teh SS and Lim YM (2011a). A new furanoxanthone from the stem bark of *Calophyllum inophyllum*. *J. Asian Nat. Prod. Res.*, **13**(10): 956-960.

Ee GCL, Mah SH, Teh SS, Rahmani M, Go R and Taufiq-Yap YH (2011b). Soulamarin, a new coumarin from stem bark of *Calophyllum soulattri*. *Molecules*, **16**(11): 9721-9727.

Ee GCL, Ng KN, Taufiq-Yap YH, Rahmani M, Ali AM and Muse R (2004). Mucigerin, a new coumarin from *Calophyllum mucigerum* (Guttiferae). *Nat. Prod. Res.*, **18**(2): 123-128.

Iinuma M, Tosa H, Tanaka T and Yonemori S (1994a). Two new xanthones in the underground part of *Calophyllum inophyllum*. *Heterocycles,* **37**(2): 833-838.

Iinuma M, Tosa H, Tanaka T and Yonemori S (1994b). Two xanthones from root bark of *Calophyllum inophyllum*. *Phytochem.*, **35**(2): 527-532.

Ito C, Itoigawa M, Mishina Y, Filho VC, Mukainaka T, Tokuda H, Nishino H and Furukawa H (2002). Chemical constituents of *Calophyllum brasiliensis*: Structure elucidation of seven new xanthones and their cancer chemopreventive activity. *J. Nat. Prod.*, **65**: 267-272.

Ito C, Itoigawa M, Miyamoto Y, Rao KS, Takayasu J, Okuda Y, Mukainaka T, Tokuda H, Nishino H and Furukawa H (1999). A new biflavonoid from *Calophyllum panciflorum* with anti-tumor-promoting activity. *J. Nat. Prod.*, **62**(12): 1668-1671.

Joshi SP, Kulkarni SR, Phalgune UD and Puranik VG. (2013). New dipyranocoumarin from the leaves of *Calophyllum apetalum* Willd. *Nat. Prod. Res.*, **27**(20): 1896-1901.

Li YZ, Li ZL, Yin SL, Shi G, Liu MS, Jing YK and Hua HM (2010). Triterpenoids from *Calophyllum inophyllum* and their growth inhibitory effects on human leukemia HL-60 cells. *Fitoterapia*, **81**: 586-590.

Mah SH, Ee GCL and Teh SS (2013). Chemistry and cytotoxic activity of essential oil from the stem Bark of *Calophyllum soulattri*. *Asian J. Chem.,* **25**(15): 8831-8832.

Mah SH, Ee GCL, Rahmani M, Taufiq-Yap YH, Go R and Teh SS (2011a). A new pyranoxanthone from the stem bark of *Calophyllum inophyllum*. *Letts. Org. Chem.,* **8**(6): 447-449.

Mah SH, Ee GCL, Rahmani M, Taufiq-Yap YH, Sukari MA and Teh SS (2011b). A new pyranoxanthone from *Calophyllum soulattri*. *Molecules,* **16**(5): 3999-4004.

Mah SH, Ee GCL, Teh SS, Rahmani M, Lim YM and Go R (2012). Phylattrin, a new cytotoxic xanthone from *Calophyllum soulattri*. *Molecules,* **17**(7): 8303-8311.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. lmmunol. Methods,* **65**: 55-63.

Ravelonjato B, Kunesch N and Poisson JE (1987). Neoflavanoids from the stem bark of *Calophyllum verticillatum*. *Phytochem,* **26**(11): 2973-2976.

Somanathan R, Sultanbawa S and Uvais M (1974). Chemical investigation of ceylonese plants. Part VIII. Trapezifolixanthone, a new di-isoprenylated xanthone from the bark of *Calophyllum trapezifolium* Thw. (Guttiferae). *J. Chem. Soc. Perkin. Trans.,* 1: 2515-2517.

Tsai SC, Liang YH, Chiang JH, Liu FC, Lin WH, Chang SJ, Lin WY, Wu CH and Weng JR (2012). Anti-inflammatory effects of *Calophyllum inophyllum* L. in RAW264.7 cells. *Oncol. Rep.,* **28**: 1096-1102.