องค์ประกอบทางเคมีจากรากตังหน *Calophyllum calaba* L.

นางสาวเฟื่องฟ้า เหลาเพียร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Calophyllum calaba* L.

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เฟื่องฟ้า เหลาเพียร : องค์ประกอบทางเคมีจากรากตังหน *Calophyllum calaba* L. (CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Calophyllum calaba* L.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. สันติ ทิพยางค์, 58 หน้า.

การศึกษาองค์ประกอบทางเคมีจากสิ่งสกัดไดคลอโรมีเทนของรากต้นตังหน สามารถแยกสารในกลุ่ม xanthone ได้ทั้งหมด 32 สาร ประกอบด้วย สารชนิดใหม่ 3 สาร ได้แก่ calaxanthones A-C (1-3) และสารที่เคยมีการรายงานโครงสร้างมาก่อนหน้านี้ 29 สาร โครงสร้างของ xanthone ทั้ง 32 ชนิด ได้พิสูจน์ทราบเอกลักษณ์ทางโครงสร้างด้วยวิธีทาง สเปกโทรสโกปี (1D และ 2D NMR) และเปรียบเทียบข้อมูลจากสารที่เคยมีการรายงานก่อนหน้านี้ จากนั้นทำการทดสอบความเป็นพิษต่อเซลล์มะเร็งชนิด KB, HeLa S-3, HT29, MCF-7 และ HepG2 ของสารบริสุทธิ์ทั้งหมดที่แยกได้ พบว่าสารส่วนใหญ่มีฤทธิ์ในการยับยั้งเซลล์มะเร็งอยู่ในระดับปานกลางไปจนถึงไม่มีฤทธิ์ ยกเว้นสาร 3 พบว่ามีความเป็นพิษต่อเซลล์มะเร็งในระดับดีทั้งเซลล์ KB, HeLa S-3, HT29, MCF-7 และ HepG2 โดยมีค่า IC_{50} เท่ากับ 1.72, 0.82, 1.17, 5.04 และ 1.65 μM ตามลำดับ สาร 6 มีความเป็นพิษต่อเซลล์ KB และ HeLa S-3 ในระดับดีเช่นกัน โดยมีค่า IC_{50} เท่ากับ 7.06 และ 5.27 μM ตามลำดับ และสุดท้ายสาร 13 ยังมีความเป็นพิษเฉพาะเซลล์ KB ในระดับดีเช่นกัน โดยมีค่า IC_{50} เท่ากับ 4.62 μM

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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FUENGFA LAOPIAN: CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Calophyllum calaba* L.. ADVISOR: ASSOC. PROF. SANTI TIP-PYANG, Ph.D., 58 pp.

Phytochemical investigation of the CH_2Cl_2 extract from the roots of *C. calaba* let to the isolation of three new xanthone derivatives, namely calaxanthones A-C (1-3), along with twenty nine known xanthones (4-32). The structures of all isolated compounds were fully characterized using spectroscopic data (1D and 2D NMR) as well as comparison with the previous literature data. Moreover, all isolated compounds were assessed for their *in vitro* cytotoxicity against the KB, HeLa S-3, HT29, MCF-7 and HepG2 human cancer cell lines. The tested compounds mostly showed moderate to inactive against these five cell lines, except compounds 3 showed potent cytotoxicity against KB, HeLa S-3, HT29, MCF-7 and HepG2 cells with IC_{50} values of 1.72, 0.82, 1.17, 5.04 and 1.65 μM, respectively. Furthermore, compound 6 showed good cytotoxicity against KB and HeLa S-3 cell with IC_{50} value of 7.06 and 5.27 μM, respectively. Moreover, compound 13 showed good cytotoxicity against only KB cell with IC_{50} value of 4.62 μM.

Department: Chemistry Student's Signature .....

Field of Study: Chemistry Advisor's Signature .....

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CONTENTS

Page

Page

Page

LIST OF FIGURES

page

LIST OF TABLES

Page

LIST OF SCHEMES

Page

LIST OF ABBREVIATIONS

IC_{50}	the molar concentration of an antagonist that reduces the response to an agonist by 50%
kg	kilogram
µg	microgram
mg	milligram
µM	micromolar
L	liter
mL	milliliter
h	hour(s)
mmol	millimole
U	unit
m/z	mass per charge number of ions (Mass Spectroscopy)
δ	chemical shift (NMR)
δ_H	chemical shift of proton (NMR)
δ_C	chemical shift of carbon (NMR)
J	coupling constant (NMR)
s	singlet (NMR)
d	doublet (NMR)
dd	doublet of doublet (NMR)
t	triplet (NMR)
brs	broad singlet (NMR)
Hz	hertz
MHz	megahertz
DMSO-d_6	deuterated dimethyl sulfoxide
$CDCl_3$	deuterated chloroform
HRESIMS	high resolution electrospray ionization mass spectroscopy

^{1}H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon-13 nuclear magnetic resonance
1D-NMR	one dimensional nuclear magnetic resonance
2D-NMR	two dimensional nuclear magnetic resonance
COSY	correlation spectroscopy
HSQC	heteronuclear single quantum correlation
HMBC	heteronuclear multiple bond correlation
calcd.	calculated
TLC	thin layer chromatography
MEM	minimum Essential Media
DCM	dichloromethane
DMSO	dimethyl sulfoxide
M	methanol
D	dichloromethane
E	ethyl acetate
H	hexane

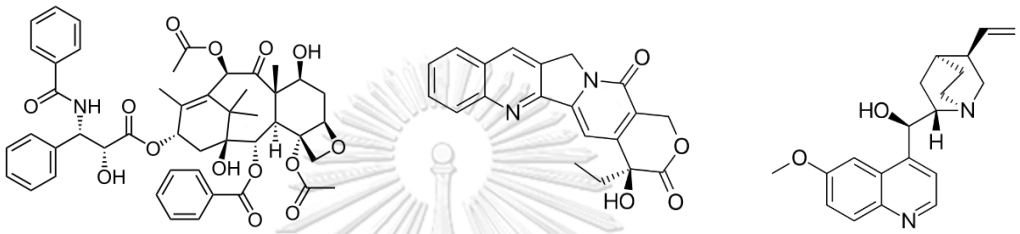
CHAPTER I

INTRODUCTION

In the present, plants are being used to treat many health concerns and conditions, including allergies, arthritis, migraines, fatigue, skin infections, wounds, burns, gastrointestinal issues and even cancer proving that is true that food is medicine. These herbs are less expensive and they are a safer means of treatment than conventional medications, which is why so many people are choosing to go back to this traditional idea of medicine.

Plants are rich in a variety of compounds. Many are secondary metabolites and include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins [1, 2]. Many of these compounds have antioxidant properties. About 200 years ago, the first pharmacologically active pure compound, morphine, was produced from opium extracted from seeds pods of the poppy *Papaver somniferum*. This discovery showed that drugs from plants can be purified and administered in precise dosages regardless of the source or age of the material [1]. This approach was enhanced by the discovery of penicillin [3]. With this continued trend, products from plants and natural sources (such as fungi and marine microorganisms) or analogs inspired by them have contributed greatly to the commercial drug preparations today. Examples include antibiotics (e.g., penicillin, erythromycin); the cardiac stimulant digoxin from foxglove (*Digitalis purpurea*); salicylic acid, a precursor of aspirin, derived from willow bark (*Salix spp.*); reserpine, an antipsychotic and antihypertensive drug from *Rauwolfia spp.*; and antimalarials such as quinine from *Cinchona* bark and lipid-lowering agents (e.g., lovastatin) from a fungus [3, 4]. Also, more than 60% of cancer therapeutics on the market or in testing are based on natural products. Of 177 drugs approved worldwide for treatment of cancer, more than 70% are based on natural products or mimetics, many of which are improved with combinatorial chemistry. Cancer therapeutics from plants include paclitaxel, isolated from the Pacific yew tree; camptothecin, derived from the Chinese “happy tree” *Camptotheca acuminata* and used to prepare irinotecan and topotecan; and

combretastatin, derived from the South African bush willow [5]. It is also estimated that about 25% of the drugs prescribed worldwide are derived from plants, and 121 such active compounds are in use [6]. Between 2005 and 2007, 13 drugs derived from natural products were approved in the United States. More than 100 natural product-based drugs are in clinical studies [3], and of the total 252 drugs in the World Health Organization's (WHO) essential medicine list, 11% are exclusively of plant origin [6].



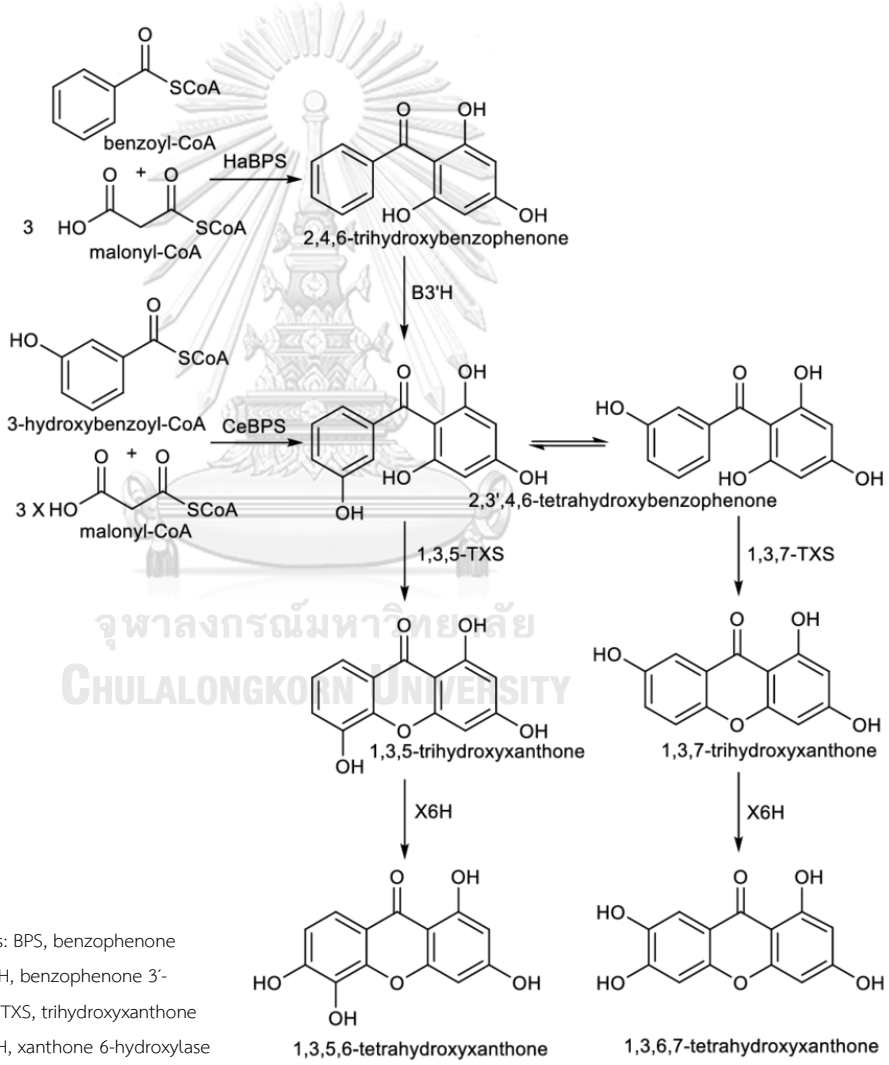
paclitaxel camptothecin quinine

Figure 1.1 Natural products derived from plants

1.1 Xanthones: biosynthesis pathway and biological activities

Xanthones (IUPAC name 9H-xanthen-9-one) are a kind of phenolic acid with a three-ring skeleton, widely distributed in herbal medicines. These constituents display a vast range of bioactitivies, including anticancer, anti- oxidative, antimicrobial, antidiabetic, antiviral, and anti- inflammatory effects. Over the past few decades, xanthones have become an important resource for drug development. For example, gambogic acid, a prenyl xanthone isolated from *Garcinia hanburyi* (Clusiaceae). A phase II clinical trial using gambogic acid in combination with anticancer drugs was carried out in China. Besides gambogic acid mentioned above, mangosteen, another of the most well- known xanthones, has been used as a dietary supplement to improve immune function, decrease serum C- reactive protein levels and increase the ratio of T helper cells. The pharmacokinetics and toxicity (PK/tox) properties of xanthones, as part of the most crucial preclinical studies, have proved that xanthones are promising drug candidates owing to their high efficacy and low toxicity.

Xanthones are mainly isolated from herbal medicines. Between 1988 and 2016, 168 species of herbal medicinal plant belonging to 58 genera, and 24 families were found to be enriched in xanthones. Among them, the *Calophyllum, Cratoxylum, Cudrania, Garcinia, Gentiana, Hypericum* and *Swertia* genera are the plant resource with the most development prospect. Xanthones display multiple bioactivities, which may be useful for new drug development for cancer, inflammation, bacterial, fungal and viral infection, diabetes, and so on [7].



Abbreviations: BPS, benzophenone synthase; B3'H, benzophenone 3′-hydroxylase; TXS, trihydroxyxanthone synthase; X6H, xanthone 6-hydroxylase

Figure 1.2 Overview of major xanthones biosynthesis pathway in plants

The genus *Calophyllum*, belonging to the family Guttiferae which comprises about 200 species that are widely distributed in Asia, America and Africa [8], [9]. This genus has been shown to possess various pharmacological activities including antiarthritic [10], antileishmanial [11], vasorelaxation [12], antiinflammatory, antioxidant [13], antibacteria [14], cytotoxicity [15], antidiabetic [16], antimicrobial [17], antiseptics, astringents, diuretics, purgatives, anti-HIV and antifungal [18]. The genus *Calophyllum* has an abundant source of secondary metabolites, especially xanthones, coumarins, flavonoids, acylphloroglucinols, terpenoids and chromanones [12], [19], [20]. *C. calaba*, commonly known as "Thunghoon" in Thai, is a tree found in the northeastern and southern parts of Thailand. Xanthones [21], [22], terpenoids [23], flavonoids [24], and fatty acids [25] have been reported from this plant.

1.2 Botanical aspect and distribution of *Calophyllum calaba*

Calophyllum calaba is a slow-growing, medium-sized evergreen tree with a spreading crown, distributed widely in the lowland tropical rain forest. It usually grows up to 10-25 meters tall, occasionally to 35 meters. All parts of the plant contain a sticky yellowish latex [26-28]. Its leaves morphologies are oval or oval-shaped, 3-6 cm wide and 4-8 cm long. The flowers are white, fragrant, 0.5 cm wide and occurs in racemose or paniculate inflorescences consisting of 5 to 15 flowers. The fruits are a round, green drupe reaching 2.5 cm (1 in) in diameter and having one-seeded drupes. When ripe, the fruit is wrinkled and its color varies from yellow to brownish-red, usually ripen the following December to April.

whole plant

stem

flower

fruit

Figure 1.3 The whole plant, stem, flower and fruit of *Calophyllum calaba*

Family	: Guttiferae
Genus	: *Calophyllum*
Species	: *Calophyllum calaba*
Common name	: Thunghoon
Local name	: Thunghoon, Pa-Ong

1.3 Chemical constituents from *Calophyllum calaba* and their biological activities

In 1981, Kumar *et al*. [22] successfully isolated two new xanthones; calocalabaxanthone and calabaxanthone from the bark of *C. calaba.*

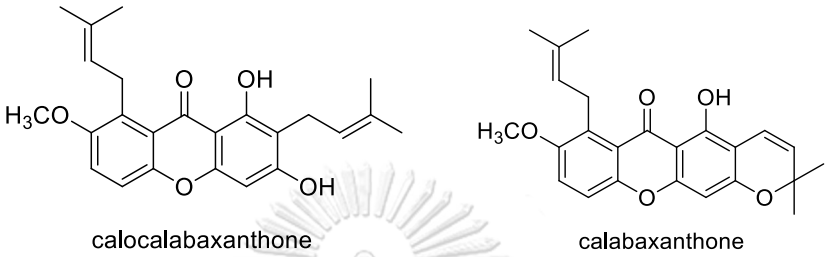
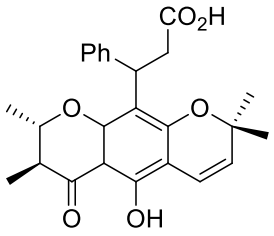
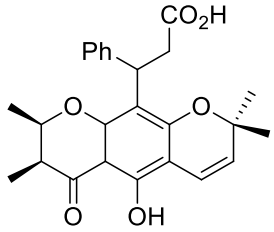


Figure 1.4 Chemical constituents from the bark of *C. calaba*

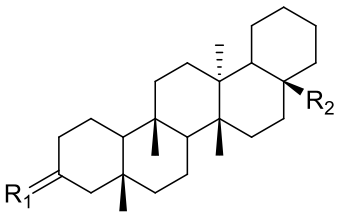
Gunatilaga *et al*. [24] in 1983 succeeded in isolating nine new acid compounds, isochapelieric acid, chapelieric acid, friwdelin, friedelan-3β-ol, canophyllal, canophyllol, friedelan-3β,28-diol, canophyllic acid and amentoilavone from leaves of *C. calaba*.

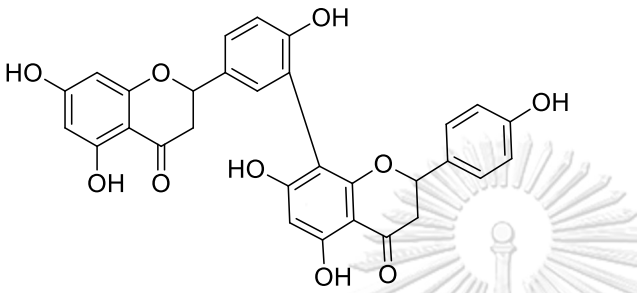


chapelieric acid



isochapelieric acid



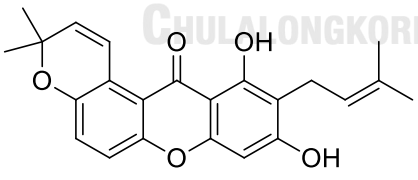


amentoilavone

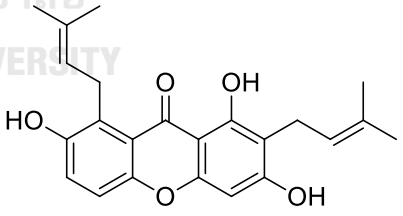
friedelin	R_1 = O, R_2 = Me
friedelan-3β-ol	R_1 = α-H, β-OH, R_2 = Me
canophyllal	R_1 = O, R_2 = CHO
canophyllol	R_1 = O, R_2 = CH_2OH
friedelan-3β,28-diol	R_1 = α-H, β-OH, R_2 = CH_2OH
canophyllic acid	R_1 = α-H, β-OH, R_2 = CO_2H

Figure 1.5 Chemical constituents from the leaves of *C. Calaba*

From the root barks of *C. calaba., C. thwaitesii* and *C. bractcurum,* Dharmaratne *et al.* [21] reported two new xanthones; calothwaitesixanthone and thwaitesixanthone, together with six known xanthones.



calothwaitesixanthone

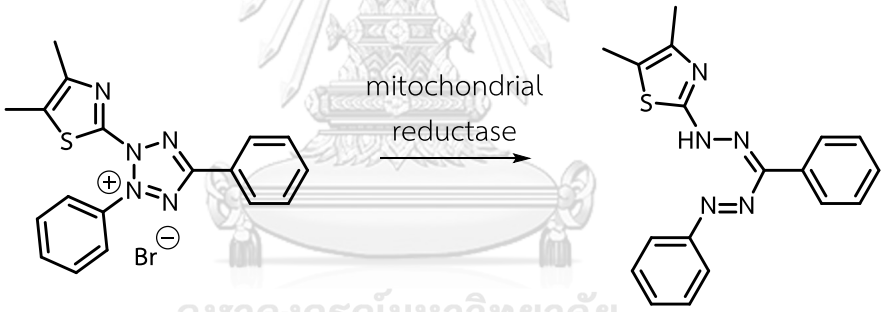


thwaitesixanthone

Figure 1.6 Chemical constituents from the root barks of *C. calaba., C. thwaitesii* and *C. bractcurum*

1.4 Cytotoxic activity against human cancer cell lines

Cancer is one of dangerous diseases caused by uncontrolled growth of the cells. The proliferation of cancer cells may invade the other tissues and organs, and disrupt the metabolic pathways of normal cells. The discovery of an anticancer agent from natural products has been developed initially through a preliminary screening of drug candidates. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is one of initial methods to screen the cytotoxicity of a substance indicated by viability of the cells. The number of viable cells are determined through the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent by mitochondrial dehydrogenase enzyme inside living cells forming a formazan dye (Figure 1.6) which is measured then using colorimetric method. The result of cytotoxic activity can be used for further investigation through *in vivo* test using an animal model to assess the metabolism properties of a drug candidate in a living organism [29].



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)
yellow color

(2*E*,4*Z*)(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (formazan)
purple color

Figure 1.7 MTT reduction in live cells by mitochondrial reductase resulting a formation of insoluble formazan

The literature review above showed no report on chemical constituent and biological activity from the roots of *C. calaba*. Therefore, those provide an insight to further investigation the bioactive compounds from the roots of this plant.

1.5 The objectives of this research

The main objectives in this investigation are as follows:

1. To isolate and purify the compounds from the roots of *C. calaba*
2. To elucidate structurally the isolated compounds by means of spectroscopic analysis, including UV, IR, 1D and 2D NMR, and HRMS.
3. To evaluate the cytotoxic activity of the isolated compounds against human cancer cell lines.

CHAPTER II

EXPERIMENTAL

Figure 2.1 The roots of *Calophyllum calaba*

2.1 Plant material

The roots of *C. calaba* were collected from Buachet district, Surin province, Thailand, in April 2016. The plant material was identified by Dr. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, and a specimen retained as a reference (Khumkratok no. 1-13).

2.2 General experiment procedures

1D and 2D NMR spectra were recorded on Bruker 400 AVANCE spectrometer. HRESIMS spectra were obtained using a Bruker MICROTOF model mass spectrometer. IR data was obtained using Nicolet 6700 FT-IR spectrometer using KBr discs. Optical rotation was detected by Jasco P-1010 Polarimeter. Melting Points were determined on a Fisher-Johns Melting Point apparatus. Column chromatography was performed by silica gel 60 (0.063-0.200 mm) and Sephadex LH-20 (25-100 μm, GE Healthcare).

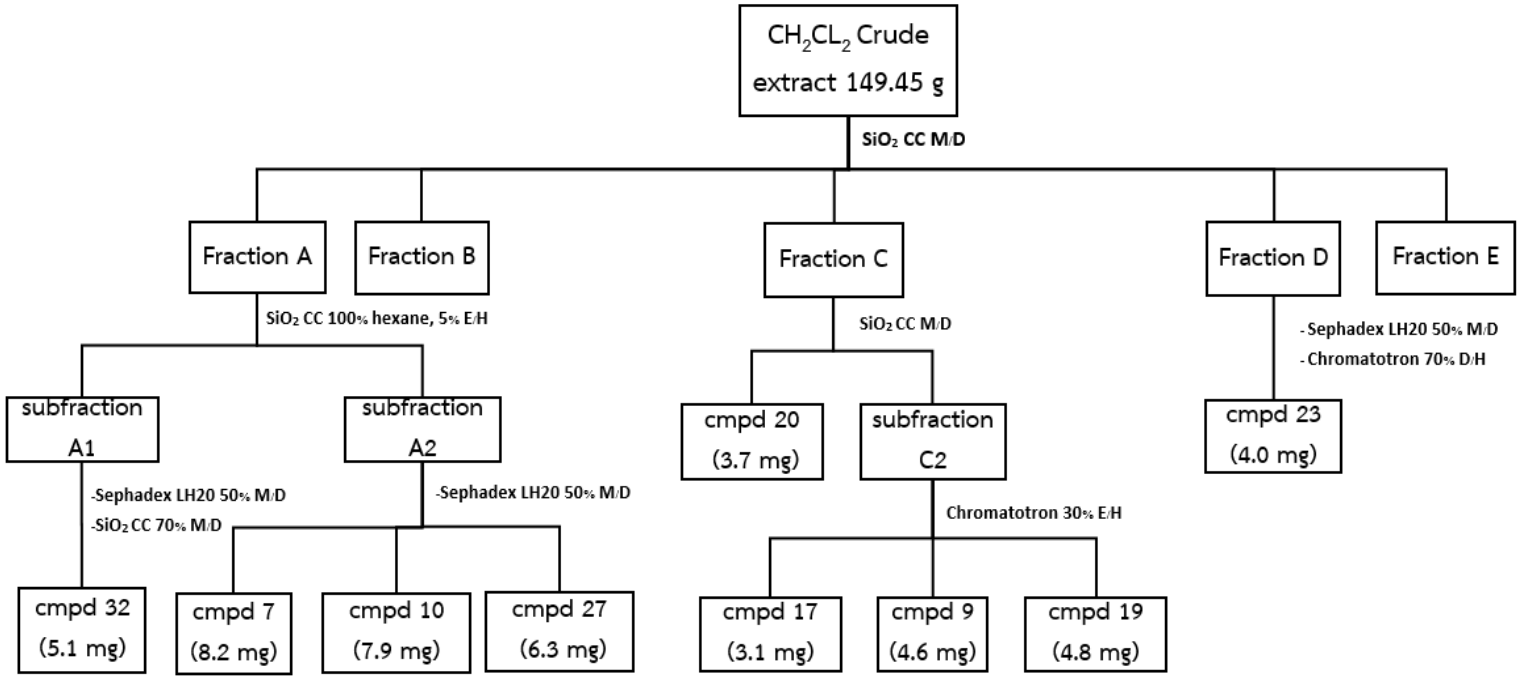
2.3 Extraction and isolation

The air-dried roots of *C. calaba* (7.0 kg) were extracted with CH_2Cl_2 over a period of 7 days at room temperature, respectively (2 × 25 L). Removal of the solvent under reduced pressure provided CH_2Cl_2 crude extract (149.45 g) that was further separated by column chromatography over silica gel (Merck Art 7734) (40×10 cm, 2.5 kg) and eluted with a gradient of Hexane-EtOAc (100% Hexane, 90%, 80%, 70%, 60%, 50% and 40% Hexane-EtOAc each 5 L) to give five fractions (A-E). Fraction A (15.0 g) was purified by silica gel column (45×5 cm, 0.5 kg) eluted with 95% hexane-EtOAc (5 L) to provide two subfractions (A1-A2). Subfraction A1 (2.0 g) was applied to a Sephadex LH-20 column (50×5 cm, 150 g) with 50% CH_2Cl_2-MeOH (2 L) to afford compound **32** (5.1 mg). Subfraction A2 (5.0 g) was separated by a Sephadex LH-20 column (50×5 cm, 150 g) eluted with 50% CH_2Cl_2-MeOH (2 L) to give compounds **7** (8.2 mg), **10** (7.9 mg) and **27** (6.3 mg). Fraction B (25.0 g) was purified over silica gel column silica gel CC (45×5 cm, 0.5 kg) eluted with 95% hexane-EtOAc (5 L) to yield four subfractions (B1-B4). Compounds **14** (2.8 mg) and **21** (2.1 mg) were separated from subfraction B1 (25.0 mg) by radial chromatography (chromatotron) using 80% hexane-EtOAc (200 mL). Subfraction B2 (2.5 g) was purified by Sephadex LH-20 column (50×5 cm, 150 g) eluted with 80% CH_2Cl_2-MeOH (2 L) to obtain compounds **3** (2.9 mg), **13** (8.2 mg) and **31** (1.9 mg). Subfraction B3 (4.0 g) was subjected to Sephadex LH-20 column (50×5 cm, 150 g) using 80% CH_2Cl_2-MeOH (2 L) and further purified by chromatotron eluted with 85% hexane-EtOAc (200 mL) to afford compounds **11** (2.2 mg), **12** (3.0 mg), **18** (1.7 mg), **28** (2.6 mg) and **29** (2.7 mg). Compound **8** (2.2 mg) was obtained from subfraction B4 (1.5 g) by Sephadex LH-20 column (50×5 cm, 150 g) with 50% CH_2Cl_2-MeOH (2 L). Fraction C (5.0 g) was separated by silica gel column (40×4 cm, 0.4 kg) eluted with 80% CH_2Cl_2-MeOH (5 L) and further purified by chromatotron with 70% hexane-EtOAc (200 mL) to obtain compounds **9** (4.6 mg), **17** (3.1 mg), **19** (4.8 mg) and **20** (3.7 mg). Compound **23** (4.0 mg) was purified from fraction D (1.0 g) by Sephadex LH-20 column (50×5 cm, 150 g) using 50% CH_2Cl_2-MeOH (2 L). Fraction E (35.0 g) was isolated by silica gel column (45×5 cm, 0.5 kg) eluted with 70% hexane-EtOAc (5L) to afford four subfractions (E1-E4). Compounds **1** (7.1 mg) and **2** (8.1 mg) were purified by chromatotron with 80%

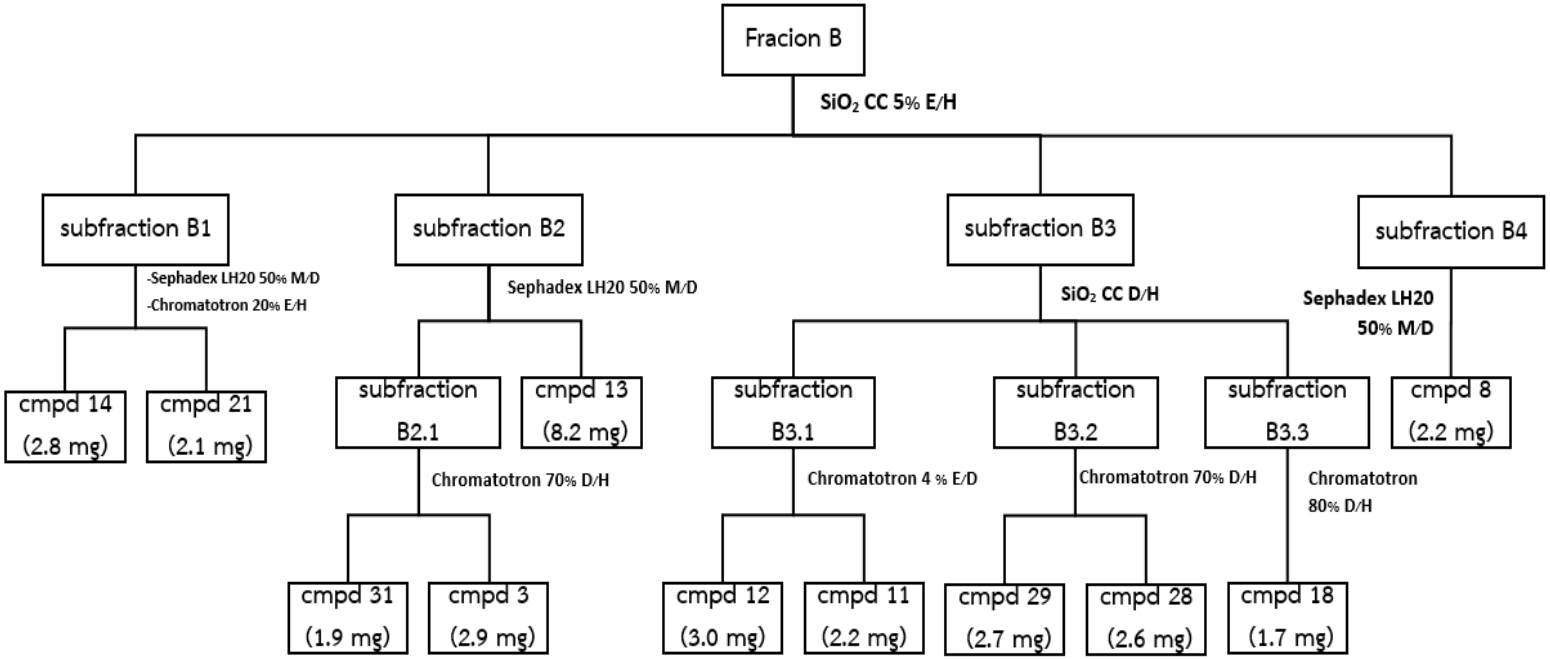
hexane-EtOAc (200 mL) from subfraction E1 (1.0 g). Subfraction E2 (2.5 g) was applied to a Sephadex LH-20 column (50×5 cm, 150 g) using 50% CH_2Cl_2-MeOH (2 L) to give compounds **4** (7.0 mg) and **6** (2.3 mg). Subfraction E3 (4.5 g) was purified by silica gel column (45×5 cm, 0.5 kg) using 80% hexane-EtOAc (5L) and further separated by chromatotron with 100% CH_2Cl_2 (200 mL) to provide compounds **5** (2.5 mg), **15** (2.0 mg), **16** (5.2 mg), **22** (6.5 mg) and **30** (6.9 mg). Finally, subfraction E4 (3.5 g) was subjected to silica gel column (45×5 cm, 0.5 kg) eluted with 100% CH_2Cl_2 and further purified by chromatotron with 60% hexane-EtOAc (200 mL) to yield compounds **24** (1.7 mg), **25** (2.4 mg) and **26** (1.7 mg).

The isolated compounds were identified by means of various spectroscopic methods including MS, 1D and 2D NMR techniques together with comparison with the previous literature data.

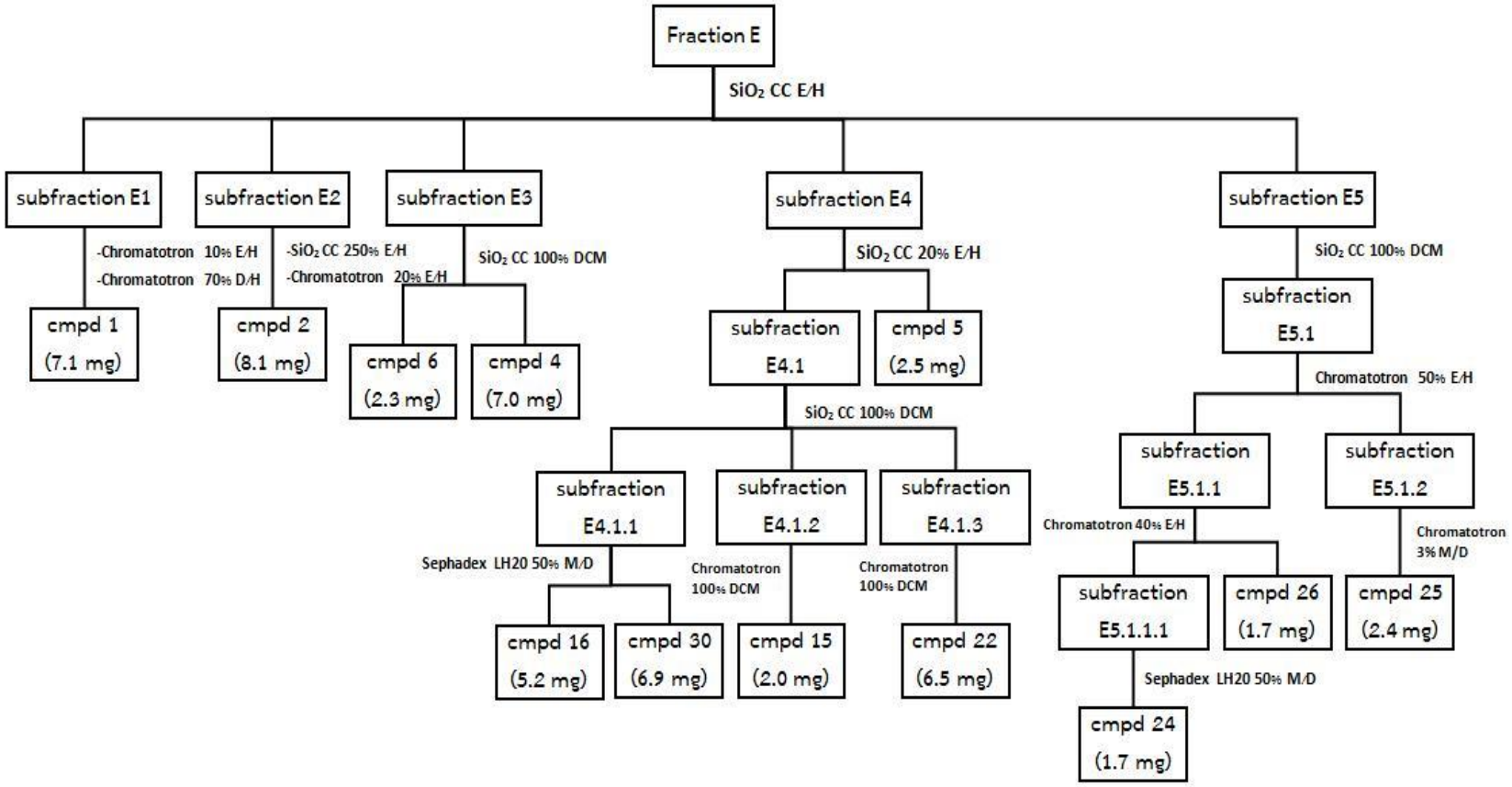
The isolation and purification of all isolated compounds from the roots of *C. calaba* were briefly summarized in **Schemes 2.1, 2.2 and 2.3.**



Scheme 2.1 Isolation procedure of subfractions A,C and D from the CH_2Cl_2 crude extract of *C. calaba* roots



Scheme 2.2 Isolation procedure of subfractions B1, B2, B3 and B4 from the CH_2Cl_2 crude extract of *C. calaba* roots



Scheme 2.3 Isolation procedure of subfractions E1, E2, E3, E4 and E5 from the CH_2Cl_2 crude extract of *C. calaba* roots

2.4 Cytotoxic activity against human cancer cell lines procedure

All isolated compounds (**1**-**32**) were subjected to cytotoxic evaluation against KB, HeLa S-3, HT29, MCF-7 and HepG2 cell lines employing the colorimetric method [30], [31]. Doxorubicin was used as the reference substance which exhibits activity against five cancer cell lines. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3×103 cells) suspended in 100 µg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 72 h pre-incubation at 37°C in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 µL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 µL of tetrazolium reagent was added into each well followed by further incubation at 37°C for 4 h. The supernatant was decanted, and DMSO (100 µL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Properties and structural elucidation of isolated compounds

The roots of *C. calaba* were grounded and extracted with CH_2Cl_2 at room temperature for six days. The crude CH_2Cl_2 extract was further subjected by various chromatographic techniques using silica gel and Sephadex LH-20 as stationary phases to afford three new xanthones, calaxanthones A-C (**1-3**) together with twenty nine known xanthones (**4-32**), including scriblitifolic acid (**4**), teysmannic acid (**5**), calophylixanthone A (**6**), 9-xanthone (**7**), 1-hydroxyxanthone (**8**), 4-hydroxyxanthone (**9**), 4-methoxyxanthone (**10**), 1,5-dihydroxyxanthone (**11**), 1-hydroxy-5-methoxyxanthone (**12**), 1,6-dihydroxyxanthone (**13**), 1-hydroxy-6-methoxy-9H-xanthen-9-one (**14**), 3-hydroxy-5-methoxy-9H-xanthen-9-one (**15**), 5-hydroxy-3-methoxy-9H-xanthen-9-one (**16**), mesuaxanthone B (**17**), buchanoxanthone (**18**), 1,5-dihydroxy-6-methoxyxanthone (**19**), 3,4-dimethoxyxanthone (**20**), 1-Hydroxy-5,6-dimethoxyxanthone (**21**), 6-hydroxy-3,4-dimethoxy-9H-Xanthen-9-one (**22**), 1,5-dihydroxy-3,6-dimethoxy-xanthen-9-one (**23**), 5-hydroxy-1,3,6-trimethoxy-9H-xanthen-9-one (**24**), 1-hydroxy-3,7-dimethoxyxanthone (**25**), 1,3,5,7-tetramethoxyxanthone (**26**), 1-hydroxy-3,5-dimethoxyxanthone (**27**), 1,5-dihydroxy-8-methoxyxanthone (**28**), cratoxyarborenone F (**29**), 3-hydroxy-2-methoxyxanthone (**30**), β-mangostin (**31**) and toxyloxanthone A (**32**). The structures of all isolated compounds were elucidated using spectroscopic methods (especially 1D and 2D NMR) and compared with their ^{1}H and ^{13}C NMR spectroscopic data of literature.

3.1.1 Calaxanthone A (1)

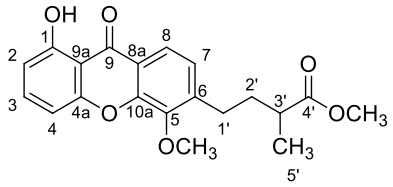


Figure 3.1 The chemical structure of compound **1**

Calaxanthone A (**1**) was obtained as a white powder and optically active $[\alpha]_D^{21}$ + 5.26 (*c* 0.3, $CHCl_3$). Its molecular formula was determined as $C_{20}H_{20}O_6$ by HRESIMS measurement through the molecular ion peak at *m/z* 357.1342 [M+ H] $^+$ (calcd. for $C_{20}H_{20}O_6H$, 357.1338). The UV spectrum displayed absorption bands at λ_{max} 314, 258 and 242 nm, which is typical of the xanthone chromophore [32]. The IR spectrum showed O-H and C=O stretching bands at 3215 and 1745 cm^{-1}, respectively. The ^{1} H NMR spectrum displayed a methyl proton at δ_H 1.24 (3H, d, *J* = 7.14 Hz, H-5′), two methoxy protons at δ_H 3.70 (3H, s, OCH_3-4′) and 4.04 (3H, s, OCH_3-5), two methylene protons at δ_H 1.76, 2.04 (2H, m, H-2′) and 2.79 (2H, t, *J* = 8.03 Hz, H-1′); six methine protons at δ_H 2.54 (1H, dd, *J* = 6.92, 13.86 Hz, H-3′), 6.81 (1H, d, *J* = 8.27 Hz, H-2), 7.00 (1H, d, *J* = 8.25 Hz, H-4), 7.20 (1H, d, *J* = 8.25 Hz, H-7), 7.60 (1H, t, *J* = 8.36 Hz, H-3), and 7.94 (1H, d, *J* = 8.23 Hz, H-8), and a hydrogen-bonded hydroxy proton at δ_H 12.65 (1H, s, OH-1). The ^{13}C NMR spectrum displayed 20 carbons, including three methyl carbons at δ_C 17.3 (C-5′), 51.7 (OCH_3-4′), and 61.8 (OCH_3-5), two methylene carbons at δ_C 28.2 (C-1′) and 34.3 (C-2′), six methine carbons at δ_C 39.3 (C-3′), 107.2 (C-4), 110.8 (C-2), 120.5 (C-8), 125.3 (C-7) and 136.8 (C-3), two carbonyl carbons at δ_C 176.8 (C-4′) and 182.3 (C-9), and seven quaternary carbons at δ_C 108.9 (C-9a), 115.7 (C-8a), 142.8 (C-6), 146.3 (C-5), 150.0 (C-10a), 156.1 (C-4a) and 162.1 (C-1). The COSY spectrum showed correlations between H-2 and H-3, H-3 and H-4 ring A, between H-7 and H-8 ring B, and between H-1′ and H-2′, H-2′ and H-3′, and H-3′ and H-5′ in methyl-2-methylbutanoate chain (**Figure 3.2**).

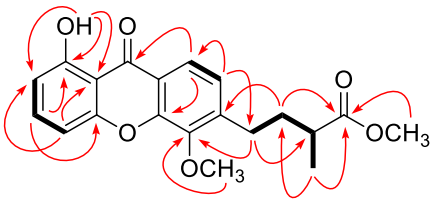


Figure 3.2 Selected HMBC (single headed arrow curves) and COSY (bold lines) correlations of **1**.

Based on the HMBC spectrum showed cross-peak between a hydroxy proton at δ_H 12.65 and carbons at C-1, C-2, and C-9a, between a methoxy proton at OCH_3-5 and carbon at C-5, and between a methylene proton at H-1′ and carbons at C-5, C-6, C-7, C-2′ and C-3′ indicated that ring A was substituted at C-1 and ring B was substituted at C-5 and C-6. The HMBC correlations (**Figure 3.2**) at H-2′ to C-6, C-1′, C-3′, C-4′ and C-5′, at H-3′ to C-1′ and C-4′, at H-5′ to C-2′ and C-4′, and at OCH_3-4′ to C-4′ showed the presence of methyl-2-methylbutanoate group located at C-6 ring B. The ^1H and ^{13}C NMR data (**Table 3.1**) of **1** were shown to be quite similar to those of known xanthone, scriblitifolic acid (**4**) [33], the difference was found at C-4′ in which the carboxylic acid of **4** was replaced to methyl ester. From these data, the structure of calaxanthone A was assigned as **1**.

Table 3.1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of compound **1** in $CDCl_3$

position	**1**		
	δ_H (*J* in Hz)	δ_C	HMBC
1		162.1	-
2	6.81, d (8.27)	110.8	C-4, C-9a
3	7.60, t (8.36)	136.8	C-1, C-4a
4	7.00, d (8.25)	107.2	C-2, C-4a, C-9a
4a		156.1	-
5		146.3	-
6		142.8	-
7	7.20, d (8.25)	125.3	C-, C-8, C-1′
8	7.94, d (8.23)	120.5	C-6, C-9, C-10a
8a		115.7	-
9		182.3	-
9a		108.9	-
10a		150.0	-
1′	2.79, t (8.03)	28.2	C-5, C-6, C-7, C-2′, C-3′
2′	2.04 (m), 1.76 (m)	34.3	C-6, C-1′, C-3′, C-4′, C-5′
3′	2.54, dd (6.92, 13.86)	39.3	C-4′, C-5′
4′		176.8	-
5′	1.24, d (7.14)	17.3	C-1′, C-2′, C3′, C-4′
1-OH	12.65		C-1, C-2, C-9a
4′-OCH_3	3.70, s	51.7	C-4′
5-OCH_3	4.04, s	61.8	C-5

3.1.2 Calaxanthone B (2)

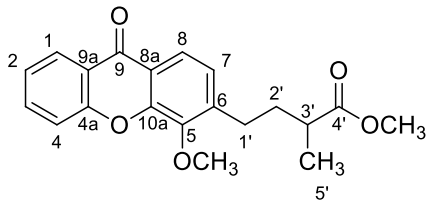


Figure 3.3 The chemical structure of compound **2**

Calaxanthone B **(2)** was obtained as a yellow viscous oil and optically active $[\alpha]_D^{21}$-8.06 (*c* 0.5, $CHCl_3$). Its molecular formula was determined as $C_{20}H_{20}O_5$ by HRESIMS measurement through the molecular ion peak at *m/z* 341.1391 [M+ H]$^+$ (calcd. for $C_{20}H_{20}O_5H$, 341.1389). The UV spectrum displayed absorption bands at λ_{max} 312, 261 and 247 nm, which is typical of the xanthone chromophore [32]. The IR spectrum showed C= O stretching bands at 1746 cm^{-1}. The ^{1}H and ^{13}C NMR spectroscopic data **(Table 3.2)** of **2** were showed to be the same with those of **1**, except for unsubstituted at C-1 ring A. The ^{1}H NMR showed the aromatic methine proton at δ_H 8.33(1H, dd, *J* = 1.42, 7.94 Hz, H-1), which were correlated in the HSQC spectrum with aromatic methine carbon at δ_C 126.8 (C-1).

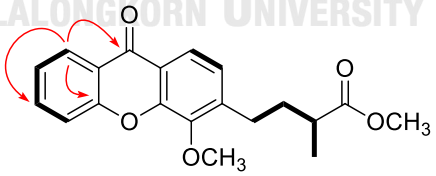


Figure 3.4 Selected HMBC (single headed arrow curves) and COSY (bold lines) correlations of **2**.

Based on the HMBC correlations (**Figure 3.4**) between H-1 and C-3 (δ_C 134.8), C-4a (δ_C 155.9) and C-9 (δ_C 176.8) confirmed unsubsituted ring A. Thus, the structure of calaxanthone B was assigned as **2**.

Table 3.2 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of compound **2** in $CDCl_3$

position	**2**		
	δ_H (*J* in Hz)	δ_C	HMBC
1	8.33, dd (1.42, 7.94)	126.8	C-3, C-4a, C-9
2	7.38, t (7.52)	124.2	C-4, C-9a
3	7.73, t (7.78)	134.8	C-1, C-4a
4	7.56, d (8.39)	118.2	C-2, C-4a, C-9a
4a		155.9	-
5		146.4	-
6		141.8	-
7	7.18, d (8.26)	125.1	C-5, C-8, C-1′
8	8.00, d (8.24)	121.3	C-6, C-9, C-10a
8a		122.1	-
9		176.8	-
9a		121.8	-
10a		150.0	-
1′	2.79, t (7.98)	28.1	C-5, C-6, C-7, C-2′, C-3′
2′	2.04 (m), 1.76 (m)	34.4	C-6, C-1′, C-3′, C-4′, C-5′
3′	2.53, dd (6.93, 13.85)	39.3	C-1′, C-2′, C-4′, C-5′
4′		177.0	-
5′	1.23, d (7.08)	17.3	C-2′, C-3′, C-4′
1-OH			-
4′-OCH_3	3.69, s	51.7	C-4′
5-OCH_3	4.05, s	61.8	C-5′

3.1.3 Calaxanthone C (3)

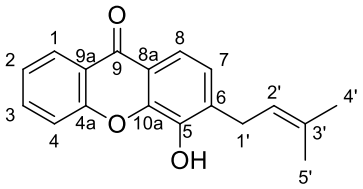


Figure 3.5 The chemical structure of compound **3**

Calaxanthone C **(3)** was obtained as a white powder. Its molecular formula was determined as $C_{18}H_{16}O_3$ by HRESIMS measurement through the molecular ion peak at *m/z* 281.1171 **[**M**+** H**]**$^{+}$ **(**calcd. for $C_{18}H_{16}O_3H$, 281.1178**).** The UV spectrum displayed absorption bands at λ_{max} 311, 263 and 245 nm, which is typical of the xanthone chromophore **[**32**].** The IR spectrum showed O-H and C=O stretching bands at 3216 and 1668 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR spectroscopic data **(Table 3.3)** were showed to be quite similar to those of **2**, except for the absence of ^{1}H NMR signal of the methoxy group at C-5 and methyl-2-methylbutanoate group at C-6 of 3. Furthermore, the substituent at C-5 was assigned as a hydroxyl group according of its ^{13}C NMR chemical shift **(**δ_C 142.2**).** The ^{1}H NMR spectrum displayed two methyl protons at δ_H 1.78 **(**3H, s, H-4′**)** and 1.78 **(**3H, s, H-5′**)**, a methylene proton at δ_H 3.52 **(**2H, d, *J* **=** 7.17 Hz, H-1′**)**, and a methine proton at δ_H 5.36 **(**1H, t, *J* **=** 7.32 Hz, H-2′**)** for prenyl unit.

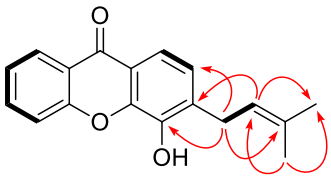


Figure 3.6 Selected HMBC (single headed arrow curves) and COSY (bold lines) correlations of **3**.

The HMBC spectrum showed cross peak **(Figure 3.6)** between H-1′ to C-2′ **(**δ_C 121.1**)**, C-7 **(**δ_C 124.9**)** and C-5 **(**δ_C 142.2**)**, and between H-2′ to C-5′ **(**δ_C 18.0**)**, C-6 **(**δ_C 134.0**)** and C-3′ **(**δ_C 134.5**)** indicated that the prenyl group was connected at C-6 ring B. From this data, the structure of calaxanthone C was therefore assigned as **3.**

Table 3.3 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of compound **3** in $CDCl_3$

position	3		
	δ_H (*J* in Hz)	δ_C	HMBC
1	8.36, d (7.91)	127.2	C-3, C-4a, C-9
2	7.41, t (7.49)	124.4	C-4, C-9a
3	7.74, t (8.05)	134.8	C-1, C-4, C-4a
4	7.53, d (8.43)	117.7	C-2, C-4a, C-9a
4a		155.5	-
5		142.2	-
6		134.0	-
7	7.17, d (8.28)	124.9	C-5, C-1′, C-2′
8	7.80, d (8.29)	117.2	C-6, C-9, C-10a
8a		120.5	-
9		176.9	-
9a		121.9	-
10a		145.3	-
1′	3.52, d (7.17)	29.0	C-5, C-6, C-7, C-2′
2′	5.36, t (7.32)	121.1	C-5′
3′		134.5	-
4′	1.78, s	25.9	C-2′, C-5′
5′	1.78, s	18.0	C-2′, C-4′

3.1.4 Scriblitifolic acid (4)

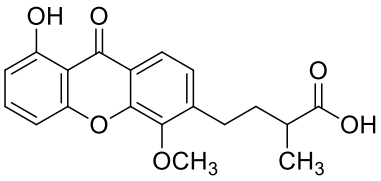


Figure 3.7 The chemical structure of compound **4**

Scriblitifolic acid (**4**) (**Figure 3.7**): The structure of compound **4** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**34**].**

3.1.5 Teysmannic acid (5)

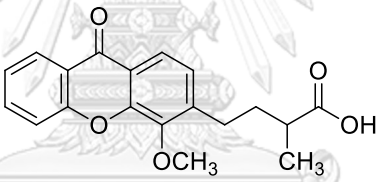


Figure 3.8 The chemical structure of compound **5**

Scriblitifolic acid (**5**) (**Figure 3.8**): The structure of compound **5** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [35].

3.1.6 Calophylixanthone A (6)

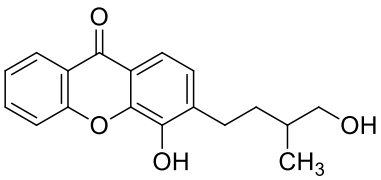


Figure 3.9 The chemical structure of compound **6**

Calophylixanthone A (**6**) (**Figure 3.9**): The structure of compound **6** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [36].

3.1.7 9-Xanthone (7)

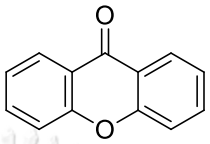


Figure 3.10 The chemical structure of compound **7**

9-Xanthone (**7**) (**Figure 3.10**): The structure of compound **7** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [37].

3.1.8 1-Hydroxyxanthone (8)

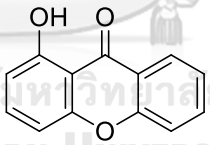


Figure 3.11 The chemical structure of compound **8**

1-Hydroxyxanthone (**8**) (**Figure 3.11**): The structure of compound **8** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [38].

3.1.9 4-Hydroxyxanthone (9)

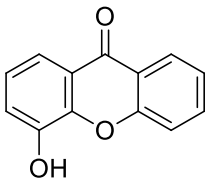


Figure 3.12 The chemical structure of compound **9**

4-Hydroxyxanthone (**9**) (**Figure 3.12**): The structure of compound **9** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [38].

3.1.10 4-Methoxyxanthone (10)

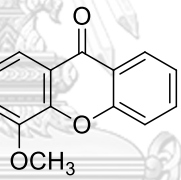


Figure 3.13 The chemical structure of compound **10**

4-Methoxyxanthone (**10**) (**Figure 3.13**): The structure of compound **10** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [39].

3.1.11 1,5-Dihydroxyxanthone (11)

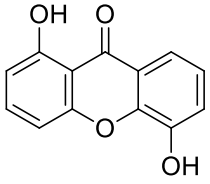


Figure 3.14 The chemical structure of compound **11**

1,5-Dihydroxyxanthone (**11**) (**Figure 3.14**): The structure of compound **11** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**40**].**

3.1.12 1-Hydroxy-5-methoxyxanthone (12)

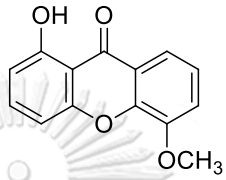


Figure 3.15 The chemical structure of compound **12**

1-Hydroxy-5-methoxyxanthone (**12**) (**Figure 3.15**): The structure of compound **12** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**41**].**

3.1.13 1,6-Dihydroxyxanthone (13)

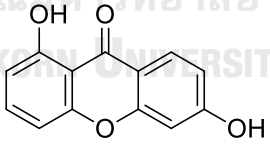


Figure 3.16 The chemical structure of compound **13**

1,6-Dihydroxyxanthone (**13**) (**Figure 3.16**): The structure of compound **13** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [42].

3.1.14 1-Hydroxy-6-methoxy-9H-xanthen-9-one (14)

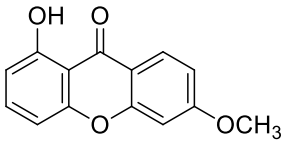


Figure 3.17 The chemical structure of compound **14**

1-Hydroxy-6-methoxy-9H-xanthen-9-one (**14**) (**Figure 3.17**): The structure of compound **14** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**43**].**

3.1.15 3-Hydroxy-5-methoxy-9H-xanthen-9-one (15)

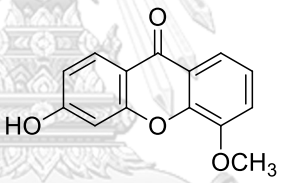


Figure 3.18 The chemical structure of compound **15**

3-Hydroxy-5-methoxy-9H-xanthen-9-one (**15**) (**Figure 3.18**): The structure of compound **15** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**43**].**

3.1.16 5-Hydroxy-3-methoxy-9H-xanthen-9-one (16)

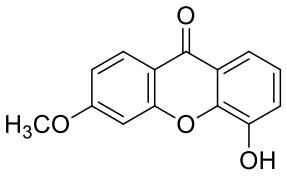


Figure 3.19 The chemical structure of compound **16**

5-Hydroxy-3-methoxy-9H-xanthen-9-one (**16**) (**Figure 3.19**): The structure of compound **16** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**43**].**

3.1.17 Mesuaxanthone B (17)

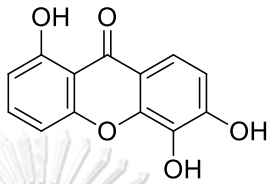


Figure 3.20 The chemical structure of compound **17**

Mesuaxanthone B (**17**) (**Figure 3.20**): The structure of compound **17** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**44**].**

3.1.18 Buchanoxanthone (18)

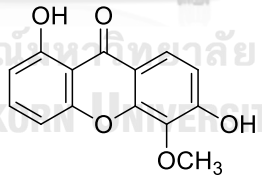


Figure 3.21 The chemical structure of compound **18**

Buchanoxanthone (**18**) (**Figure 3.21**): The structure of compound **18** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**45**].**

3.1.19 1,5-Dihydroxy-6-methoxyxanthone (19)

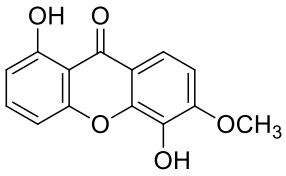


Figure 3.22 The chemical structure of compound **19**

1,5- Dihydroxy- 6- methoxyxanthone (**19**) (**Figure 3. 22**) : The structure of compound **19** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**46**].**

3.1.20 3,4-Dimethoxyxanthone (20)

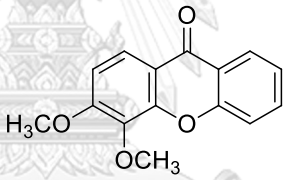


Figure 3.23 The chemical structure of compound **20**

3,4-Dimethoxyxanthone (**20**) (**Figure 3.23**): The structure of compound **20** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**47**].**

3.1.21 1-Hydroxy-5,6-dimethoxyxanthone (21)

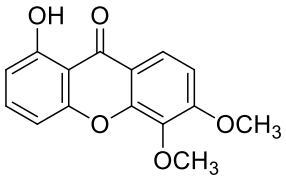


Figure 3.24 The chemical structure of compound **21**

1- Hydroxy- 5,6- dimethoxyxanthone (**21**) (**Figure 3. 24**) : The structure of compound **21** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**45**].**

3.1.22 6-Hydroxy-3,4-dimethoxy- 9H-xanthen-9-one (22)

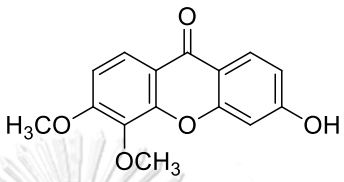


Figure 3.25 The chemical structure of compound **22**

6-Hydroxy-3,4-dimethoxy- 9H-xanthen-9-one (**22**) (**Figure 3.25**): The structure of compound **22** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**48**].**

3.1.23 1,5-Dihydroxy-3,6-dimethoxy-xanthen-9-one (23)

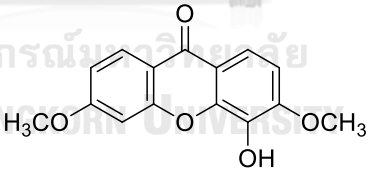


Figure 3.26 The chemical structure of compound **23**

1,5-Dihydroxy-3,6-dimethoxy-xanthen-9-one (**23**) (**Figure 3.26**): The structure of compound **23** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report **[**49**].**

3.1.24 5-Hydroxy-1,3,6-trimethoxy-9H-xanthen-9-one (24)

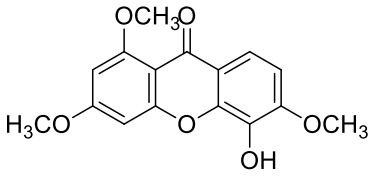


Figure 3.27 The chemical structure of compound **24**

5-Hydroxy-1,3,6-trimethoxy-9H-xanthen-9-one (**24**) (**Figure 3.27**): The structure of compound **24** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [50].

3.1.25 1-Hydroxy-3,7-dimethoxyxanthone (25)

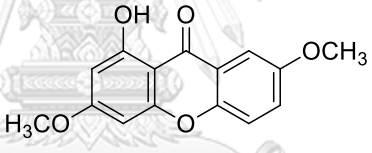


Figure 3.28 The chemical structure of compound **25**

1-Hydroxy-3,7-dimethoxyxanthone (**25**) (**Figure 3.28**): The structure of compound **25** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [51].

3.1.26 1,3,5,7-Tetramethoxyxanthone (26)

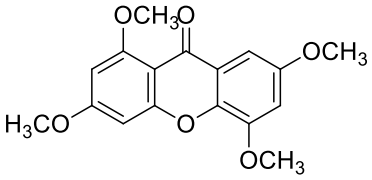


Figure 3.29 The chemical structure of compound **26**

1,3,5,7-Tetramethoxyxanthone (**26**) (**Figure 3.29**): The structure of compound **26** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [52].

3.1.27 1-Hydroxy-3,5-dimethoxyxanthone (27)

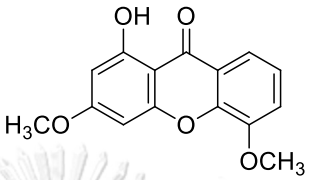


Figure 3.30 The chemical structure of compound **27**

1- Hydroxy- 3,5- dimethoxyxanthone (**27**) (**Figure 3. 30**) : The structure of compound **27** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [53].

3.1.28 1,5-Dihydroxy-8-methoxyxanthone (28)

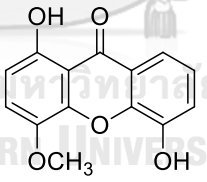


Figure 3.31 The chemical structure of compound **28**

1,5- Dihydroxy- 8- methoxyxanthone (**28**) (**Figure 3. 31**) : The structure of compound **28** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [54].

3.1.29 Cratoxyarborenone F (29)

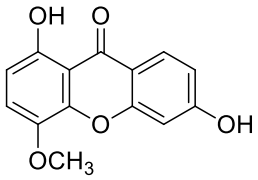


Figure 3.32 The chemical structure of compound **29**

Cratoxyarborenone F (**29**) (**Figure 3.32**): The structure of compound **29** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [55].

3.1.30 3-Hydroxy-2-methoxyxanthone (30)

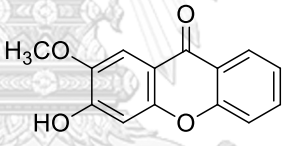


Figure 3.33 The chemical structure of compound **30**

3-Hydroxy-2-methoxyxanthone (**30**) (**Figure 3.33**): The structure of compound **30** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [56].

3.1.31 β-Mangostin (31)

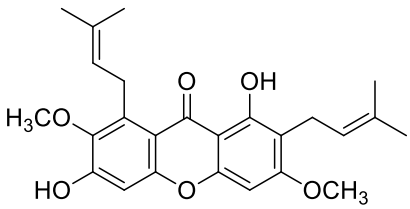


Figure 3.34 The chemical structure of compound **31**

β-Mangostin (**31**) (**Figure 3.34**): The structure of compound **31** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [57].

3.1.32 Toxyloxanthone A (32)

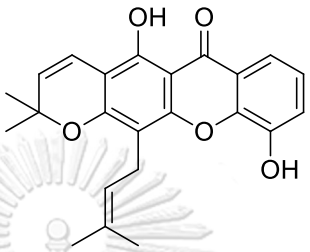


Figure 3.35 The chemical structure of compound **32**

Toxyloxanthone A (**32**) (**Figure 3.35**): The structure of compound **32** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [58].

3.2 Cytotoxic activity of isolated compounds (1-31) against human cancer cell lines

All isolated compounds were *in vitro* evaluated for their cytotoxic potential against KB, HeLa S-3, HT29, MCF-7 and HepG2 cell lines using the modified MTT method with doxorubicin as the positive control. The results are summarized in Table 3.4. The test compounds mostly showed moderate to inactive against these five cell lines, except compounds **3** showed potent cytotoxicity against KB, HeLa S-3, HT29, MCF-7 and HepG2 cells with IC_{50} values of 1.72, 0.82, 1.17, 5.04 and 1.65 μM, respectively. Furthermore, compound **6** showed good cytotoxicity against KB and HeLa S-3 cell with IC_{50} value of 7.06 and 5.27 μM, respectively. Moreover, compound **13** showed good cytotoxicity against only KB cell with IC_{50} value of 4.62 μM. Compounds **1**, **2**, **16** and **32** showed moderate cytotoxicity against KB cell with IC_{50} values in the range of 16.32-28.19 μM. Compounds **13**, **31** and **32** showed moderate cytotoxicity against HeLa S-3 cell with IC_{50} values in the range of 17.49-27.91 μM. Compounds **6** and **32** showed moderate cytotoxicity against MCF-7 and HepG2 cell with IC50 values in the range of 17.49-27.91 μM. The SAR studied data from **Figure 4.1** and **Table 3.4** suggested that the appearance of C-5 hydroxy and C-6 side chain of xanthones might improve the cytotoxicity, as inferred from the comparison of their cytotoxicity from compounds **3**, **6**, **11**, **17** and **19.**

Table 3.4 The *in vitro* cytotoxic activity of compouds **1-32** against KB, HeLa S-3, HT29, MCF-7 and HepG2 human cancer cell lines

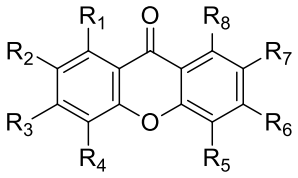
Compound	IC_{50}(μM) ± SD; 72h					Compound	IC_{50}(μM) ± SD; 72h				
	KB	Hela S-3	HT29	MCF-7	HepG2		KB	Hela S-3	HT29	MCF-7	HepG2
1	26.32 ± 1.29	30.19 ± 1.61	55.22 ± 2.18	53.17 ± 1.26	>100	**17**	>100	>100	-	-	-
2	27.64 ± 1.47	51.62 ± 0.65	>100	>100	>100	**18**	>100	>100	-	-	-
3	1.72 ± 0.23	0.82 ± 0.16	1.17 ± 0.19	5.04 ± 0.67	1.65 ± 0.18	**19**	52.57 ± 2.92	63.27 ± 1.45	-	-	-
4	56.88 ± 2.81	77.67 ± 6.14	-	-	-	**20**	>100	>100	-	-	-
5	47.12 ± 4.53	93.82 ± 5.13	-	-	-	**21**	>100	>100	-	-	-
6	7.06 ± 0.18	5.27 ± 0.39	46.60 ± 3.37	20.04 ± 1.55	20.44 ± 0.84	**22**	>100	>100	-	-	-
7	-	-	-	-	-	**23**	>100	>100	-	-	-
8	>100	>100	-	-	-	**24**	40.44 ± 2.93	63.90 ± 2.82	-	-	-
9	>100	>100	-	-	-	**25**	63.90 ± 2.26	64.53 ± 1.96	-	-	-
10	-	-	-	-	-	**26**	89.88 ± 3.21	61.52 ± 0.27	-	-	-
11	>100	>100	-	-	-	**27**	-	-	-	-	-
12	>100	>100	-	-	-	**28**	99.30 ± 1.14	>100	-	-	-
13	4.62 ± 0.22	17.49 ± 3.03	-	-	-	**29**	>100	>100	-	-	-
14	>100	>100	-	-	-	**30**	69.13 ± 3.78	66.46 ± 4.73	-	-	-
15	36.35 ± 1.32	61.53 ± 8.54	-	-	-	**31**	36.34 ± 2.28	23.88 ± 0.28	-	-	-
16	28.19 ± 0.94	83.20 ± 11.88	>100	>100	>100	**32**	16.32 ± 1.11	17.66 ± 0.19	34.90 ± 2.17	17.49 ± 0.73	27.91 ± 2.37
Doxorubicin	0.16 ± 0.01	0.03 ± 0.01	0.34 ± 0.03	0.43± 0.03	1.43 ± 0.08						

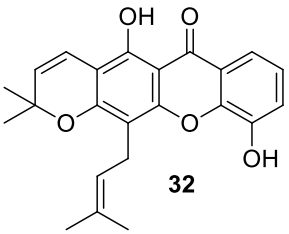
$IC_{50} \leq 10$ μM= active; $10 < IC_{50} \leq 30$ μM= moderate; $30 < IC_{50} \leq 100$ μM= weak; $IC_{50} > 100$ μM= inactive

CHAPTER IV

CONCLUSION

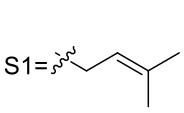
In conclusion, compounds **1-32** were successfully isolated and purified from the CH_2Cl_2 crude extract from the roots of *C. calaba* by silica gel and Sephadex LH-20 column chromatographies. The isolated compounds consisted of three new xanthones, calaxanthones A-C (**1-3**) together with twenty nine known xanthones (**4-32**), including scriblitifolic acid (**4**), teysmannic acid (**5**), calophylixanthone A (**6**), 9-xanthone (**7**), 1-hydroxyxanthone (**8**), 4-hydroxyxanthone (**9**), 4-methoxyxanthone (**10**), 1,5- dihydroxyxanthone (**11**), 1- hydroxy- 5- methoxyxanthone (**12**), 1,6-dihydroxyxanthone (**13**), 1-hydroxy-6-methoxy-9H-xanthen-9-one (**14**), 3-hydroxy-5-methoxy- 9H- xanthen- 9- one (**15**), 5- hydroxy- 3- methoxy- 9H- xanthen- 9- one (**16**), mesuaxanthone B (**17**), buchanoxanthone (**18**), 1,5-dihydroxy-6-methoxyxanthone (**19**), 3,4-dimethoxyxanthone (**20**), 1-Hydroxy-5,6-dimethoxyxanthone (**21**), 6-hydroxy-3,4-dimethoxy- 9H-Xanthen-9-one (**22**), 1,5-dihydroxy-3,6-dimethoxy-xanthen-9-one (**23**), 5-hydroxy-1,3,6-trimethoxy-9H-xanthen-9-one (**24**), 1-hydroxy-3,7-dimethoxyxanthone (**25**), 1,3,5,7-tetramethoxyxanthone (**26**), 1-hydroxy-3,5-dimethoxyxanthone (**27**), 1,5-dihydroxy- 8- methoxyxanthone (**28**), cratoxyarborenone F (**29**), 3- hydroxy- 2-methoxyxanthone (**30**), β-mangostin (**31**) and toxyloxanthone A (**32**). The structures of all isolated compounds were elucidated using spectroscopic methods especially 1D and 2D NMR spectroscopies and compared with their ^{1}H and ^{13}C NMR spectroscopic data of literature. Moreover, the cytotoxic activity against KB and HeLa S-3 cancer cell lines were performed to evaluate the bioactivity of all 32 compounds.

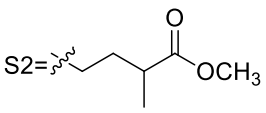


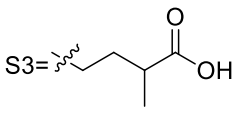


32

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	OH	H	H	H	OCH_3	S2	H	H
2	H	H	H	H	OCH_3	S2	H	H
3	H	H	H	H	OH	S1	H	H
4	OH	H	H	H	OCH_3	S3	H	H
5	H	H	H	H	OCH_3	S3	H	H
6	H	H	H	H	OH	S4	H	H
7	H	H	H	H	H	H	H	H
8	OH	H	H	H	H	H	H	H
9	H	H	H	OH	H	H	H	H
10	H	H	H	OCH_3	H	H	H	H
11	OH	H	H	H	OH	H	H	H
12	OH	H	H	H	OCH_3	H	H	H
13	OH	H	H	H	H	OH	H	H
14	OH	H	H	H	H	OCH_3	H	H
15	H	H	OH	H	OCH_3	H	H	H
16	H	H	OCH_3	H	OH	H	H	H
17	OH	H	H	H	OH	OH	H	H
18	OH	H	H	H	OCH_3	OH	H	H
19	OH	H	H	H	OH	OCH_3	H	H
20	H	H	OCH_3	OCH_3	H	H	H	H
21	OH	H	H	H	OCH_3	OCH_3	H	H
22	H	H	OCH_3	OCH_3	H	OH	H	H
23	OH	H	OCH_3	H	OH	OCH_3	H	H
24	OCH_3	H	OCH_3	H	OH	OCH_3	H	H
25	OH	H	OCH_3	H	H	H	OCH_3	H
26	OCH_3	H	OCH_3	H	OCH_3	H	OCH_3	H
27	OH	H	OCH_3	H	OCH_3	H	H	H
28	OH	H	H	OCH_3	OH	H	H	H
29	OH	H	H	OCH_3	H	OH	H	H
30	H	OCH_3	OH	H	H	H	H	H
31	OH	S1	OCH_3	H	H	OH	OCH_3	S1







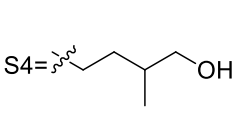


Figure 4.1 Structures of xanthones **1-32** from the roots of *C. calaba*

The results of the cytotoxicity against human cancer cell lines showed the tested compounds mostly showed moderate to inactive against these five cell lines, except compounds **3** showed potent cytotoxicity against KB, HeLa S-3, HT29, MCF-7 and HepG2 cells with IC_{50} values of 1.72, 0.82, 1.17, 5.04 and 1.65 μM, respectively. Furthermore, compound **6** showed good cytotoxicity against KB and HeLa S-3 cell with IC_{50} value of 7.06 and 5.27 μM, respectively. Moreover, compound **13** showed good cytotoxicity against only KB cell with IC_{50} value of 4.62 μM. Compounds **1**, **2**, **16** and **32** showed moderate cytotoxicity against KB cell with IC_{50} values in the range of 16.32-28.19 μM. Compounds **13**, **31** and **32** showed moderate cytotoxicity against HeLa S-3 cell with IC_{50} values in the range of 17.49-27.91 μM. Compounds **6** and **32** showed moderate cytotoxicity against MCF-7 and HepG2 cell with IC50 values in the range of 17.49-27.91 μM

The future works may involve the modification and synthesis of active compounds for a new potent drug. In addition, these results might provide basic knowledge to study the mechanism of active compounds toward disease for the drug improvement.

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APPENDIX

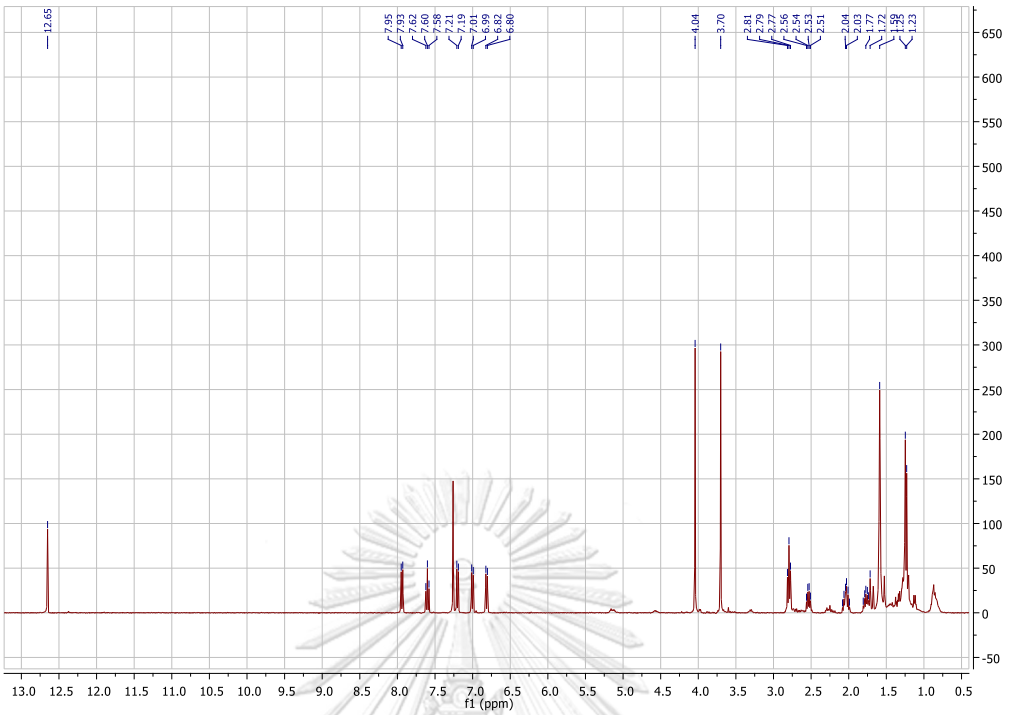


Figure A-1 ^{1}H NMR spectrum of **1** in $CDCl_3$

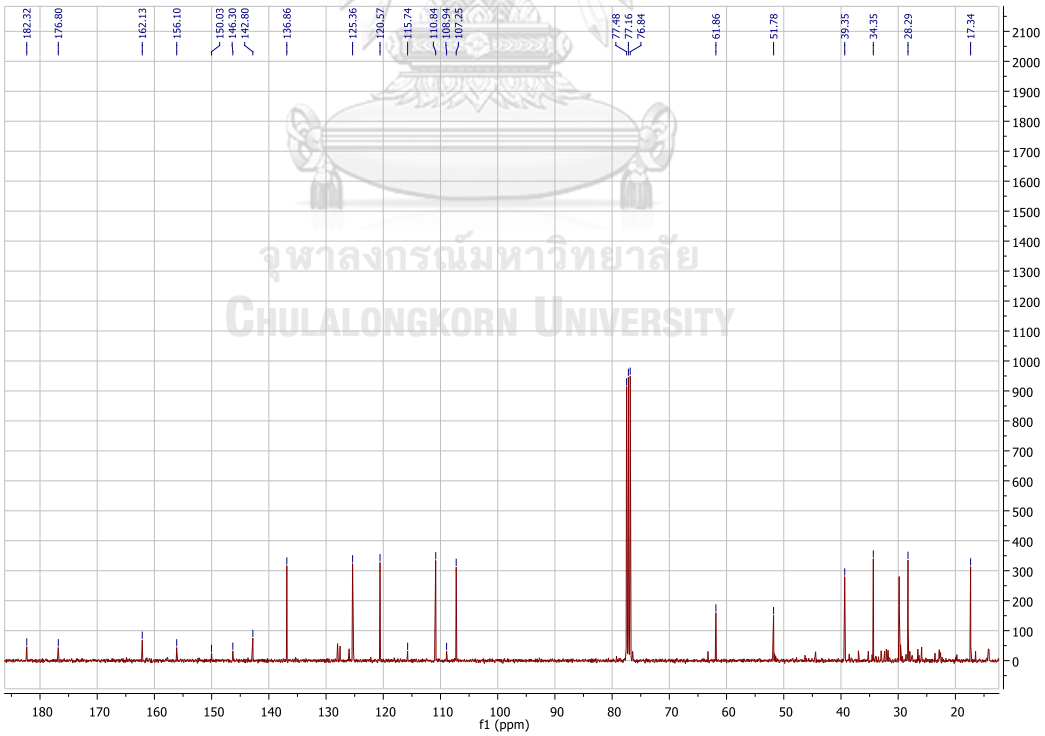


Figure A-2 ^{13}C NMR spectrum of **1** in $CDCl_3$

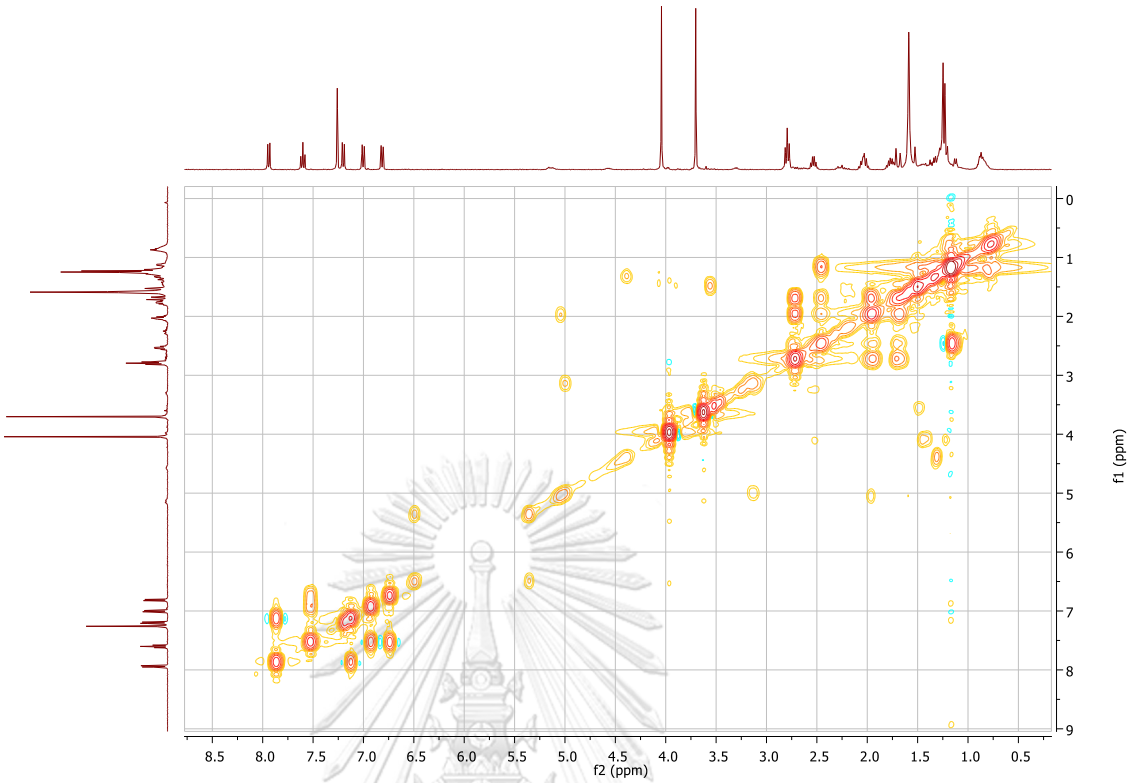


Figure A-3 COSY NMR spectrum of **1** in $CDCl_3$

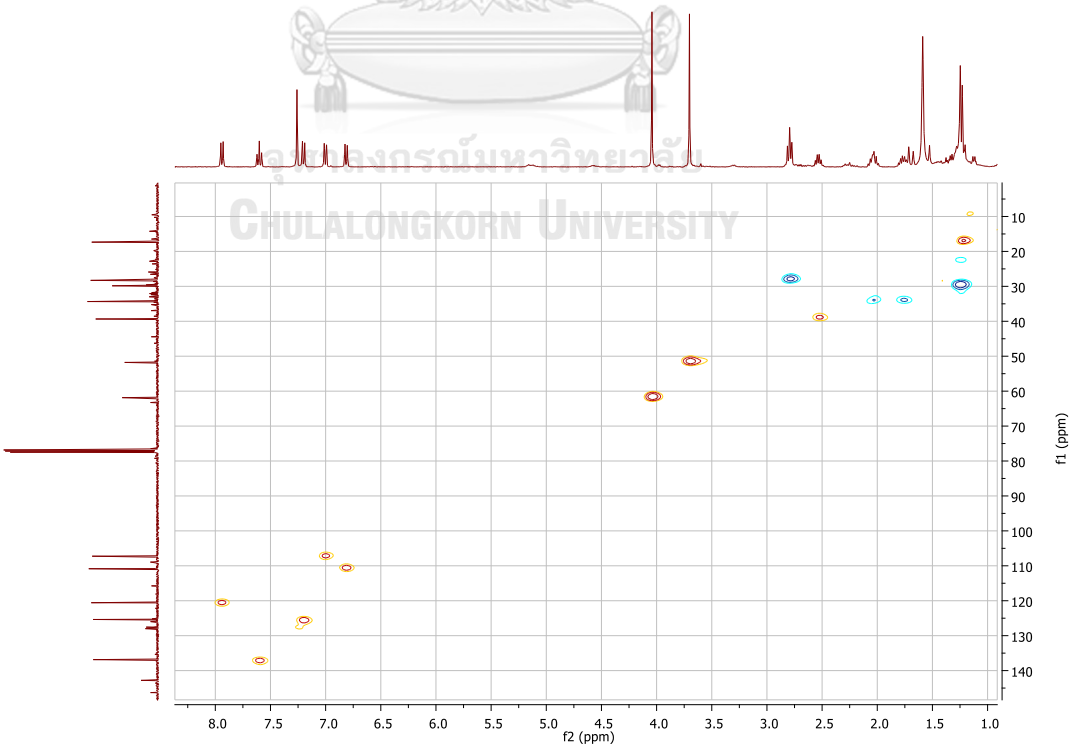


Figure S1.4 HSQC NMR spectrum of **1** in $CDCl_3$

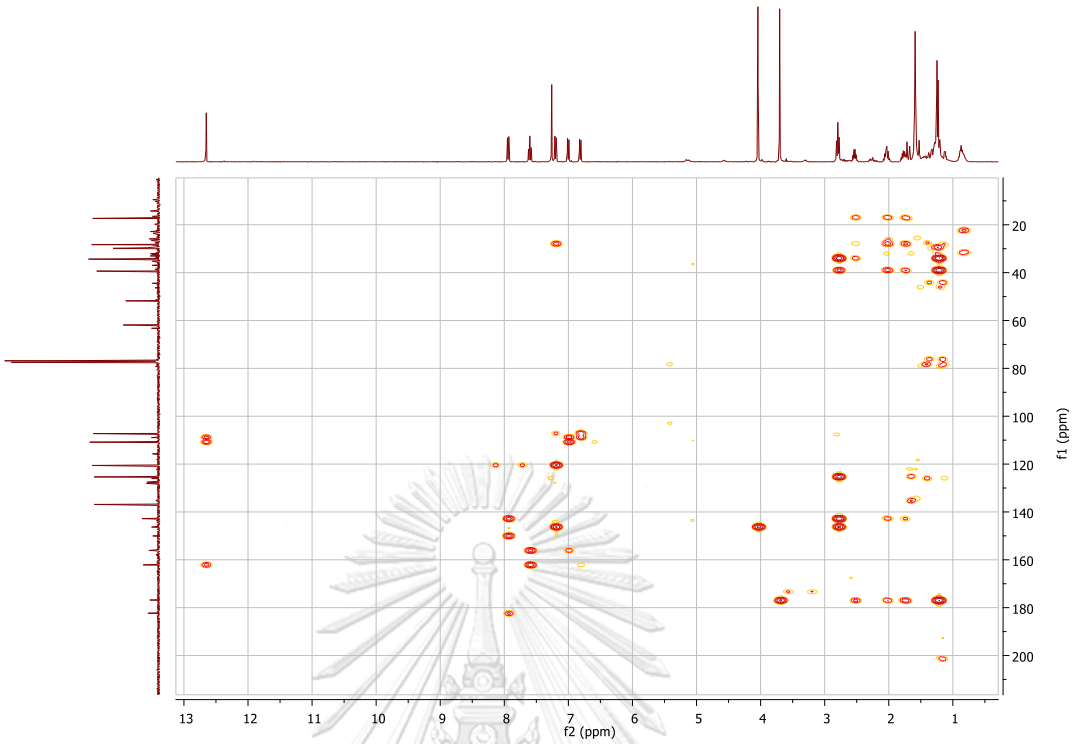
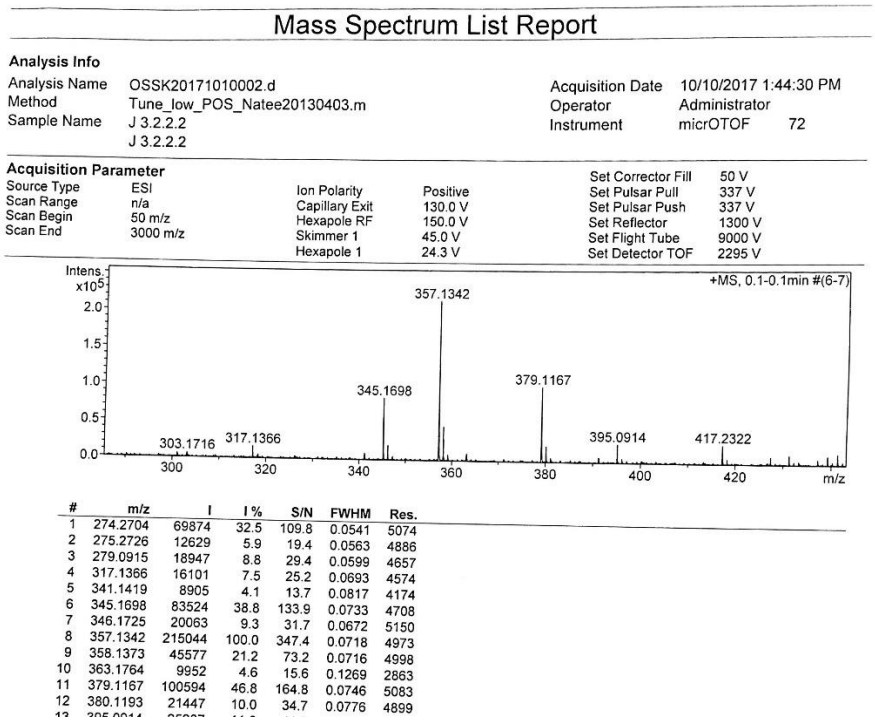


Figure A-5 HMBC NMR spectrum of **1** in $CDCl_3$



Mass Spectrum List Report

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	J 3.2.2.2		

Acquisition Parameter

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Scan Begin	50 m/z	Hexapole RF	150.0 V	Set Pulsar Push	337 V
Scan End	3000 m/z	Skimmer 1	45.0 V	Set Reflector	1300 V
		Hexapole 1	24.3 V	Set Flight Tube	9000 V
				Set Detector TOF	2295 V

#	m/z	I	I %	S/N	FWHM	Res.
1	274.2704	69874	32.5	109.8	0.0541	5074
2	275.2726	12629	5.9	19.4	0.0563	4886
3	279.0915	18947	8.8	29.4	0.0599	4657
4	317.1366	16101	7.5	25.2	0.0693	4574
5	341.1419	8905	4.1	13.7	0.0817	4174
6	345.1698	83524	38.8	133.9	0.0733	4708
7	346.1725	20063	9.3	31.7	0.0672	5150
8	357.1342	215044	100.0	347.4	0.0718	4973
9	358.1373	45577	21.2	73.2	0.0716	4998
10	363.1764	9952	4.6	15.6	0.1269	2863
11	379.1167	100594	46.8	164.8	0.0746	5083
12	380.1193	21447	10.0	34.7	0.0776	4899

Figure A-6 HRESIMS spectrum of **1**

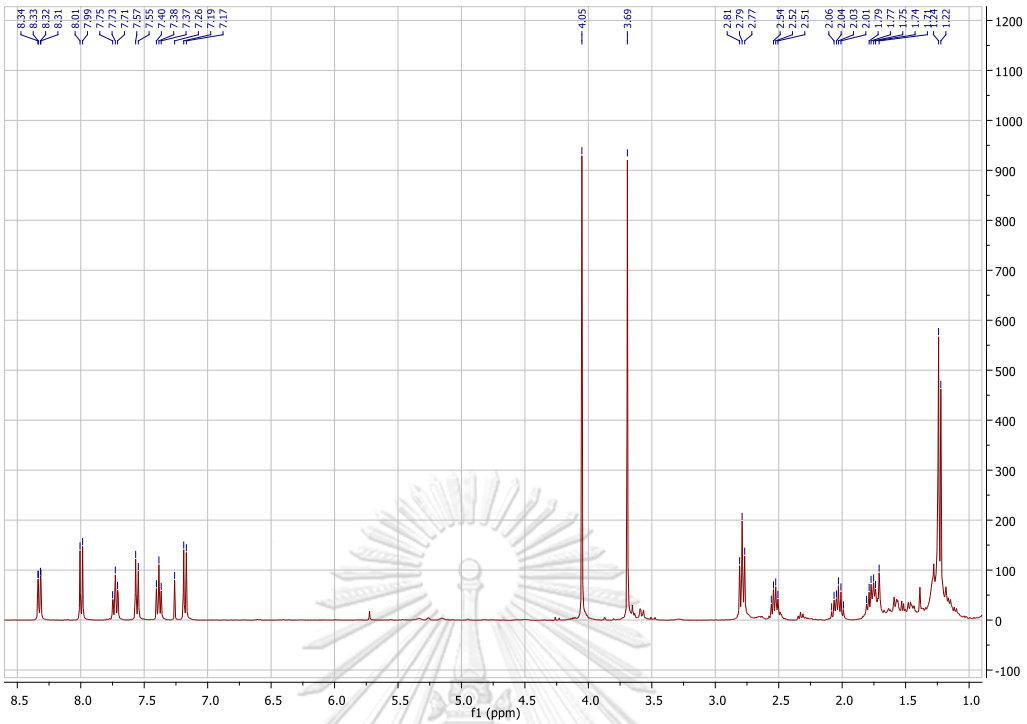

Figure A-7 ^{1}H NMR spectrum of **2** in $CDCl_3$

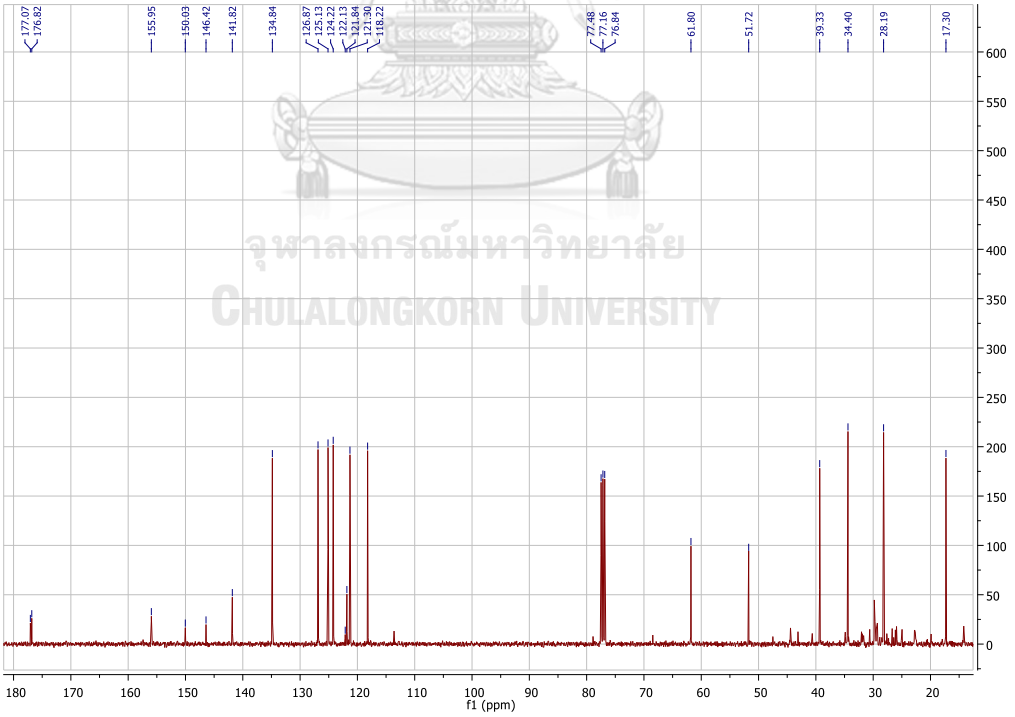

Figure A-8 ^{13}C NMR spectrum of **2** in $CDCl_3$

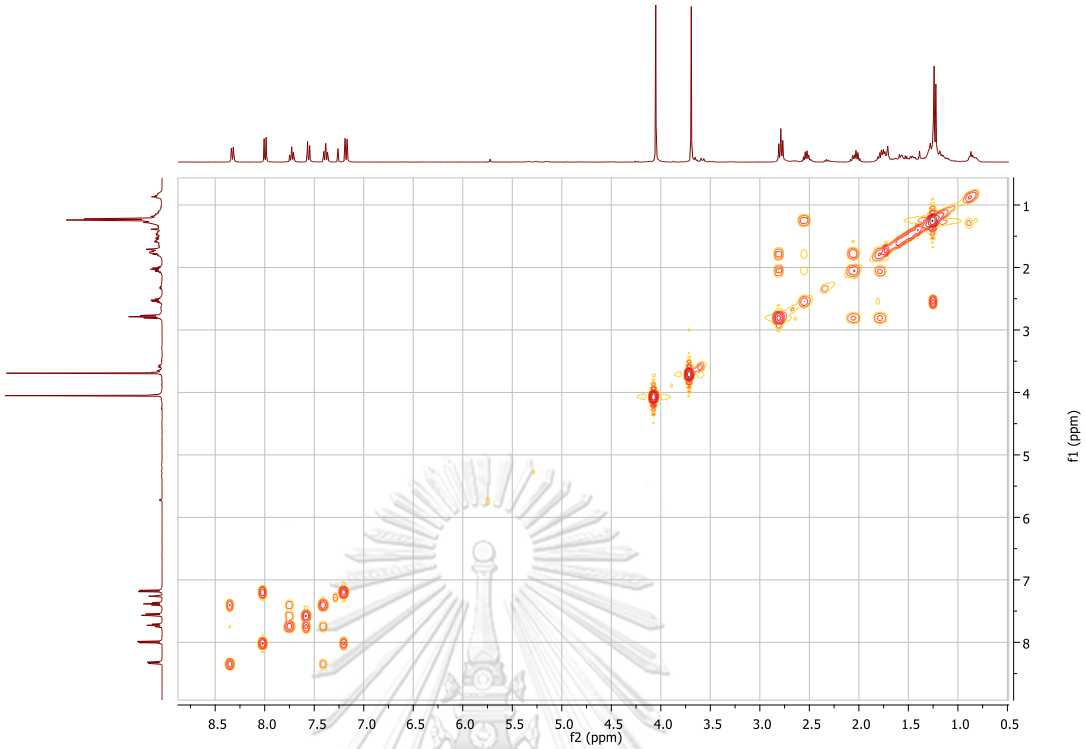

Figure A-9 COSY NMR spectrum of **2** in $CDCl_3$

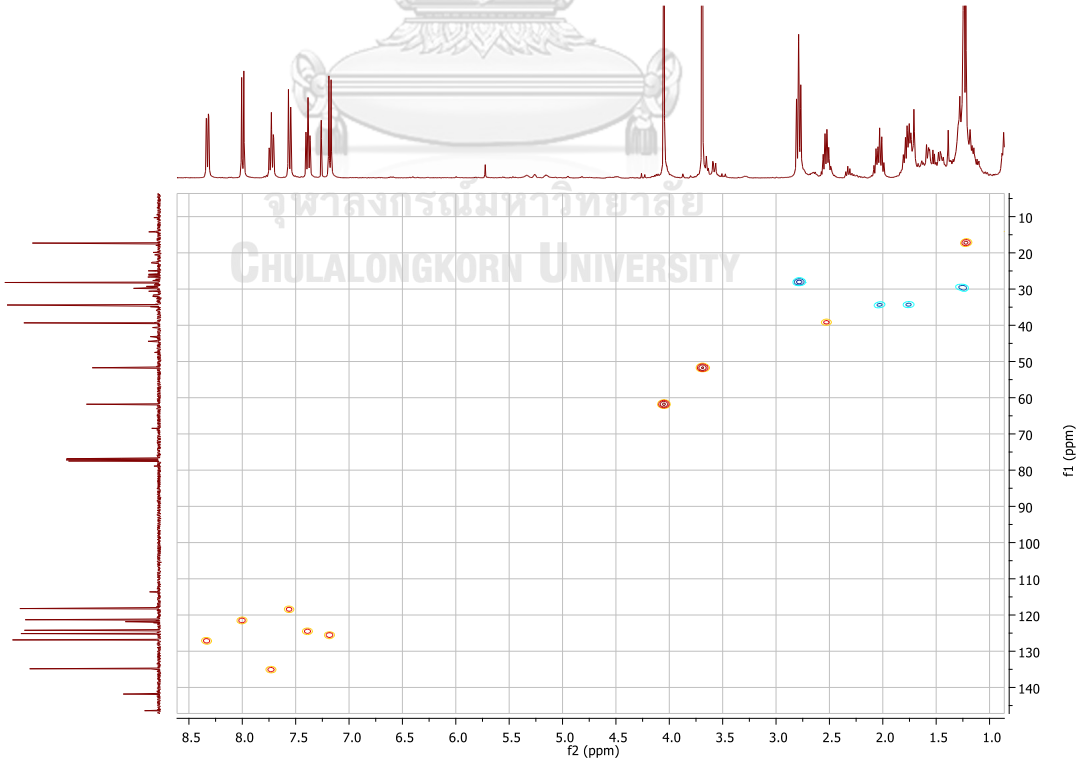

Figure A-10 HSQC NMR spectrum of **2** in $CDCl_3$

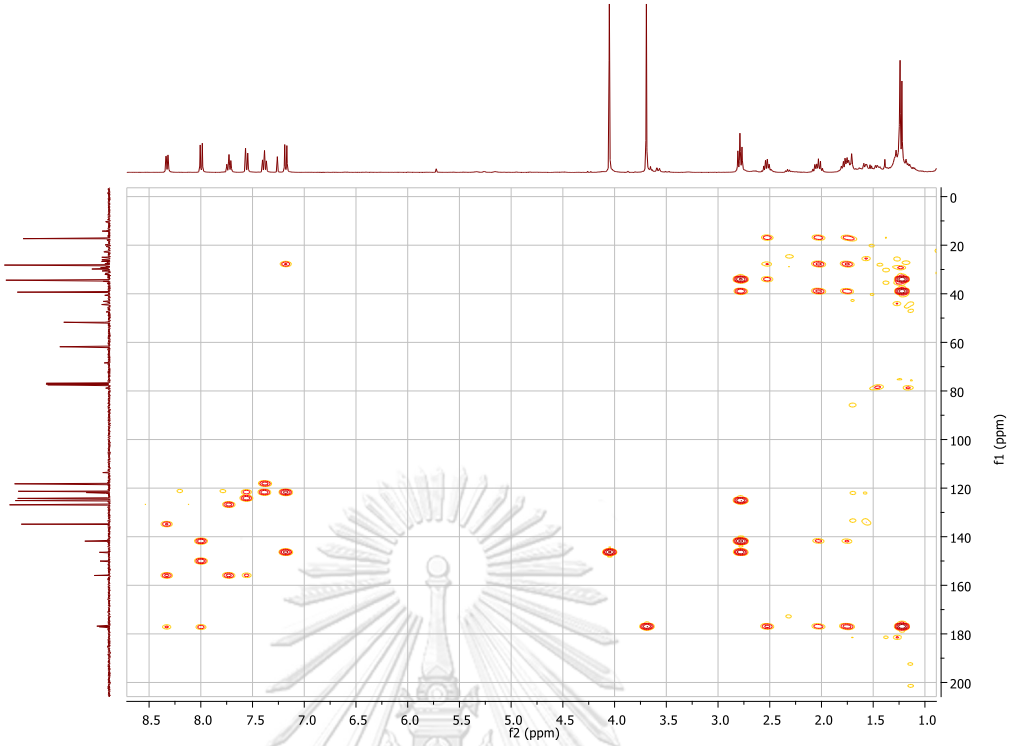

Figure A-11 HMBC NMR spectrum of **2** in $CDCl_3$

Mass Spectrum List Report

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	J 3.3.3.2		

Acquisition Parameter

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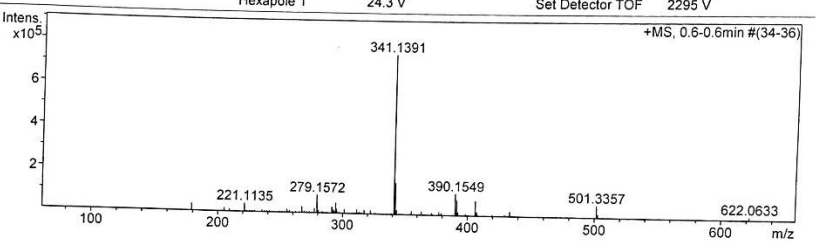
Scan Range	n/a	Capillary Exit	130.0 V	Set Pulsar Pull	337 V
Scan Begin	50 m/z	Hexapole RF	150.0 V	Set Pulsar Push	337 V
Scan End	3000 m/z	Skimmer 1	45.0 V	Set Reflector	1300 V
		Hexapole 1	24.3 V	Set Flight Tube	9000 V
				Set Detector TOF	2295 V



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2	205.0827	21046	2.8	30.1	0.0455	4506
3	221.1135	42642	5.7	61.1	0.0482	4583
4	267.1252	31328	4.2	44.0	0.0650	4110
5	277.1790	24355	3.3	33.9	0.0631	4390
6	279.1572	87404	11.7	123.7	0.0596	4687
7	291.1978	32755	4.4	45.7	0.0791	3683
8	292.1923	20764	2.8	28.6	0.0704	4152
9	294.2044	51572	6.9	72.3	0.0614	4791
10	295.1997	23242	3.1	32.1	0.0848	3480
11	301.1420	22287	3.0	30.7	0.0745	4044
12	311.1769	22316	3.0	30.6	0.0859	3623

Figure A-12 HRESIMS spectrum of **2**

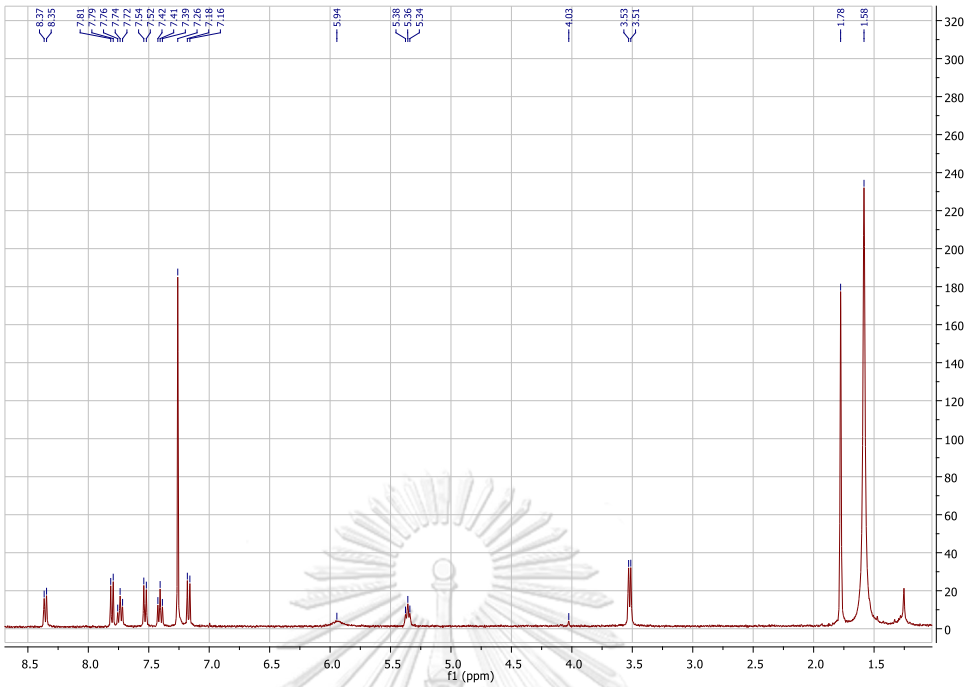

Figure A-13 ^{1}H NMR spectrum of **3** in $CDCl_3$

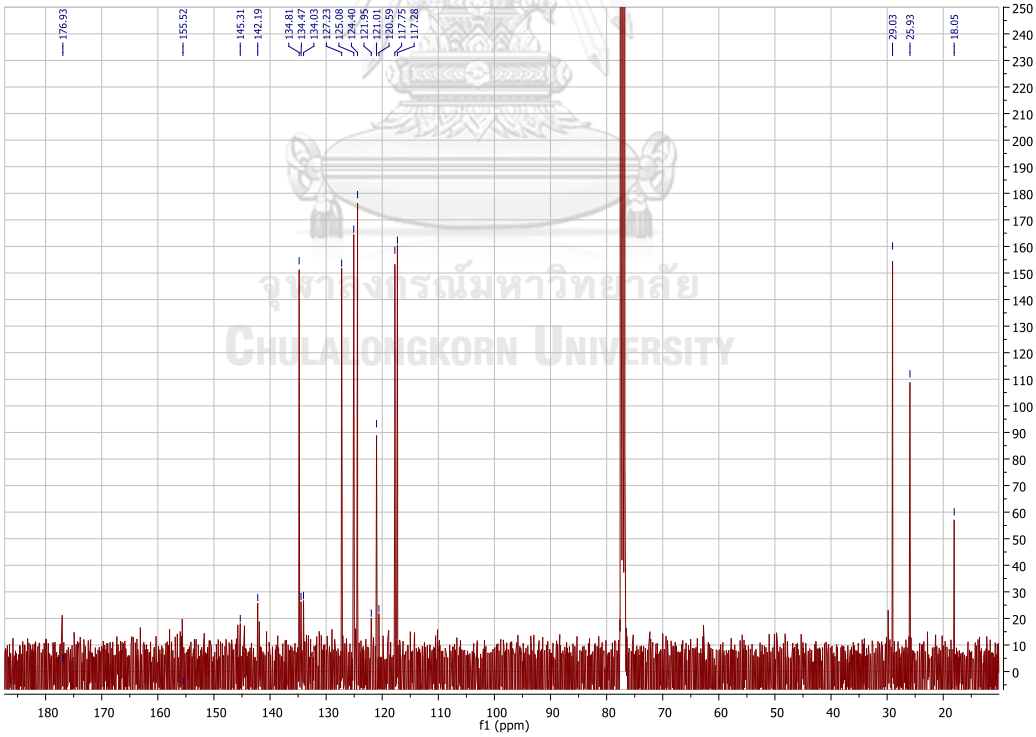

Figure A-14 ^{13}C NMR spectrum of **3** in $CDCl_3$

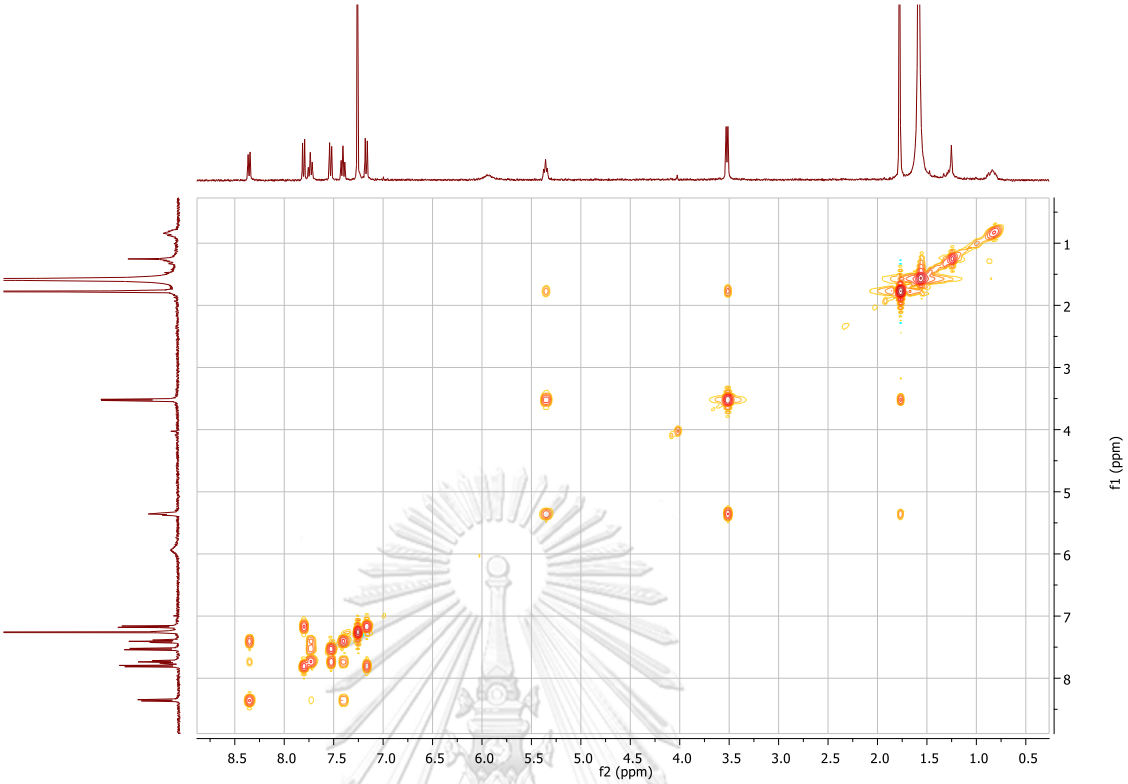

Figure A-15 COSY NMR spectrum of **3** in $CDCl_3$

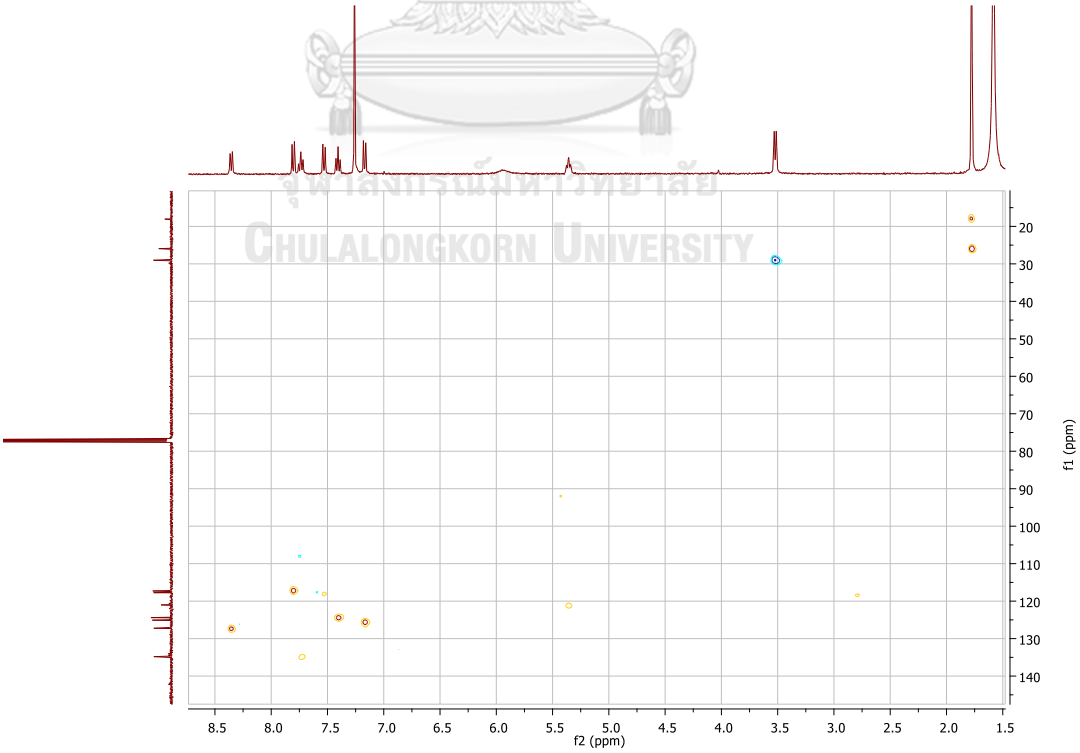

Figure A-16 HSQC NMR spectrum of **3** in $CDCl_3$

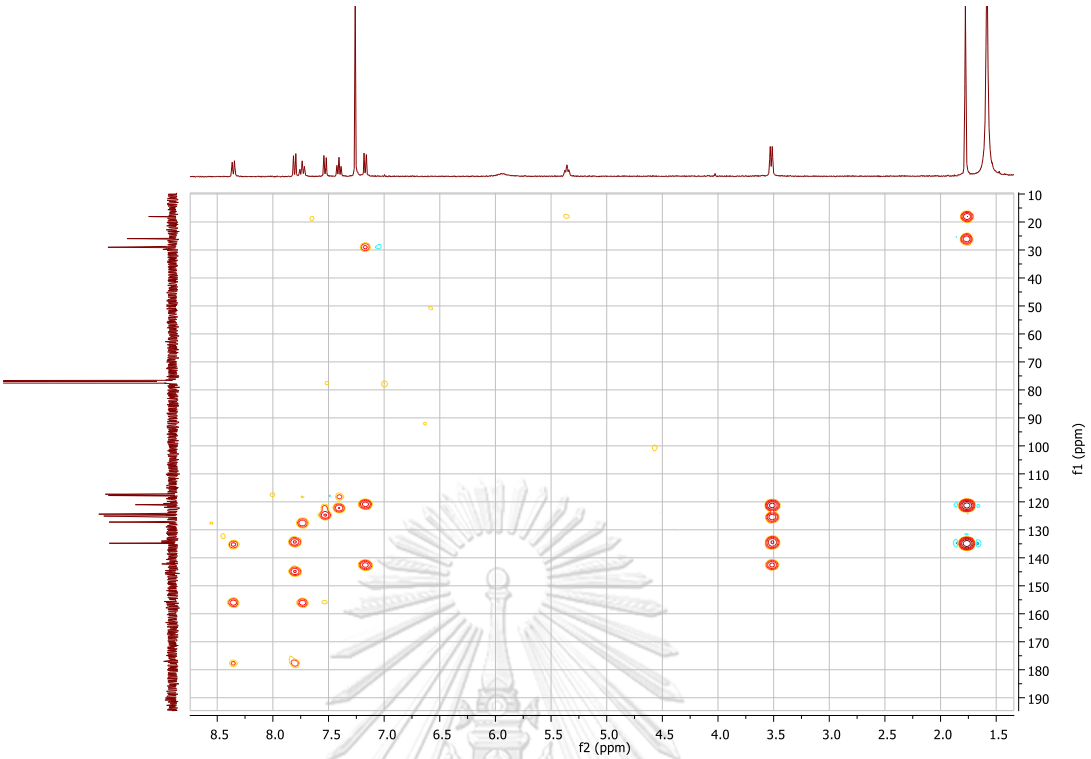

Figure A-17 HMBC NMR spectrum of **3** in $CDCl_3$

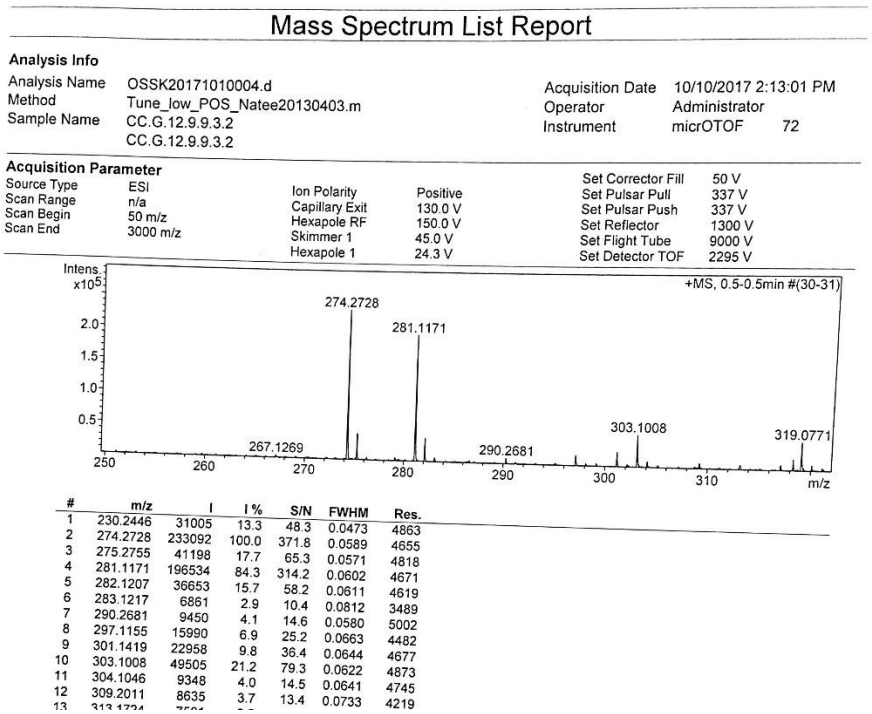

Figure A-18 HRESIMS spectrum of **3**

VITA

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