องค์ประกอบทางเคมีของรากมะดัน *Garcinia schomburgkiana* Pierre

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Garcinia schomburgkiana* Pierre

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Chemistry

Department of Chemistry

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Thesis Title	CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Garcinia schomburgkiana* Pierre
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Field of Study	Chemistry
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เอ็ดวิน ริสกี้ ซูกานดาร์ : องค์ประกอบทางเคมีของรากมะดัน *Garcinia schomburgkiana* Pierre (CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Garcinia schomburgkiana* Pierre) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.สันติ ทิพยางค์, 54 หน้า.

จากการศึกษาองค์ประกอบทางเคมีของสิ่งสกัดไดคลอโรมีเทนจากรากมะดัน พบว่าสามารถแยกสารใหม่ในกลุ่มเดปซิโดน (depsidone) ได้ 2 ชนิด คือ schomburgdepsidone A และ B (GS1 และ GS2) พร้อมกับสารใหม่ในกลุ่มแซนโทน (xanthone) อีก 1 ชนิด ได้แก่ schomburgxanthone A (GS3) นอกจากนี้ยังพบสารที่มีรายงานมาก่อนหน้านี้อีก 8 ชนิด คือ oliveridepsidone A (GS4), oliveridepsidone D (GS5), 1,5-dihydroxyxanthone (GS6), nigrolineaxanthone E (GS7), 6-desoxyjacareubin (GS8), aucuparin (GS9), 3-hydroxy-5-methoxybiphenyl (GS10) และ methyl-2,6-dihydroxy-4-methoxy-3(3'-methyl-2'-butenyl)-benzoate (GS11) โดยโครงสร้างของสารใหม่ทั้งหมดนี้ (GS1–GS3) ได้พิสูจน์ทราบด้วยข้อมูลทาง 1D และ 2D เอ็นเอ็มอาร์ สเปกโทรสโกปี ส่วนโครงสร้างของสารที่มีรายงานมาก่อนหน้านี้ (GS4–GS11) ได้พิสูจน์ทราบด้วยข้อมูลทาง ^{1}H และ ^{13}C เอ็นเอ็มอาร์ สเปกโทรสโกปี ร่วมกับการเปรียบเทียบกับข้อมูลที่มีการรายงานมาก่อนหน้านี้

สารทั้งหมดได้นำไปทดสอบฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB, HeLa S-3, HT-29, MCF-7 และ Hep G2 พร้อมกับทดสอบฤทธิ์ยับยั้งเอนไซม์ไลเปส จากผลการทดสอบฤทธิ์ในการยับยั้งเซลล์มะเร็ง พบว่า สารส่วนใหญ่มีฤทธิ์ในการยับยั้งเซลล์มะเร็งอยู่ในระดับปานกลางไปจนถึงไม่มีฤทธิ์ในการยับยั้งเซลล์มะเร็ง ยกเว้นสาร GS7 ที่มีฤทธิ์ในการยับยั้งเซลล์มะเร็งชนิด KB, Hela S-3 และ MCF-7 อยู่ในระดับที่ดี เนื่องจากมีค่า IC_{50} อยู่ในช่วง 3.17–6.07 μM และสาร GS3 ยังมีฤทธิ์ในการยับยั้งเฉพาะเซลล์มะเร็งชนิด KB ได้ดี เนื่องจากมีค่า IC_{50} เท่ากับ 8.14 μM จากผลการทดสอบฤทธิ์ยับยั้งเอนไซม์ไลเปสของสารทั้งหมดนี้ พบว่า สาร GS1–GS3, GS7, GS8 และ GS11 มีฤทธิ์ในการยับยั้งเอนไซม์ไลเปส อยู่ในระดับปานกลาง เนื่องจากมีค่า IC_{50} อยู่ในช่วง 23.71–80.78 μM ส่วนสารตัวอื่น ๆ ไม่มีฤทธิ์ยับยั้งเอนไซม์ไลเปส เนื่องจากมีค่า IC_{50} มากกว่า 100 μM

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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EDWIN RISKY SUKANDAR: CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Garcinia schomburgkiana* Pierre. ADVISOR: ASSOC. PROF. SANTI TIP-PYANG, Ph.D., 54 pp.

Phytochemical investigation of the CH_2Cl_2 extract from the roots of *Garcinia schomburgkiana* led to the isolation of two new depsidones, schomburgdepsidones A and B (GS1 and GS2), and one new xanthone, schomburgxanthone A (GS3), along with eight known compounds; oliveridepsidone A (GS4), oliveridepsidone D (GS5), 1,5-dihydroxyxanthone (GS6), nigrolineaxanthone E (GS7), 6-desoxyjacareubin (GS8), aucuparin (GS9), 3-hydroxy-5-methoxybiphenyl (GS10) and methyl-2,6-dihydroxy-4-methoxy-3(3'-methyl-2'-butenyl)-benzoate (GS11). The structures of the three new isolated compounds (GS1–GS3) were elucidated by using 1D- and 2D-NMR spectroscopy. The structures of the known compounds (GS4–GS11) were determined and confirmed by comparison of their ^{1}H and ^{13}C NMR spectroscopic data with previously published data. All 11 compounds were evaluated on the *in vitro* cytotoxicity against the KB, HeLa S-3, HT-29, MCF-7 and Hep G2 human cancer cell lines, and pancreatic lipase inhibitory activity. The tested compounds mostly showed moderate activities to inactive against the five human cancer cell lines, except for compound GS7 exhibited a good cytotoxicity against the KB, Hela S-3 and MCF-7 cell lines with IC_{50} values in the range of 3.17–6.07 μM. Compound GS3 showed a good cytotoxic activity against the KB cell line only, with an IC_{50} value of 8.14 μM. The result of the pancreatic lipase inhibitory activity of 11 compounds possessed moderately active of compounds GS1–GS3, GS7, GS8 and GS11 with IC_{50} values in the range of 23.71–80.78 μM, while the other tested compounds showed a weak activity (IC_{50} values >100 μM).

Department: Chemistry Student's Signature .....
Field of Study: Chemistry Advisor's Signature .....
Academic Year: 2015

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LIST OF ABBREVIATIONS

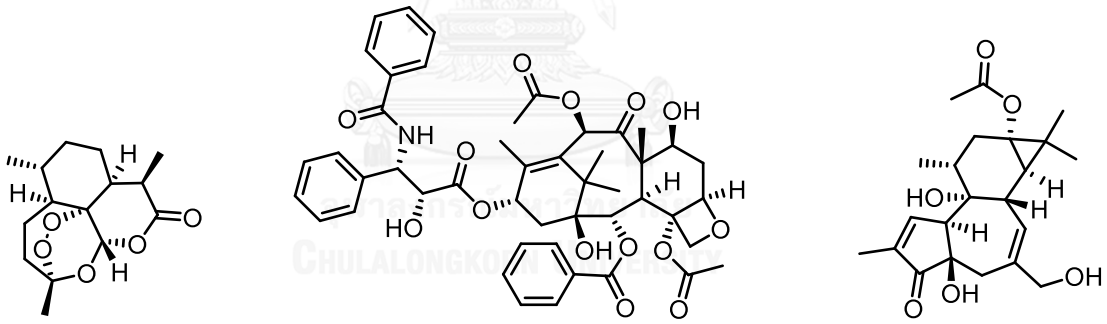
EC_{50}	the molar concentration of an agonist that produces 50% of the maximal possible effect of that agonist
IC_{50}	the molar concentration of an antagonist that reduces the response to an agonist by 50%
SW620	human colon cancer cell lines
KATO-III	human stomach cancer cell lines
CHAGO	human lung cancer cell lines
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	tirplet (NMR)
brs	broad singlet (NMR)
Hz	hertz
MHz	megahertz
DMSO-d_6	deuterated dimethyl sulfoxide
$CDCl_3$	deuterated chloroform
HR-ESI-MS	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

CHAPTER I

INTRODUCTION

Herbal medicines have long been used for centuries to prevent and treat several serious diseases, including cancer, metabolic disorders such as diabetes mellitus and obesity, and infectious diseases from bacteria and parasites [1]. The raw materials were particularly obtained from medicinal plants and consumed traditionally as a decoction or a powdered herb for topical use. The formulation of herbal products have also been developed into many form of standardized herbal extracts such as a capsule, a liquid formation and an ointment for external use [2]. Their remarkable medical action have been a great attention to be further investigated. The presence of secondary metabolite constituents which have been successfully isolated might contribute for their pharmacological properties [3].



artemisinin paclitaxel prostratin

Figure 1.1 Natural products derived from plants

Secondary plant metabolites are natural products generated biosynthetically through several metabolic pathways. Plants produce these compounds with limited abundance in nature to adapt in an environment and protect themselves from predators. These substances are also used to attract insects for plant pollinating [4]. Interestingly, the plant-derived natural products also give a benefit for human as health

therapies. More than a thousand natural products have been hitherto designed as potential drug candidates, and some of those even have been distributed as new drugs (Figure 1.1). For example, artemisinin as an antimalarial drug was isolated from a Chinese herbal plant *Artemisia annua*. From the bark of *Taxus brevifolia*, paclitaxel (Taxol®), a breast cancer drug, was firstly isolated. In addition, prostratin obtained from a plant extract *Homalanthus nutans* was carried out in phase I human clinical trial for AIDS treatment [5].

Genus *Garcinia* belonging to the family Clusiaceae (Guttiferae) is distributed widely in the lowland tropical rain forest, especially in the Southeast Asia, West Africa and South America. Genus *Garcinia*, well known as a mangosteen family, is a tropical plant, evergreen, shrub, mostly edible, and comprise more than 300 species. Twenty nine species of this genus have been identified in Thailand and mostly used for herbal medication [6, 7]. *Garcinia* plants are rich in secondary metabolites, including xanthones as a chemotaxonomic marker of this genus, flavonoids, benzophenones, depsidones and triterpenoids [7, 8]. These type of compounds also performed a broad spectrum of pharmacological properties, including an anticancer, antidibetes, antiobesity and antioxidant [9-15]. As part of phytochemical investigation for bioactive compounds, this research performed the isolation and structure elucidation of chemical constituents from the roots of *G. schomburgkiana*, one of *Garcinia* species collected from Thailand, and biological activities study of isolated compounds.

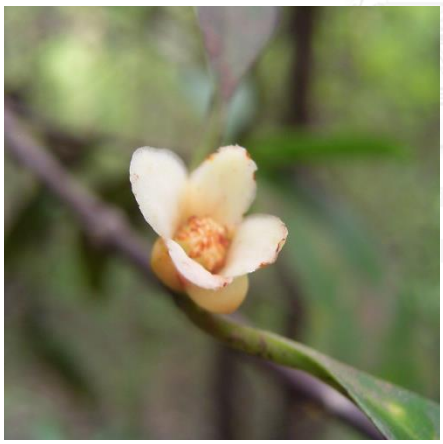
1.1 Botanical aspect and distribution of *Garcinia schomburgkiana* Pierre

Garcinia schomburgkiana Pierre is a small evergreen tree which can grow about 3-7 meter high. This species, locally named “Ma Dan” in Thai can be found in Thailand, Laos and Vietnam as an indigenous plant (Figure 1.2). Ma Dan plant commonly grows along rivers, streams, swamps, or cultivated in the garden [16, 17]. Its leaves morphologies are simple, opposite, elliptic-lanceolate or elliptic-ovate, apex subacute, base cuneate, margin entire, glossy dark green, 2–3 cm wide and 5–9 cm long. The flowers of this plant are unisexual and monoecious borne in 3-6-flowered axillary

clusters, and consist of four pinkish petals, which are 3 mm wide and 6.5 mm long with stamens in four fascicles. Ma Dan fruits are obliquely ellipsoid to ellipsoid-oblong, 5–7 cm long, 2–3 cm wide, glossy green and ripening yellow [16].

Whole plant

Stem



Flower

Fruit

Figure 1.2 The whole plant, stem, flower and fruit of *Garcinia schomburgkiana*

Family	: Clusiaceae
Genus	: *Garcinia*
Species	: *Garcinia schomburgkiana* Pierre

Common name : Ma Dan

Local name : Ma Dan (Thai)

Tro-Meng, Tro-Moung (Khmer)

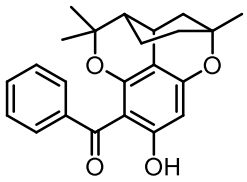
Bứa Dồng, Cây Bứa Dồng, Cây Thuốc (Vietnamese)

Ma Dan fruits are edible with sour taste and may be consumed directly or processed first. Thai people mix these fruits into a shrimp paste with chili and can be served together with fish and vegetables. The fruits also can be salted and dried being preserved fruits, and treated with salty crab being a Thai salad. The young leaves of Ma Dan are often used as a vegetable accompaniment to many Thai dishes [16].

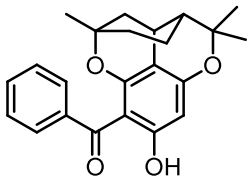
1.2 Chemical constituents from *Garcinia schomburgkiana* and their biological activities

Garcinia schomburgkiana has been long used by Thai people in folk medicine as an expectorant, laxative, menstrual treatment and diabetes prevention [16, 18]. Previous phytochemical studies on *G. schomburgkiana* have revealed the presence of xanthones, biflavonoids, biphenyl derivatives, benzophenones phenolic aldehyde, quinones and steroids, and some of these exhibited an antimalarial activity or cytotoxicity against cancer cell lines [17-22].

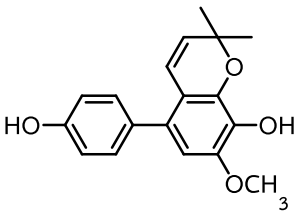
In 2006, Fun *et al*. [21] reported the remakable antimalaria activity of the hexane extract of *G. schomburgkiana* stems with EC_{50} value of 2.2 μg/mL. A further investigation of this extract led to the isolation of two benzophenones, clusicitrans A and B (**1** and **2**). Ito *et al*. [20] in 2013 worked in the same part of this plant and achieved two new biphenyls, schomburgbiphenyls A and B (**3** and **4**), and five known compounds (**5**–**9**). On the other hand, a phytochemical study from the acetone extract of *G. schomburgkiana* led to the isolation of three biflavonoids, named as volkensiflavone (**10**), morelloflavone (**11**) and fukugeside (**12**) (Figure 1.3) [19].



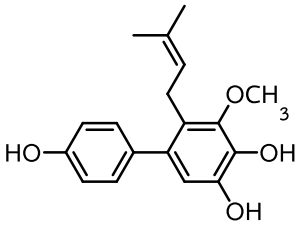
clusicitran A (**1**)



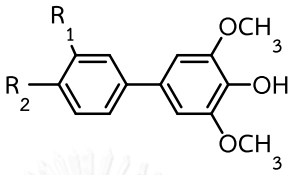
clusicitran B (**2**)



schomburgbiphenyl A (**3**)



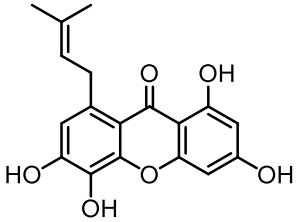
schomburgbiphenyl B (**4**)



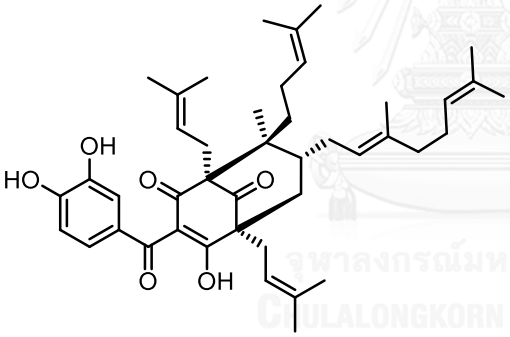
garcibiphenyl C (**5**) : R_1=H ; R_2=OH

nigrolineabiphenyl A (**6**) : R_1=R_2=OH

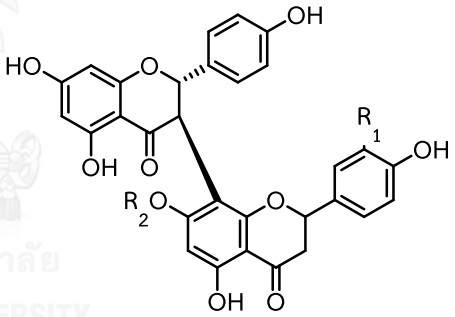
nigrolineabiphenyl B (**7**) : R_1=OCH_3 ; R_2=OH



garcinexanthone C (**8**)



oblongifolin C (**9**)



volkensiflavone (**10**) : R_1=R_2=H

morelloflavone (**11**) : R_1=OH ; R_2=H

fukugeside (**12**) : R_1=OH ; R_2 = β-D-gluc

Figure 1.3 Chemical constituents (**1**–**12**) from the stems and heartwoods of *G. schomburgkiana*

Moreover, isolation of the CH_2Cl_2 extract from the woods of *G. schomburgkiana* yielded three xanthones (**13**–**15**) and three biphenyls (**5**, **7**, **16**). Of isolated compounds, isojacareubin (**13**) revealed highly cytotoxic activity against five human

cancer cell lines, especially SW620 cell lines with IC_{50} value of <0.001 μg/mL [18]. In continuing phytochemical investigation from the woods of this plant, Mungmee *et al*. [17] in 2013 succeeded in isolating a new biphenyl, schomburgbiphenyl (**17**), together with three known biphenyls (**5**, **7** and **16**), three xanthones (**13**–**15**), three biflavonoids (**10**–**12**), two benzophenones (**9** and **18**) and three triterpenoids (**19**–**21**) (Figure 1.4). Oblongifolin C (**9**) and guttiferone K (**18**) showed a potent cytotoxicity against SW620 cell line with IC_{50} values of <0.0015 and 0.0017 μM. Guttiferone K (**18**) also had lower IC_{50} (strongly active) compared with doxorubicin as the positive control towards KATO-III, Hep G2 and CHAGO cell lines.

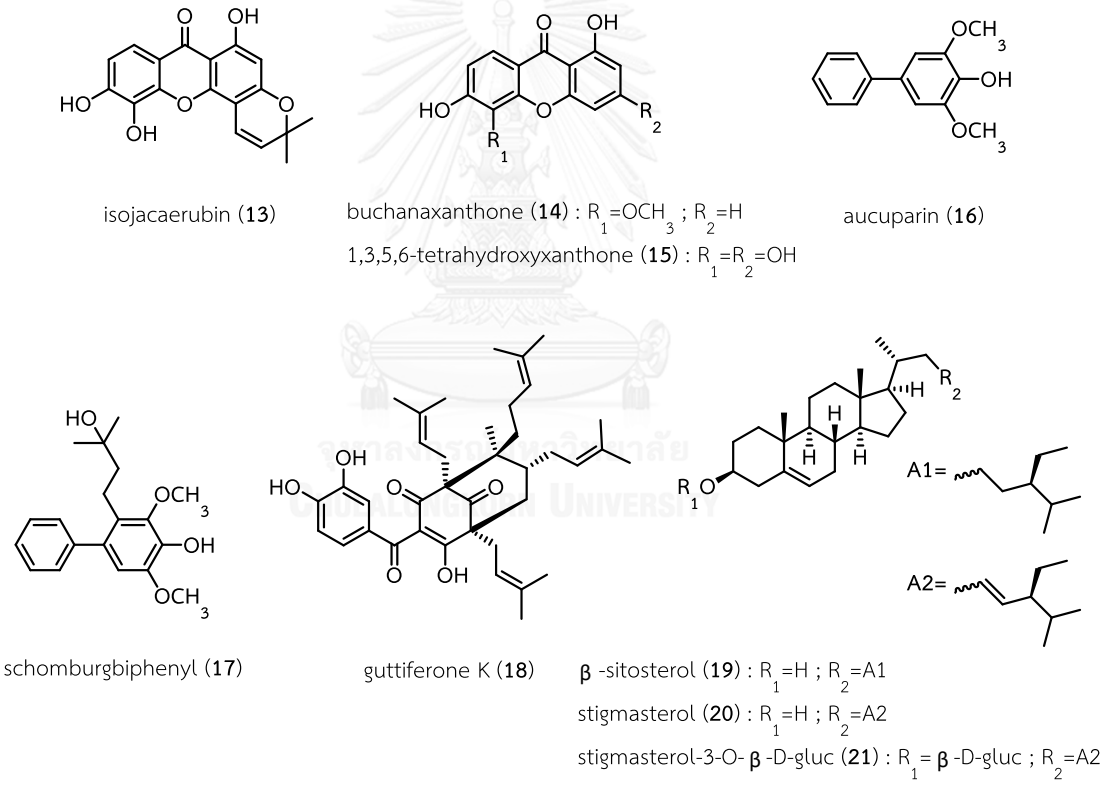


Figure 1.4 Chemical constituents (**13**–**21**) from the woods of *G. schomburgkiana*

From the bark of *G. schomburgkiana*, Vo *et al*. [22] in 2012 successfully isolated two new xanthones; 6-O-demethyloliverixanthone (**22**) and schomburgxanthone (**23**), together with six known xanthones (**24**–**29**). A remarkable cytotoxic activity against the

HeLa cell line of fuscaxanthone B (**27**) and cowanin (**28**) was observed with IC_{50} values of 2.4 and 2.7 μg/mL (Figure 1.5).

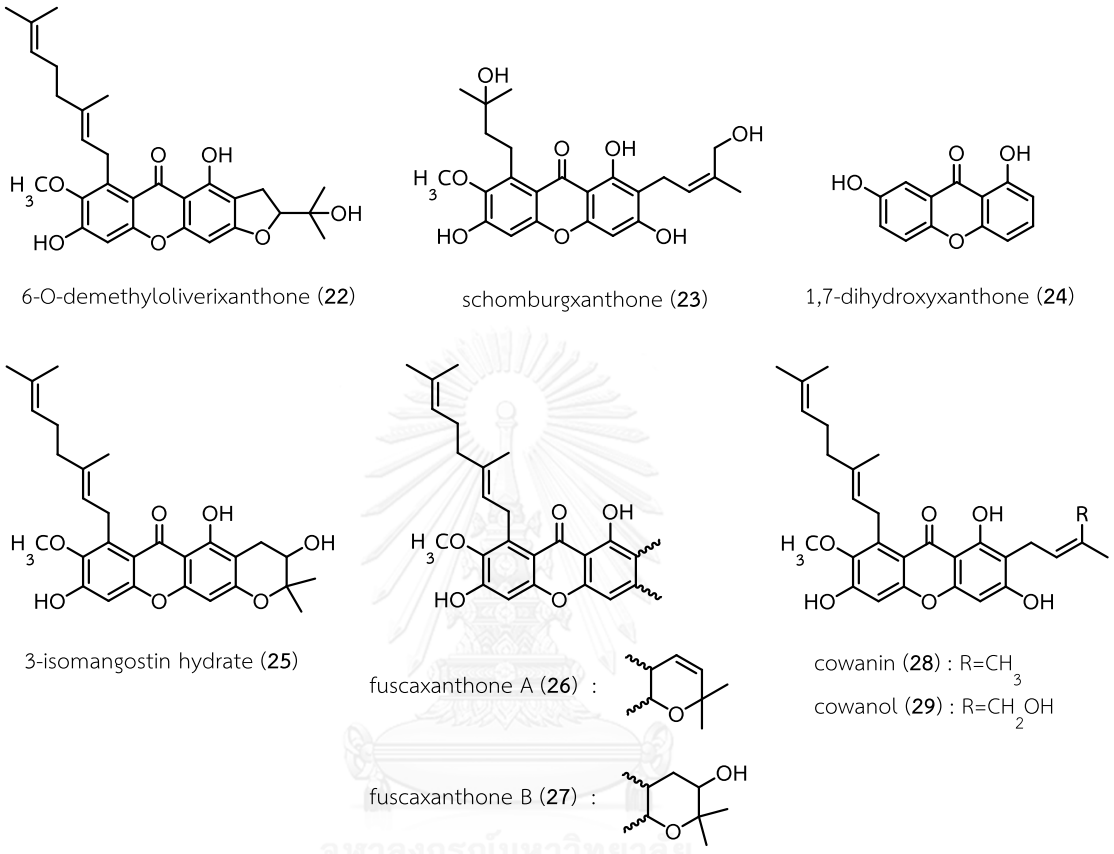
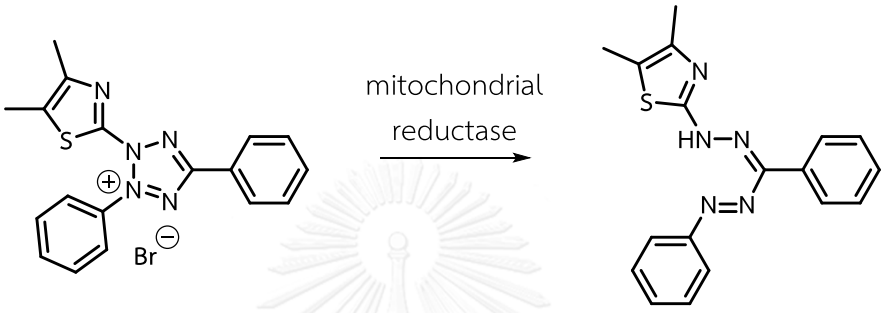


Figure 1.5 Chemical constituents (**22**–**29**) from the bark of *G. schomburgkiana*

1.3 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 [23].



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

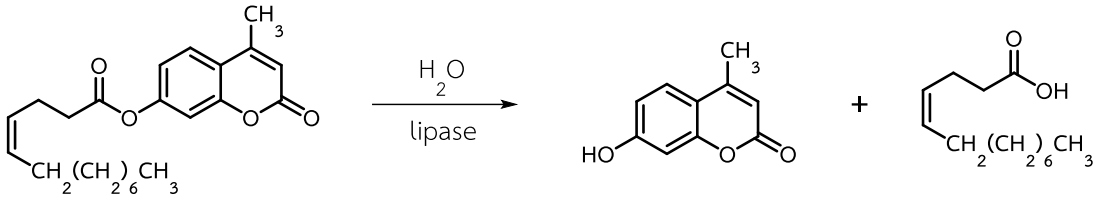
(2*E*,4*Z*)(4,5-dimethylthiazol-2-yl)-
3,5-diphenylformazan
(formazan)
purple color

Figure 1.6 MTT reduction in live cells by mitochondrial reductase resulting a formation of insoluble formazan

1.4 pancreatic lipase inhibitory activity

Pancreatic lipase plays the key role in digestion of dietary fat by hydrolizing the triglicerides to monoglicerides and free fatty acids (FFA). The excessive accumulation of fat in adipose tissue from FFA absoption may cause an obesity problem. Orlistat, a lipase inhibitory drug, is used widely for treating the obese people to lose their weight. However, orlistat may cause several side effects, including flatulence, steatorrhea, abdominal cramping and fat-soluble vitamin deficiencies. Natural products have been alternatively used as an atiobesity treatment by their abilities to inhibit the enzyme work [24]. The pancreatic lipase inhibitory assay is one of the biological testing to screen potential lipase inhibitor agents. In this method, the lipase enzyme hydrolyze

the substrate 4-methylumbelliferyl oleate to form a fluorescent substance 4-methylumbelliferone (Figure 1.7), which can be measured quantitatively by a fluorescence machine [25].



4-methylumbelliferyl oleate (4-MU oleate)

4-methylumbelliferone **fluorescent**

oleic acid

Figure 1.7 Hydrolysis reaction on which the fluorescent lipase assay is based.

The literature review above showed the use of *G. schomburgkiana* as herbal treatments and the biologically active components which have been isolated. However, there has been no report on chemical constituents from the roots of this plant. Therefore, those provide an insight to further investigate the bioactive compounds from the roots of *G. schomburgkiana* with the main objectives of the research as follows:

1. To isolate and purify the chemical constituents from the roots of *Garcinia schomburgkiana*.
2. To elucidate structurally the isolated compounds by means of spectroscopy analysis, including UV-vis, IR, 1D and 2D NMR, and HRMS.
3. To evaluate the cyototoxic activity of the isolated compounds against human cancer cell lines.
4. To evaluate the pancreatic lipase inhibitory activity of isolated compounds.

CHAPTER II

EXPERIMENTAL

2.1 Plant Material

The roots of *G. schomburgkiana* were collected from a riparian zone along the Chi River, Mahasarakham Province, Thailand. The plant was identified and deposited with a voucher specimen (Khumkratok no. 92-08) by Dr. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, Thailand.

2.2 General Experiment Procedures

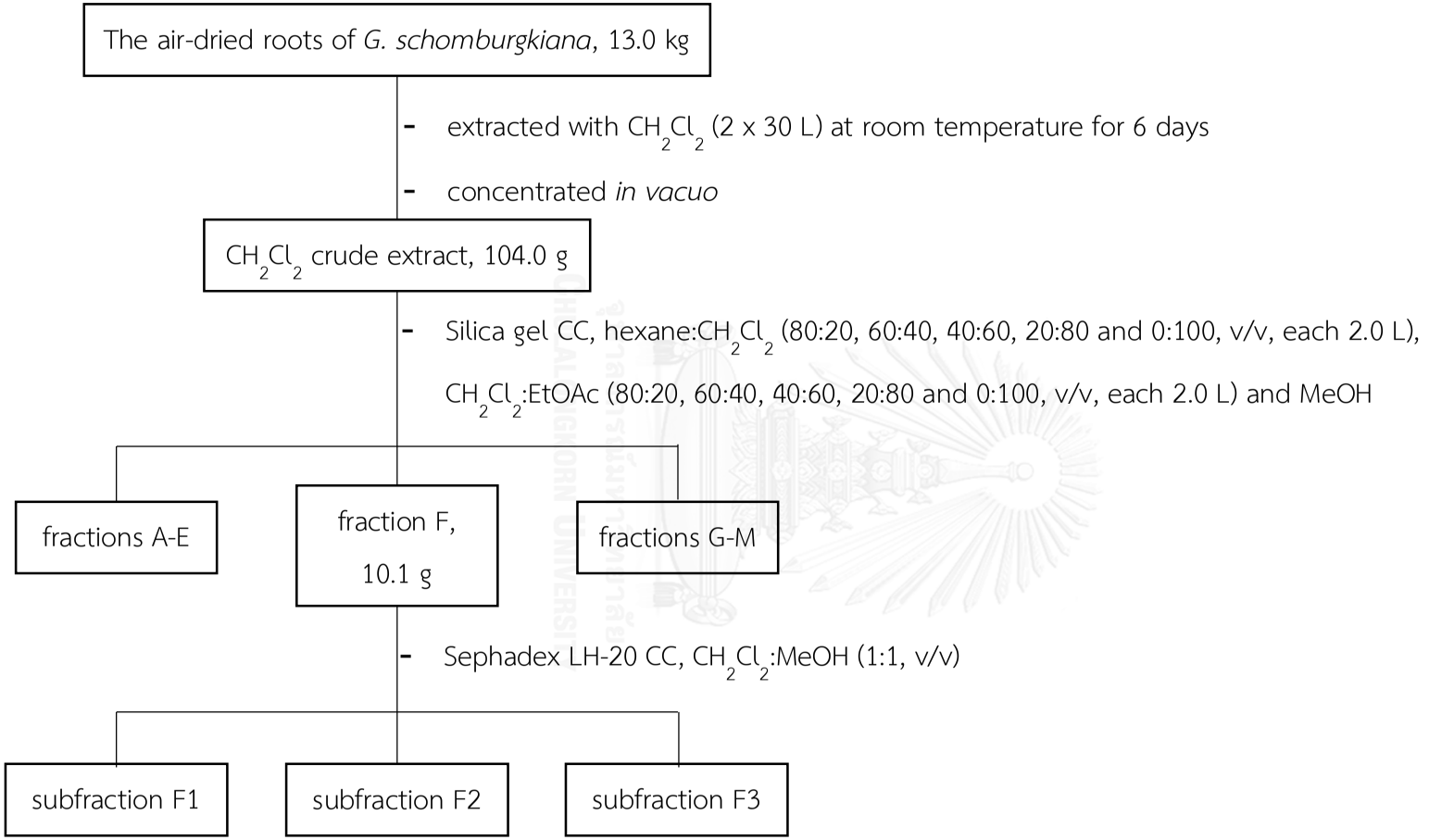
The UV spectra were analyzed using a UV-2550 UV–vis spectrometer (Shimadzu, Kyoto, Japan), while IR data were obtained on Nicolet 6700 FT-IR spectrometer using the KBr disc method. The 1D- and 2D-NMR spectra were measured on a Bruker 400 AVANCE spectrometer in $CDCl_3$. The HR-ESI-MS were analyzed using a Bruker MICROTOF model mass spectrometer. Column chromatography (CC) was performed using silica gel 60 (Merck) and Sephadex LH-20. For TLC analysis, precoated silica gel plates (Merck silica gel 60 GF_{254}, 0.25 mm) were used. Spots were visualized under UV light and sprayed with anisaldehyde solution followed by heating.

2.3 Extraction and Isolation

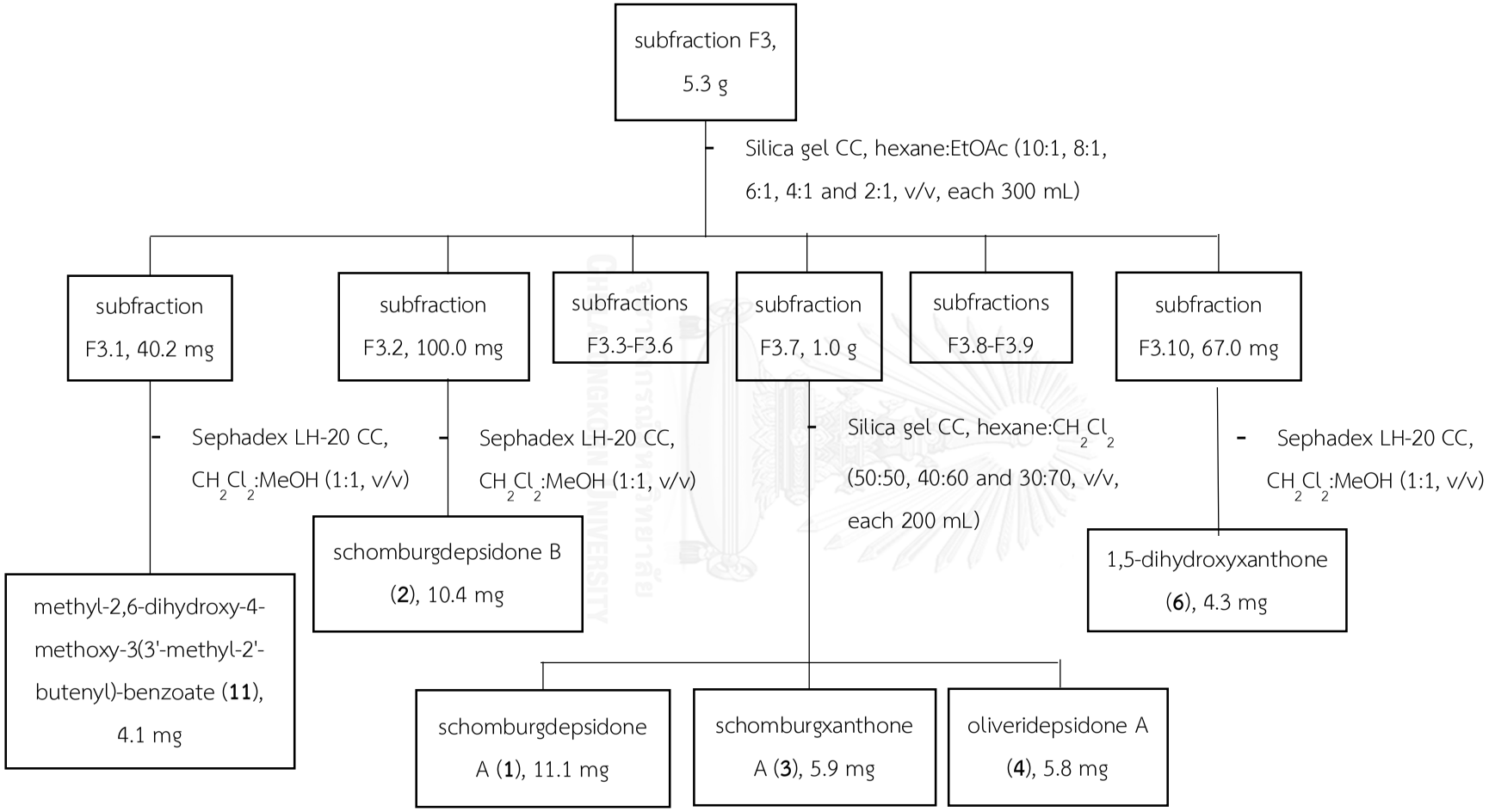
The air-dried roots of *G. schomburgkiana* (13.0 kg) were ground into powder and extracted twice with 30 L each time of dichloromethane (CH_2Cl_2) at room temperature for six days. The solvent was removed from the extract *in vacuo* to yield 104.0 g of crude extract. The crude CH_2Cl_2 extract was then fractionated by CC on silica gel (1.5 kg) with a step gradient elution of hexane:CH_2Cl_2 (80:20, 60:40, 40:60, 20:80 and 0:100, *v/v*, each 2.0 L), CH_2Cl_2:EtOAc (80:20, 60:40, 40:60, 20:80 and 0:100, *v/v*, each 2.0 L) and MeOH, respectively, to obtain 13 fractions (A–M). Fraction F (10.1 g) was further subjected to Sephadex LH-20 CC (300.0 g) eluted with CH_2Cl_2:MeOH (1:1, *v/v*) to yield

three subfractions (F1–F3). Subfraction F3 (5.3 g) was separated by using silica gel CC (200.0 g) with a gradient of hexane:EtOAc (10:1, 8:1, 6:1, 4:1 and 2:1, *v/v*, each 300.0 mL) to give ten subfractions (F3.1–F.3.10).

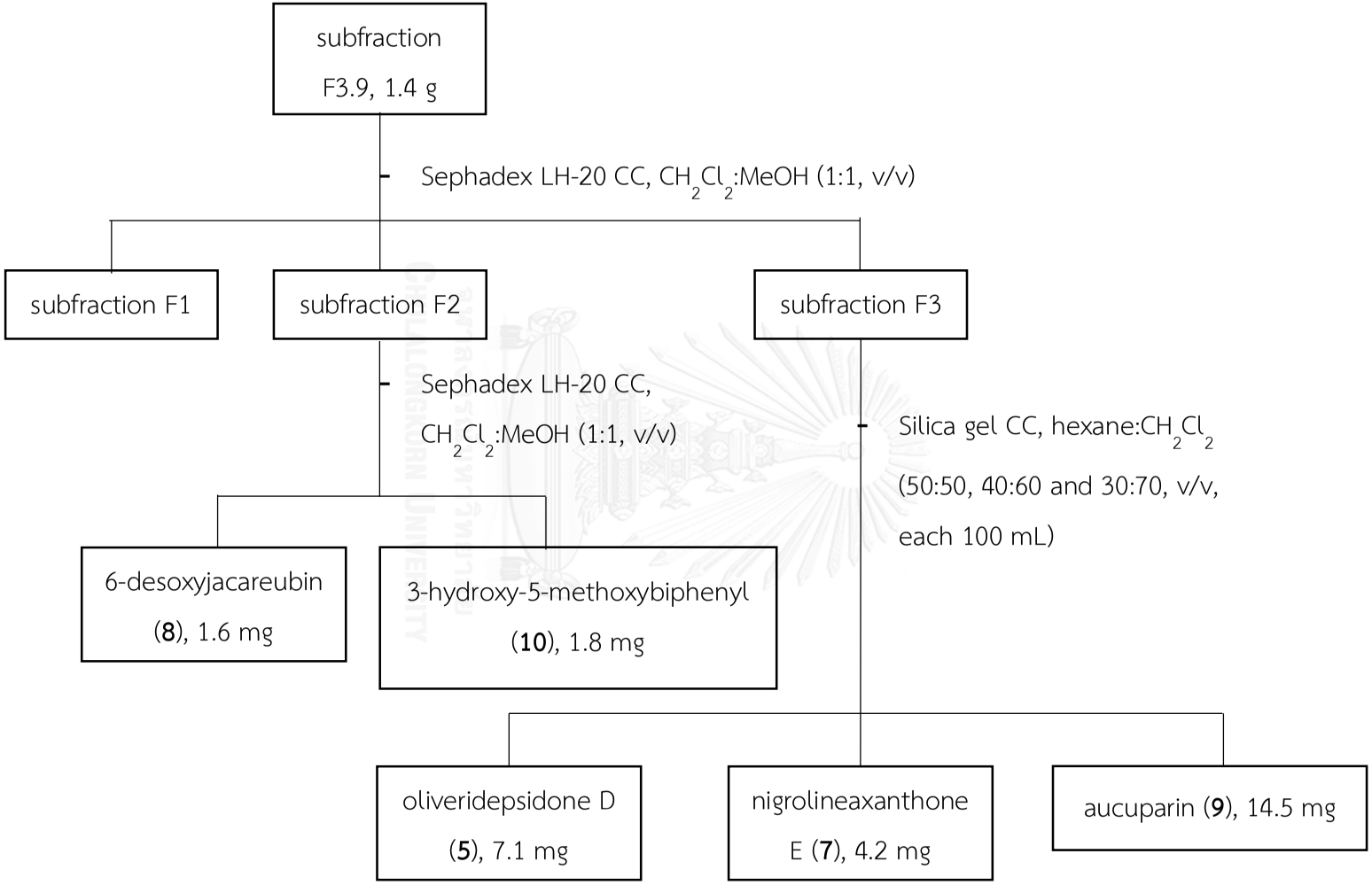
Subfraction F3.1 (40.2 mg) was subjected to Sephadex LH-20 CC (30.0 g) eluted by CH_2Cl_2:MeOH (1:1, *v/v*) to afford **GS11** (4.1 mg). Compound **GS2** (10.4 mg) was obtained by separation of subfraction F3.2 (150.0 mg) on Sephadex LH-20 CC (50.0 g) with CH_2Cl_2:MeOH (1:1, *v/v*). Subfraction F3.7 (1.0 g) was applied to CC over silica gel (50.0 g) with a hexane:CH_2Cl_2 gradient solvent system (50:50, 40:60 and 30:70, *v/v*, each 200.0 mL) to obtain **GS1** (11.1 mg), **GS3** (5.9 mg) and **GS4** (5.8 mg). Compound **GS6** (4.3 mg) was afforded by purification of subfraction F3.10 (67.0 mg) on Sephadex LH-20 CC (30.0 g) eluted with CH_2Cl_2:MeOH (1:1, *v/v*). Subfraction F3.9 (1.4 g) was chromatographed into Sephadex LH-20 CC (100.0 g) with CH_2Cl_2:MeOH (1:1, *v/v*) to give three subfractions (F3.9.1–F3.9.3). Compound **GS8** (1.6 mg) and **GS10** (1.8 mg) were achieved from subfraction F3.9.2 (106.8 mg) by repeated Sephadex LH-20 CC (50.0 g) with a CH_2Cl_2:MeOH (1:1, *v/v*) solvent system. Subfraction F3.9.3 (570.0 mg) was treated by silica gel CC (50.0 g) eluted with a step gradient of hexane:CH_2Cl_2 (50:50, 40:60 and 30:70, *v/v*, each 100.0 mL) to yield **GS5** (7.1 mg), **GS7** (4.2 mg) and **GS9** (14.5 mg).



Scheme 2.1 Extraction procedure of *Garcinia schomburgkiana* roots and fractionation procedure of the CH_2Cl_2 crude extract



Scheme 2.2 Isolation procedure of subfractions F3.1, F3.2, F3.7 and F3.10 from the CH_2Cl_2 crude extract of *G. schomburgkiana* roots



Scheme 2.3 Isolation procedure of subfractions F3.9 from the CH_2Cl_2 crude extract of *G. schomburgkiana* roots

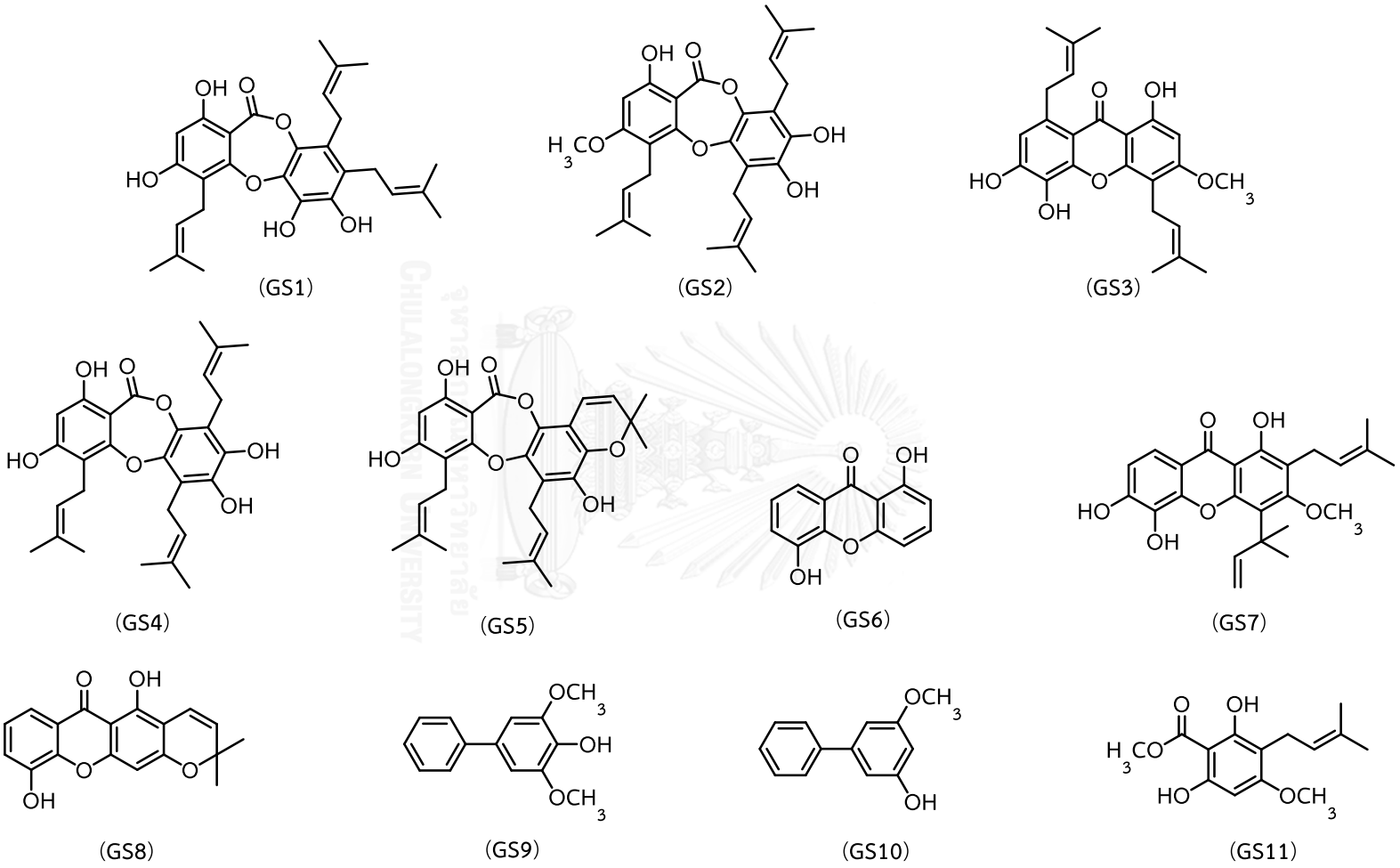


Figure 2.1 Chemical constituents from the CH_2Cl_2 crude extract of *Garcinia schomburgkiana* roots

2.4 Cytotoxic activity against human cancer cell lines procedure

All isolated compounds (**GS1**–G**S11**) were evaluated for their *in vitro* cytotoxic activities against the KB (epidermoid carcinoma), HeLa S-3 (servix adenocarcinoma), HT-29 (colon adenocarcinoma), MCF-7 (breast adenocarcinoma) and Hep G2 (hepatocellular carcinoma) human cancer cell lines using the MTT colorimetric method [26]. Doxorubicin was used as the reference substance.

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 24 h of 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.

2.5 Pancreatic lipase inhibitory activity procedure

The pancreatic lipase activity was performed using 4-MU oleate as a substrate by the method of Duangjai *et al*. [27] with a slight modification. A reaction mixture containing 25 μL of sample (in DMSO) in various contentrations; 40 μL phosphate buffer (pH 8.0) consisting of 13 mmol Tris-HCl, 150 mmol NaCl and 1.3 mmol $CaCl_2$; and 25 μL pancreatic lipase (50 U/mL) was pre-incubated for 10 min at 37°C. The 10 μL substrate (1 mmol 4-MU oleate) was then added to the mixture and further

incubated at 37°C for 30 min. The product was measured with a fluorometrical microplate reader at an excitation wavelength of 535 nm and an emission wavelength of 595 nm. Orlistat was used as a positive control. The percentage of enzyme inhibition by the sample was calculated by:

$$\% \text{ inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the tested sample. The IC_{50} value was determined from a plot of percentage inhibition versus sample concentration.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Properties and structural elucidation of isolated compounds

The roots of *G. schomburgkiana* 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 two new depsidones, schomburgdepsidones A and B (**GS1** and **GS2**) [28], and one new xanthone, schomburgxanthone A (**GS3**) [28], along with two known depsidones; oliveridepsidone A (**GS4**) [29] and oliveridepsidone D (**GS5**) [29], three known xanthones; 1,5-dihydroxyxanthone (**GS6**) [30], nigrolineaxanthone E (**GS7**) [31] and 6-desoxyjacareubin (**GS8**) [32], two biphenyl derivatives; aucuparin (**GS9**) [33] and 3-hydroxy-5-methoxybiphenyl (**GS10**) [34], and a known prenylated benzoic acid derivative; methyl-2,6-dihydroxy-4-methoxy-3(3'-methyl-2'-butenyl)-benzoate (**GS11**) [35]. The structures of the three new isolated compounds (**GS1**–**GS3**) were elucidated by using 1D- and 2D-NMR spectroscopy. The structures of the known compounds (**GS4**–**GS11**) were determined and confirmed by comparison of their ^{1}H and ^{13}C NMR spectroscopic data with previously literature data.

3.1.1 Schomburgdepsidone A (GS1)

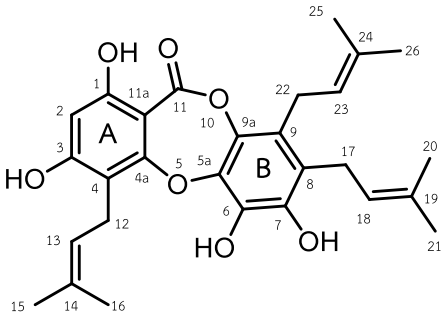


Figure 3.1 The chemical structure of compound **GS1**

Schomburgdepsidone A (**GS1**) was obtained as a brownish gum. Its molecular formula was determined as $C_{28}H_{32}O_7$ by HR-ESI-MS measurement through the sodium adduct ion at *m/z* 503.2051 $[M + Na]^+$ (calcd. for $C_{28}H_{32}O_7Na$, 503.2046). The UV spectrum displayed absorption bands at λ_{max} 320, 272 and 217 nm. The IR spectrum showed characteristic absorption bands for hydroxy and lactone carbonyl stretching bonds at 3415 and 1656 cm^{-1}, indicating the presence of a depsidone chromophore [29]. The ^1H NMR spectrum (Table 3.1) consisted of signals for a hydrogen-bonded hydroxy proton at δ_H 10.99 (1H, s, 1-OH); an aromatic proton at δ_H 6.28 (1H, s, H-2); three hydroxy protons at δ_H 6.28 (1H, brs, 3-OH), 5.83 (1H, brs, 6-OH) and 5.42 (1H, brs, 7-OH); and three 3-methylbut-2-enyl units, as deduced from the resonances of the three olefinic protons at δ_H 5.24 (1H, t, *J* = 6.0 Hz, H-13), 5.08 (1H, t, *J* = 6.4 Hz, H-18) and 5.01 (1H, t, *J* = 6.4 Hz, H-23); three sets of methylene protons at δ_H 3.64 (2H, d, *J* = 6.0 Hz, H-12), 3.35 (2H, d, *J* = 6.4 Hz, H-17) and 3.44 (2H, d, *J* = 6.4 Hz, H-22); and six methyl protons at δ_H 1.79 (3H, s, H-15), 1.86 (3H, s, H-16), 1.71 (3H, s, H-20), 1.79 (3H, s, H-21), 1.67 (3H, s, H-25) and 1.79 (3H, s, H-26).

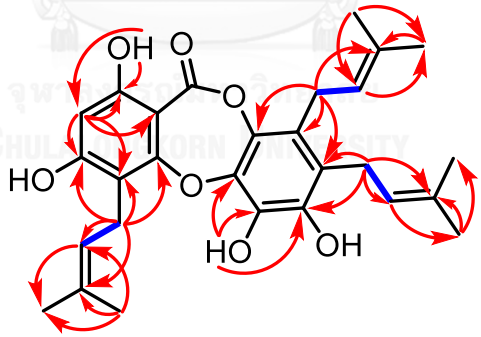


Figure 3.2 The key of HMBC and COSY of compound **GS1**

Based on the HMBC spectrum (Table 3.1), a hydrogen-bonded hydroxy proton at δ_H 10.99 (1-OH) correlated to C-1 (δ_C 163.9) and C-2 (δ_C 100.8), showing that the hydroxy group was attached to C-1. An aromatic proton was placed at C-2 by the observed crosspeaks of H-2 to the oxygenated quaternary aromatic carbons C-1 and

C-3 (δ_C 162.1), and substituted aromatic carbons C-4 (δ_C 111.2) and C-11a (δ_C 99.5). Furthermore, one isoprenyl moiety was attached to C-4 from the correlations of its methylene protons at H-12 to C-3, C-4 and C-4a (δ_C 158.9), supporting the assignment of the A-ring. The ^{1}H and ^{13}C NMR spectroscopic data of **GS1** (Table 3.1) were similar to those of oliveridepsidone A (**GS4**) [29]. The major difference was the position of the remaining two prenyl units and two hydroxy groups in the B-ring. The HMBC spectrum of **GS1** (Figure 3.2) assigned the correlations of methylene protons at H-17 to C-7 (δ_C 141.0) and C-8 (δ_C 123.7), and H-22 to C-8, C-9 (δ_C 123.6) and C-9a (δ_C 136.8), suggesting that the two isoprenyl units were connected to C-8 and C-9 in the B-ring. Consequently, the two remaining hydroxy groups were placed at C-6 (δ_C 133.6) and C-7 (δ_C 141.0).

Table 3.1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of compound **GS1** in $CDCl_3$

Position	GS1		
	δ_H (*J* in Hz)	δ_C	HMBC
1		163.9	
2	6.28 (1H, s)	100.8	1, 3, 4, 11a
3		162.1	
4		111.2	
4a		158.9	
5a		136.5	
6		133.6	
7		141.0	
8		123.7	
9		123.6	
9a		136.8	
11		168.6	
11a		99.5	
12	3.64 (2H, d, 6.0)	22.5	3, 4, 4a, 13, 14

13	5.24 (1H, t, 6.0)	122.2	15, 16
14		137.1	
15	1.79 (3H, s)	25.9	14, 16
16	1.86 (3H, s)	18.2	13, 14, 15
17	3.35 (2H, d, 6.4)	25.8	7, 8, 18, 19
18	5.08 (1H, t, 6.4)	121.9	20, 21
19		134.3	
20	1.71 (3H, s)	25.9	18, 19, 21
21	1.79 (3H, s)	18.1	19, 20
22	3.44 (2H, d, 6.4)	25.6	8, 9, 9a, 24
23	5.01 (1H, t, 6.4)	122.4	25, 26
24		132.4	
25	1.67 (3H, s)	25.9	23, 24, 26
26	1.79 (3H, s)	18.2	24, 25
1-OH	10.99 (1H, s)		1, 2
3-OH	6.28 (1H, brs)		
6-OH	5.83 (1H, brs)		5a, 6, 7
7-OH	5.42 (1H, brs)		

The linkage between two phenyl moieties (the A- and B-ring) was determined by the following spectroscopic data. The carbon signal at δ_C 168.6 (C-11) in the ^{13}C NMR spectrum and the observed strong absorption at ν_{max} 1656 cm^{-1} in the IR spectrum indicated a characteristic of lactone carbonyl from a depsidone [29, 36]. The ^{13}C NMR spectrum also assigned the signals of oxygenated quaternary aromatic carbons C-4a, C-5a (δ_C 136.5) and C-9a, and substituted aromatic carbon C-11a bonded to the carbonyl carbon of depsidone. Based on the HMBC experiment (Figure 3.2), the correlations of H-2 to C-11a and H-12 to C-4a suggested that the A-ring was connected to the C-ring. Furthermore, the correlations of 6-OH (δ_H 5.83) to C-5a and H-22 to C-9a suggested that the B-ring was also connected to the C-ring. Therefore, both phenyl moieties were linked by a seven-membered ring (the C-ring) containing an ester linkage

and an ether bridge. From the above evidences and by comparison with the literature [29], compound **GS1** (Figure 3.1) was determined as 1,3,6,7-tetrahydroxy-4,8,9-tris(3-methylbut-2-enyl)-11*H*-dibenzo[*b*,*e*][1,4]-dioxepin-11-one.

3.1.2 Schomburgdepsidone B (GS2)

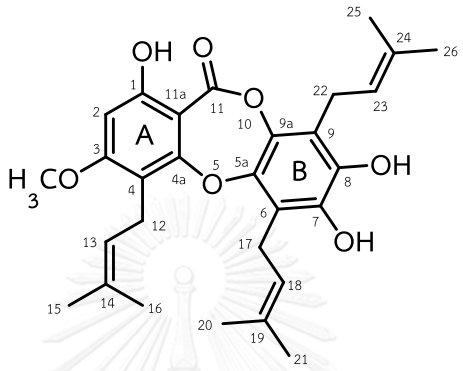


Figure 3.3 The chemical structure of compound **GS2**

Schomburgdepsidone B (**GS2**) was isolated as a brownish gum. Its HR-ESI-MS exhibited a molecular ion peak at *m/z* 495.2390 $[M + H]^+$ (calcd. for $C_{29}H_{35}O_7$, 495.2383), which corresponded to the molecular formula of $C_{29}H_{34}O_7$. The UV spectrum revealed maximum absorption bands at λ_{max} 316, 279 and 212 nm. The IR spectrum showed hydroxy and lactone carbonyl stretching bands at 3408 and 1660 cm^{-1}, suggesting the presence of a depsidone derivative [29]. The ^1H and ^{13}C NMR spectroscopic data of **GS2** (Table 3.2) were comparable to an analogue, oliveridepsidone A (**GS4**) [29]. The difference was the replacement of the ^1H NMR signal for the hydroxy group at C-3 of **GS4** with a methoxy group at δH 3.85 (3H, s, 3-OCH_3) in **GS2**. This structural assignment was further confirmed by the HMBC correlation of the methoxy signal (δH 3.85) to C-3 (δC 165.0) (Figure 3.4). From these data, the structure of **GS2** (Figure 3.3) was established as 1,7,8-trihydroxy-3-methoxy-4,6,9-tris(3-methylbut-2-enyl)-11*H*-dibenzo[*b*,*e*][1,4]dioxepin-11-one.

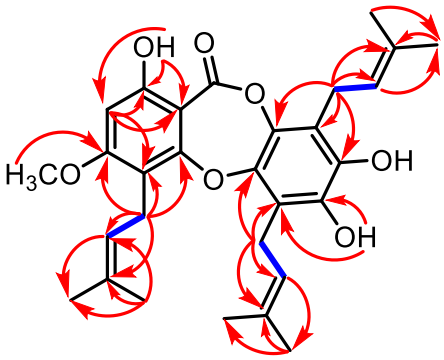


Figure 3.4 The key HMBC and COSY of compound **GS2**

Table 3.2 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of compound **GS2** in $CDCl_3$

Position	GS2		
	δ_H (J in Hz)	δ_C	HMBC
1		164.9	
2	6.32 (1H, s)	96.2	1, 4, 11a
3		165.0	
4		113.7	
4a		158.6	
5a		141.9	
6		117.4	
7		140.2	
8		140.1	
9		118.1	
9a		136.5	
11		169.4	
11a		98.7	
12	3.49 (2H, d, 6.0)	22.6	3, 4, 4a, 13, 14
13	5.14 (1H, t, 6.0)	122.8	15, 16
14		132.3	

15	1.67 (3H, s)	25.8	13, 14, 16
16	1.73 (3H, s)	18.0	13, 14, 15
17	3.57 (2H, d, 6.4)	23.9	5a, 6, 18, 19
18	5.20 (1H, t, 6.4)	121.6	20, 21
19		135.5	
20	1.74 (3H, s)	25.9	19
21	1.81 (3H, s)	18.1	18, 19, 20
22	3.55 (2H, d, 7.2)	23.8	8, 9, 9a, 23, 24
23	5.27 (1H, t, 7.2)	121.2	25, 26
24		135.0	
25	1.73 (3H, s)	25.9	26
26	1.84 (3H, s)	18.1	24
1-OH	11.30 (1H, s)		1, 2, 11a
3-OH			
6-OH			
7-OH	5.54 (1H, s)		6, 7
3-OCH_3	3.85 (3H, s)	56.2	3

3.1.3 Schomburgxanthone A (GS3)

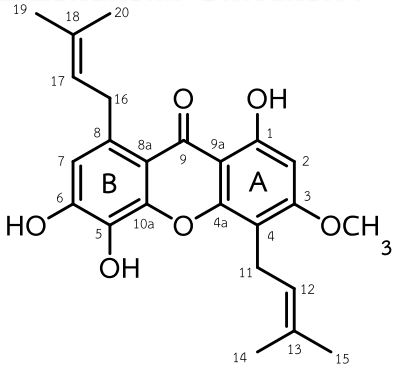


Figure 3.5 The chemical structure of compound **GS3**

Schomburgxanthone A (**GS3**) was obtained as a yellow powder. The molecular formula was measured by HR-ESI-MS as $C_{24}H_{26}O_6$ (*m/z* 411.1818 $[M + H]^+$, calcd. for $C_{24}H_{27}O_6$ 411.1808). The UV spectrum displayed absorption bands at λ_{max} 325, 281 and 224 nm, which were typical of a xanthone chromophore [37]. The IR spectrum showed absorption bands at 3337 and 1636 cm^{-1} due to hydroxy and carbonyl stretching bonds, respectively. The ^1H NMR spectrum (Table 3.3) displayed a hydrogen-bonded hydroxy proton at δ_H 13.44 (1H, s, 1-OH); two hydroxy protons at δ_H 5.47 (1H, brs, 5-OH) and 5.90 (1H, brs, 6-OH); two singlet aromatic protons at δ_H 6.35 (1H, s, H-2) and δ_H 6.78 (1H, s, H-7); a methoxy signal at δ_H 3.90 (3H, s, 3-OCH_3); and two 3-methylbut-2-enyl units, as determined by the resonances of the two olefinic protons at δ_H 5.22 (1H, t, *J* = 6.8 Hz, H-12) and 5.35 (1H, t, *J* = 7.2 Hz, H-17); two sets of methylene protons at δ_H 3.48 (2H, d, *J* = 6.8 Hz, H-11) and 3.97 (2H, d, *J* = 7.2 Hz, H-16); and four methyl protons at δ_H 1.73 (3H, s, H-14), 1.85 (3H, s, H-15), 1.75 (3H, s, H-19), and 1.73 (3H, s, H-20).

In the HMBC spectrum of **GS3** (Figure 3.6), the two isoprenyl groups were located at C-4 and C-8 by the crosspeaks of H-11 (δ_H 3.48) to C-3 (δ_C 163.4), C-4 (δ_C 106.6) and C-4a (δ_C 153.2), and H-16 (δ_H 3.97) to C-7 (δ_C 113.4) and C-8 (δ_C 137.8). In addition, the crosspeak of the methoxy signal (δ_H 3.90) to C-3 indicated that the methoxy group was attributed at C-3 in the A-ring. The ^1H and ^{13}C NMR data of **GS3** (Table 3.3) were similar to those of dulxanthone C [36], except for the absence of the ^1H NMR signal of the methoxy group at C-6 in **GS3**. Furthermore, the substituent at C-6 was assigned as a hydroxy group according to its ^{13}C NMR chemical shift (δ_C 148.2). Thus, the structure of **GS3** (Figure 3.5) was identified as 1,5,6-trihydroxy-3-methoxy-4,8-bis(3-methylbut-2-enyl)-9*H*-xanthen-9-one.

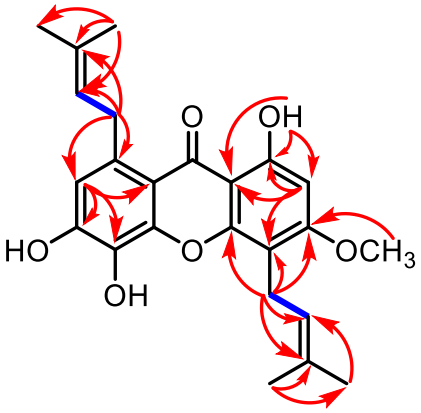


Figure 3.6 The key HMBC and COSY of compound **GS3**

Table 3.3 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopic data of compound **GS3** in $CDCl_3$

Position	GS3		
	δ_H (J in Hz)	δ_C	HMBC
1		162.6	
2	6.35 (1H, s)	94.6	1, 4, 9a
3		163.4	
4		106.6	
4a		153.2	
5		128.9	
6		148.2	
7	6.78 (1H, s)	113.4	5, 6, 8a, 16
8		137.8	
8a		111.9	
9		182.8	
9a		103.7	
10a		146.4	
11	3.48 (2H, d, 6.8)	21.9	3, 4, 4a, 12, 13
12	5.22 (1H, t, 6.8)	123.5	

13		131.7	
14	1.73 (3H, s)	25.7	12, 13, 15
15	1.85 (3H, s)	18.0	12, 13, 14
16	3.97 (2H, d, 7.2)	33.1	7, 8, 17, 18
17	5.35 (1H, t, 7.2)	122.7	
18		133.3	
19	1.73 (3H, s)	18.1	20
20	1.75 (3H, s)	26.0	17, 18, 19
1-OH	13.44 (1H, s)		1, 2, 9a
5-OH	5.47 (1H, brs)		
6-OH	5.90 (1H, brs)		
3-OCH_3	3.90 (3H, s)		3

3.1.4. The known isolated compounds (GS4–GS11)

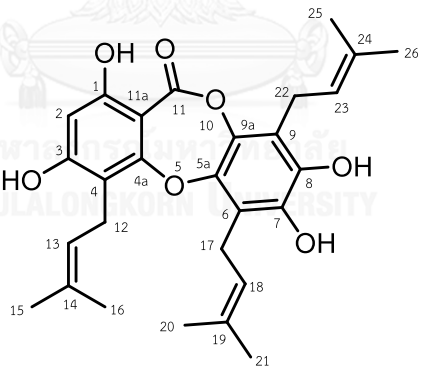


Figure 3.7 The chemical structure of compound **GS4**

Oliveridepsidone A (**GS4**) (Figure 3.7): a pale yellow gum, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 11.09 (1H, s, 1-OH), 6.29 (1H, s, H-2), 5.57 (1H, brs, 7-OH), 5.52 (1H, brs, 8-OH), 5.26 (1H, t, *J* = 7.2 Hz, H-18), 5.22 (1H, t, *J* = 6.8 Hz, H-23), 5.18 (1H, t, *J* = 6.4 Hz, H-13), 3.61 (2H, d, *J* = 6.4 Hz, H-12), 3.55 (2H, d, *J* = 7.2 Hz, H-17), 3.53 (2H, d, *J* = 6.8 Hz, H-22), 1.84 (3H, s, H-15), 1.82 (3H, s, H-20), 1.79 (3H, s, H-25), 1.77 (3H, s, H-26), 1.74

(3H, s, H-16), 1.74 (3H, s, H-21); ^{13}C NMR (100 MHz, $CDCl_3$); δ_C 169.2 (C-11), 164.4 (C-1), 162.9 (C-3), 159.3 (C-4a), 142.1 (C-5a), 140.2 (C-7), 140.1 (C-8), 136.7 (C-9a), 136.4 (C-24), 135.5 (C-14), 135.4 (C-19), 121.5 (C-18), 121.4 (C-13), 121.0 (C-23), 118.1 (C-9), 117.4 (C-6), 110.5 (C-4), 100.9 (C-2), 99.3 (C-11a), 25.9 (C-16), 25.9 (C-21), 25.9 (C-26), 23.8 (C-17), 23.8 (C-22), 22.9 (C-12), 18.1 (C-15), 18.1 (C-20), 18.1 (C-25). The structure of compound **GS4** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [29].

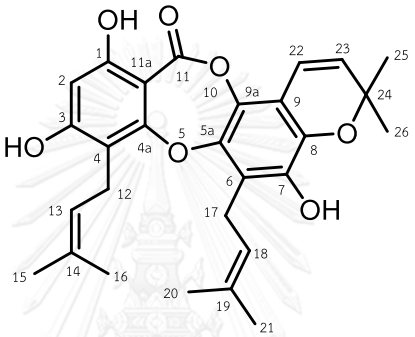


Figure 3.8 The chemical structure of compound **GS5**

Oliveridepsidone D (**GS5**) (Figure 3.8): a pale yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 11.09 (1H, s, 1-OH), 6.76 (1H, d, *J* = 10.0 Hz, H-22), 6.37 (1H, brs, 3-OH), 6.29 (1H, s, H-2), 5.69 (1H, d, *J* = 10.0 Hz, H-23), 5.47 (1H, brs, 7-OH), 5.25 (1H, t, *J* = 6.8 Hz, H-18), 5.15 (1H, t, *J* = 6.4 Hz, H-13), 3.62 (2H, d, *J* = 6.4 Hz, H-12), 3.48 (2H, d, *J* = 6.8 Hz, H-17), 1.82 (3H, s, H-15), 1.78 (3H, s, H-20), 1.76 (3H, s, H-21), 1.70 (3H, s, H-16), 1.45 (3H, s, H-25), 1.45 (3H, s, H-26); ^{13}C NMR (100 MHz, $CDCl_3$); δ_C 169.1 (C-11), 164.5 (C-1), 163.2 (C-3), 159.2 (C-4a), 142.3 (C-5a), 140.3 (C-7), 136.8 (C-9a), 136.5 (C-8), 136.5 (C-14), 133.3 (C-19), 131.3 (C-23), 121.9 (C-18), 121.5 (C-13), 120.1 (C-6), 116.4 (C-22), 111.6 (C-9), 110.7 (C-4), 100.9 (C-2), 99.2 (C-11a), 77.8 (C-24), 28.0 (C-25), 28.0 (C-26), 26.0 (C-21), 25.8 (C-16), 23.5 (C-12), 22.9 (C-17), 18.1 (C-15), 18.1 (C-20). The structure of compound **GS5** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [29].

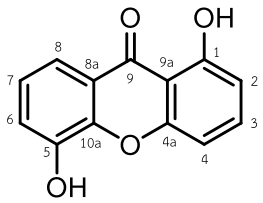


Figure 3.9 The chemical structure of compound **GS6**

1,5-dihydroxyxanthone (**GS6**) (Figure 3.9): a yellow solid, ^{1}H NMR (400 MHz, DMSO-d_6); δ_H 12.61 (1H, s, 1-OH), 10.56 (1H, s, 5-OH), 7.73 (1H, t, *J* = 8.4 Hz, H-3), 7.59 (1H, dd, *J* = 7.6, 1.2 Hz, H-8), 7.36 (1H, dd, *J* = 7.6, 1.2 Hz, H-6), 7.29 (1H, t, *J* = 7.6 Hz, H-7), 7.09 (1H, d, *J* = 8.4 Hz, H-4), 6.81 (1H, d, *J* = 8.4 Hz, H-2); ^{13}C NMR (100 MHz, DMSO-d_6); δ_C 182.1 (C-9), 161.0 (C-1), 155.6 (C-4a), 146.4 (C-5), 145.2 (C-1a), 137.4 (C-3), 124.3 (C-7), 121.1 (C-6), 120.9 (C-8a), 114.6 (C-8), 110.0 (C-2), 108.2 (C-9a), 107.3 (C-4). The structure of compound **GS6** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [30].

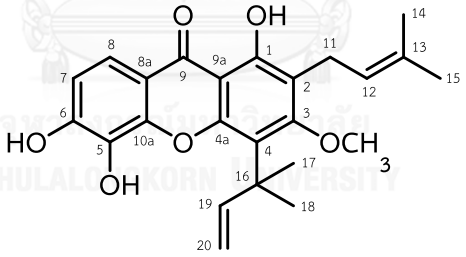


Figure 3.10 The chemical structure of compound **GS7**

Nigrolineaxanthone E (**GS7**) (Figure 3.10): a pale yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 13.34 (1H, s, 1-OH), 7.73 (1H, d, *J* = 8.8 Hz, H-8), 6.96 (1H, d, *J* = 8.8 Hz, H-7), 6.63 (1H, dd, *J* = 17.6, 10.4 Hz, H-19), 6.15 (1H, s, 6-OH), 5.99 (1H, s, 5-OH), 5.30 (1H, t, *J* = 6.4 Hz, H-12), 5.25 (1H, dd, *J* = 17.6, 0.8 Hz, H-20), 5.05 (1H, dd, *J* = 17.6, 0.8 Hz, H-20), 3.78 (3H, s, 3-OCH_3), 3.42 (2H, d, *J* = 6.4 Hz, H-11), 1.80 (3H, s, H-15), 1.72 (3H, s, H-14), 1.68 (3H, s, H-17), 1.68 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$); δ_C 181.4 (C-9), 164.6

(C-3), 160.2 (C-1), 156.9 (C-19), 152.9 (C-4a), 149.4 (C-6), 144.9 (C-10a), 132.3 (C-13), 131.0 (C-5), 122.7 (C-12), 118.8 (C-4), 118.5 (C-2), 118.0 (C-8), 114.0 (C-8a), 113.0 (C-7), 105.8 (C-9a), 104.5 (C-20), 62.8 (3-OCH_3), 42.1 (C-16), 28.9 (C-17), 28.9 (C-18), 25.9 (C-14), 23.1 (C-11), 18.2 (C-15). The structure of compound **GS7** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [31].

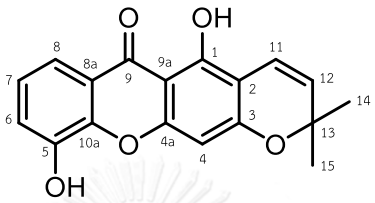


Figure 3.11 The chemical structure of compound **GS8**

6-desoxyjacareubin (**GS8**) (Figure 3.11): a pale yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 13.16 (1H, s, 1-OH), 7.76 (1H, dd, *J* = 8.0, 1.2 Hz, H-8), 7.30 (1H, t, *J* = 8.0 Hz, H-7), 7.27 (1H, dd, *J* = 8.0, 1.2 Hz, H-6), 6.73 (1H, d, *J* = 10.4 Hz, H-11), 6.38 (1H, s, H-4), 6.38 (1H, s, 5-OH), 5.62 (1H, d, *J* = 10.4 Hz, H-12), 1.49 (3H, s, H-14), 1.49 (3H, s, H-15); ^{13}C NMR (100 MHz, $CDCl_3$); δ_C 180.7 (C-9), 161.1 (C-1), 157.9 (C-3), 156.7 (C-4a), 145.3 (C-10a), 144.7 (C-5), 127.9 (C-12), 124.3 (C-7), 121.5 (C-8a), 120.3 (C-6), 117.1 (C-8), 115.5 (C-11), 105.2 (C-2), 103.7 (C-9a), 95.1 (C-4), 78.7 (C-13), 28.6 (C-14), 28.6 (C-15). The structure of compound **GS8** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [32].

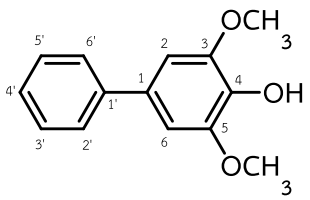


Figure 3.12 The chemical structure of compound **GS9**

Aucuparin (**GS9**) (Figure 3.12): a brown solid, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 7.55 (1H, d, *J* = 7.6, 0.8 Hz, H-2′), 7.55 (1H, d, *J* = 7.6, 0.8 Hz, H-6′), 7.42 (1H, t, *J* = 7.6 Hz, H-3′), 7.42 (1H, t, *J* = 7.6 Hz, H-5′), 7.32 (1H, t, *J* = 7.6 Hz, H-4′), 6.81 (1H, s, H-2), 6.81 (1H, s, H-6), 3.96 (3H, s, 3-OCH_3), 3.96 (3H, s, 5-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$); δ_C 147.5 (C-3), 147.5 (C-5), 141.6 (C-1′), 134.7 (C-4), 133.1 (C-1), 128.9 (C-3′), 128.9 (C-5′), 127.1 (C-2′), 127.1 (C-4′), 127.1 (C-6′), 104.4 (C-2), 104.4 (C-6), 56.6 (3-OCH_3), 56.6 (5-OCH_3). The structure of compound **GS9** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [33].

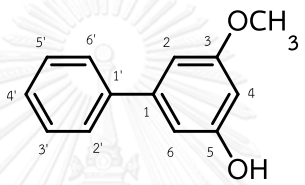


Figure 3.13 The chemical structure of compound **GS10**

3-hydroxy-5-methoxybiphenyl (**GS10**) (Figure 3.13): a pale yellow solid, ^{1}H NMR (400 MHz, $CDCl_3$); δ_H 7.55 (1H, d, *J* = 7.6 Hz, H-2′), 7.55 (1H, d, *J* = 7.6 Hz, H-6′), 7.42 (1H, t, *J* = 7.6 Hz, H-3′), 7.42 (1H, t, *J* = 7.6 Hz, H-5′), 7.35 (1H, t, *J* = 7.6 Hz, H-4′), 6.72 (1H, s, H-6), 6.66 (1H, s, H-2), 6.41 (1H, s, H-4), 3.84 (3H, s, 3-OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$); δ_C 160.3 (C-5), 157.1 (C-3), 143.5 (C-1′), 140.9 (C-1), 128.9 (C-2′), 128.9 (C-6′), 127.8 (C-4′), 127.3 (C-3′), 127.3 (C-5′), 107.0 (C-2), 105.9 (C-6), 100.6 (C-4), 55.6 (3-OCH_3). The structure of compound **GS10** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [34].

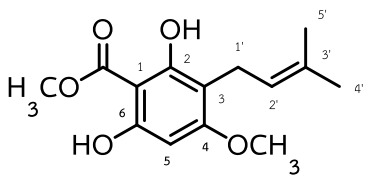


Figure 3.14 The chemical structure of compound **GS11**

Methyl-2,6-dihydroxy-4-methoxy-3(3'-methyl-2'-butenyl)-benzoate (**GS11**) (Figure 3.14): a pale yellow oil, ^{1}H NMR (400 MHz, DMSO-d_6); δ_H 10.78 (1H, s, 2-OH), 10.01 (1H, s, 6-OH), 6.11 (1H, s, H-5), 5.07 (1H, t, *J* = 7.2 Hz, H-2′), 3.89 (3H, s, 1-$COOCH_3$), 3.78 (3H, s, 4-OCH_3), 3.13 (2H, d, *J* = 7.2 Hz, H-1′), 1.68 (3H, s, H-4′), 1.60 (3H, s, H-5′); ^{13}C NMR (100 MHz, DMSO-d_6); δ_C 170.5 (1-**C**$OOCH_3$), 162.8 (C-4), 159.8 (C-2), 158.6 (C-6), 130.0 (C-3′), 122.9 (C-2′), 107.3 (C-3), 94.3 (C-1), 91.6 (C-5), 55.6 (4-OCH_3), 52.4 (1-COO**C**H_3), 25.4 (C-4′), 21.1 (C-1′), 17.5 (C-5′). The structure of compound **GS11** was determined and confirmed by comparison of the physical and spectroscopic data with a previous report [35].

Depsidones are mainly formed in lichens through polyketide pathway which usually contain 3,8-dioxygenation and methyl groups attached to C-1, C-9 and/or C-6 [8, 29]. However, a number of prenylated depsidones were reported from the genus *Garcinia* [8, 10, 12, 28, 29, 36, 38], which well known to have a rich source of shikimate and mevalonate-derived aromatic compounds [8], including xanthones as chemotaxonomic marker of this genus [7]. It is possible that the biosynthesis of the prenylated depsidones lies in Baeyer-Villiger rearrangements from hydroperoxylation reactions of the precursor xanthones, with incorporation of the mevalonate-derived prenyl units [8, 39].

3.2 Cytotoxic activity of isolated compounds (GS1–GS11) against human cancer cell lines

The *in vitro* cytotoxicity assay of all isolated compounds (**GS1**–**GS11**) against five human cancer cell lines (KB, HeLa S-3, HT-29, MCF-7 and Hep G2) was evaluated by using a modified MTT method with doxorubicin as the positive control [26]. The results are summarized in Table 3.4. The tested compounds mostly showed moderate activities to inactive against the five cancer cell lines, except for compound **GS7** showed a good cytotoxicity against the KB, Hela S-3 and MCF-7 cell lines with IC_{50} values of 3.17, 5.46, and 6.07 μM, respectively. Compound **GS3** showed a good

cytotoxic activity against the KB cell line only, with an IC_{50} value of 8.14 μM. Interestingly, the xanthone-type compounds (**GS3**, **GS7** and **GS8**) showed a better cytotoxicity (IC_{50} < 100 μM) than the other tested compounds. Among the depsidones (**GS1**, **GS2**, **GS4** and **GS5**), only compound **GS5** had a slightly higher cytotoxicity with IC_{50} values in the range of 11.14–30.40 μM.

3.3 Pancreatic lipase inhibitory activity of isolated compounds (GS1–GS11)

The pancreatic lipase inhibitory activity of all isolated compounds (**GS1**–**GS11**) was evaluated using 4-MU oleate as a substrate. Orlistat was used as the positive control [27]. The results are summarized in Table 3.5. The tested compounds mostly showed moderately to weakly active against pancreatic lipase inhibition. Compounds **GS1**–**GS3**, **GS7**, **GS8** and **GS11** performed a moderate activity with IC_{50} values of 36.35, 28.28, 23.71, 37.28, 61.47 and 80.78 μM, respectively. Comparison of the lipase inhibitory activities of compound **GS2** with those of compounds **GS4** and **GS5** indicated that the replacement of methoxy group at position C-3 to a hydroxy group in compound **GS4** and the cyclization of prenyl group at C-9 with a hydroxy group at C-8 in **GS5** might reduce the inhibitory activity against pancreatic lipase enzyme. Among the xanthones (**GS3**, **GS6**–**GS8**), only compound **GS6** showed a weak activity to inhibit the lipase work (IC_{50} > 100 μM).

Table 3.4 The *in vitro* cytotoxic activity of compounds **GS1**–**GS11** against five human cancer cell lines

Compound	IC_{50} (μM) ± SD				
	KB	Hela S-3	HT-29	Hep G2	MCF-7
Schomburgdepsidone A (**GS1**)	43.39 ± 1.95	>100	>100	47.86 ± 0.58	43.87 ± 6.68
Schomburgdepsidone B (**GS2**)	52.33 ± 1.99	95.96 ± 6.06	>100	>100	46.97 ± 0.97
Schomburgxanthone A (**GS3**)	8.14 ± 0.61	14.03 ± 0.26	>100	48.90 ± 0.88	21.56 ± 0.19
Oliveridepsidone A (**GS4**)	55.94 ± 5.63	>100	58.74 ± 0.11	39.27 ± 0.54	36.46 ± 0.76
Oliveridepsidone D (**GS5**)	11.14 ± 0.86	19.27 ± 0.27	30.40 ± 1.78	27.83 ± 0.92	13.42 ± 0.10
1,5-dihydroxyxanthone (**GS6**)	25.50 ± 0.96	>100	>100	>100	>100
Nigrolineaxanthone E (**GS7**)	3.17 ± 0.08	5.46 ± 0.08	34.08 ± 0.73	16.08 ± 0.22	6.07 ± 0.11
6-desoxyjacareubin (**GS8**)	18.56 ± 0.15	19.69 ± 0.46	69.29 ± 0.79	39.35 ± 1.05	17.53 ± 0.06
Aucuparin (**GS9**)	>100	>100	>100	>100	>100
3-hydroxy-5-methoxybiphenyl (**GS10**)	59.78 ± 1.01	>100	>100	>100	>100
Methyl-2,6-dihydroxy-4-methoxy-3(3'-methyl-2'-butenyl)-benzoate (**GS11**)	>100	>100	>100	>100	69.45 ± 0.34
Doxorubicin	0.22 ± 0.05	0.11 ± 0.03	0.44 ± 0.08	2.80 ± 0.09	0.42 ± 0.03

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

Table 3.5 The *in vitro* pancreatic lipase inhibitory activity of compounds **GS1**–**GS11**

Compound	IC_{50} (μM)
Schomburgdepsidone A (**GS1**)	36.35
Schomburgdepsidone B (**GS2**)	28.28
Schomburgxanthone A (**GS3**)	23.71
Oliveridepsidone A (**GS4**)	> 100
Oliveridepsidone D (**GS5**)	> 100
1,5-dihydroxyxanthone (**GS6**)	> 100
Nigrolineaxanthone E (**GS7**)	37.28
6-desoxyjacareubin (**GS8**)	61.47
Aucuparin (**GS9**)	> 100
3-hydroxy-5-methoxybiphenyl (**GS10**)	> 100
Methyl-2,6-dihydroxy-4-methoxy-3(3'-methyl-2'-butenyl)-benzoate (**GS11**)	80.78
Orlistat	0.09

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

CHAPTER IV
CONCLUSION

In conclusion, compounds **GS1**–**GS11** were successfully isolated and purified from the CH_2Cl_2 extract of *Garcinia schomburgkiana* roots by silica gel and Sephadex LH-20 column chromatographies. The isolated compounds consisted of two new depsidones, schomburgdepsidones A and B (**GS1** and **GS2**), and one new xanthone, schomburgxanthone A (**GS3**), along with eight known compounds; oliveridepsidone A (**GS4**), oliveridepsidone D (**GS5**), 1,5-dihydroxyxanthone (**GS6**), nigrolineaxanthone E (**GS7**), 6-desoxyjacareubin (**GS8**), aucuparin (**GS9**), 3-hydroxy-5-methoxybiphenyl (**GS10**) and methyl-2,6-dihydroxy-4-methoxy-3(3'-methyl-2'-butenyl)-benzoate (**GS11**). The structure of all isolated compounds were structurally elucidated by means of spectroscopic analysis as well as comparison with the literature data. In addition, the cytotoxic activity against five human cancer cell lines (KB, HeLa S-3, HT-29, MCF-7 and Hep G2) and pancreatic lipase inhibitory activity were performed to evaluate the bioactivity of all 11 compounds.

The results of the cytotoxicity against human cancer cell lines showed that compound **GS7** performed a good cytotoxic activity against the KB, Hela S-3 and MCF-7 cell lines with IC_{50} values in the range of 3.17–6.07 μM and compound **GS3** exhibited a good cytotoxic activity against the KB cell line only, with an IC_{50} value of 8.14 μM. On the other hand, the inhibitory activity of 11 compounds against pancreatic lipase enzyme resulted a moderately active of compounds **GS1**–**GS3**, **GS7**, **GS8** and **GS11** with IC_{50} values in the range of 23.71–80.78 μM, while the other compounds showed a weak activity with IC_{50} values which were more than 100 μM.

This research presented the bioactive components isolated successfully from the CH_2Cl_2 extract of the roots of *G. schomburgkiana* as an anticancer and antiobesity agents through the preliminary screening on cytotoxic activity and pancreatic lipase inhibitory activity. The future works might involve a further investigation of the most active compounds for an *in vivo* experiment and chemical constituents exploration of

the MeOH extract (polar part) from the roots of *G. schomburgkiana*. Moreover, these results might provide an knowledge to modify and synthesize natural product-derived compounds for achieving a potent drug candidate.

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APPENDIX

CONTENT OF SUPPORTING INFORMATION

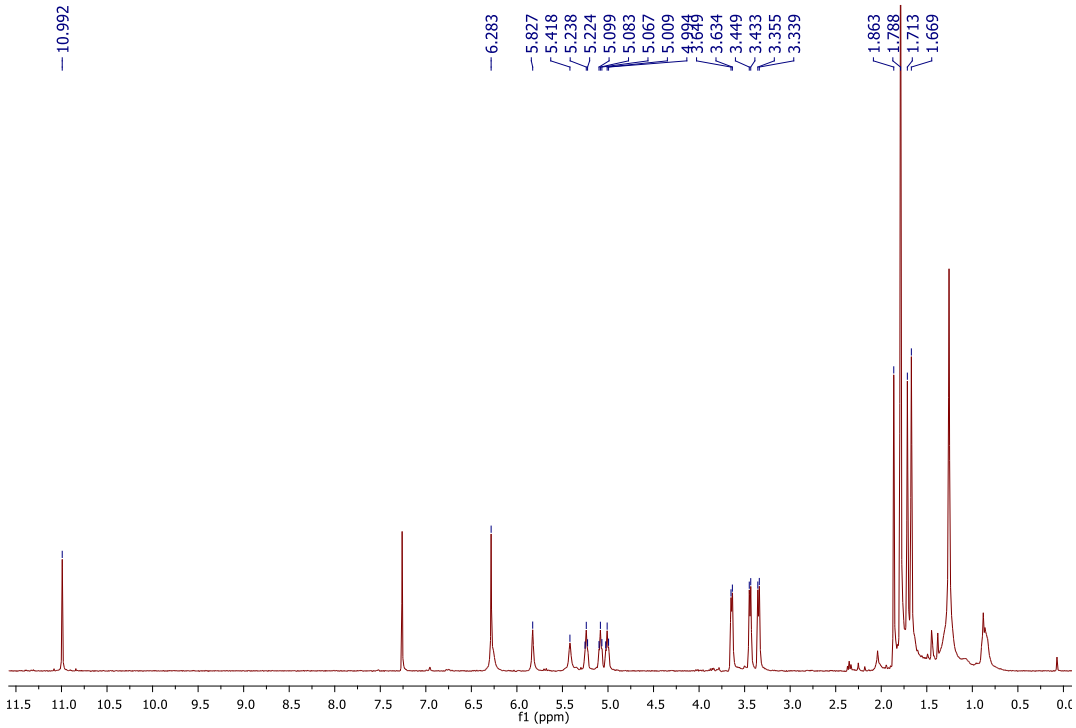


Figure A.1 ^{1}H NMR spectrum of compound **GS1** in $CDCl_3$

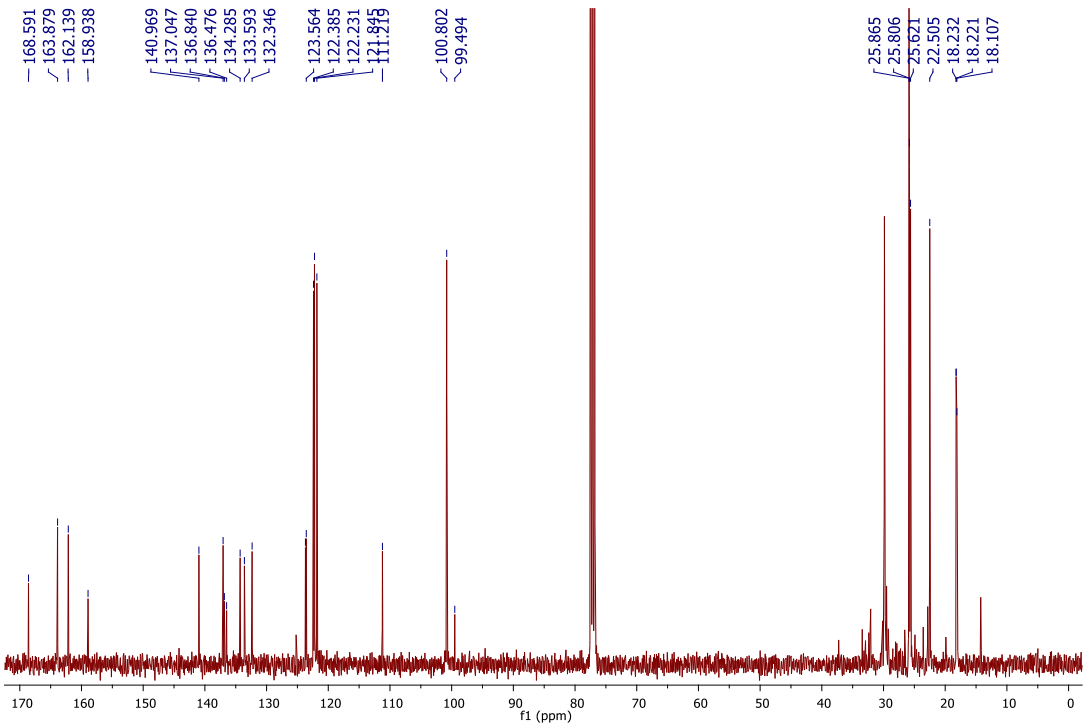
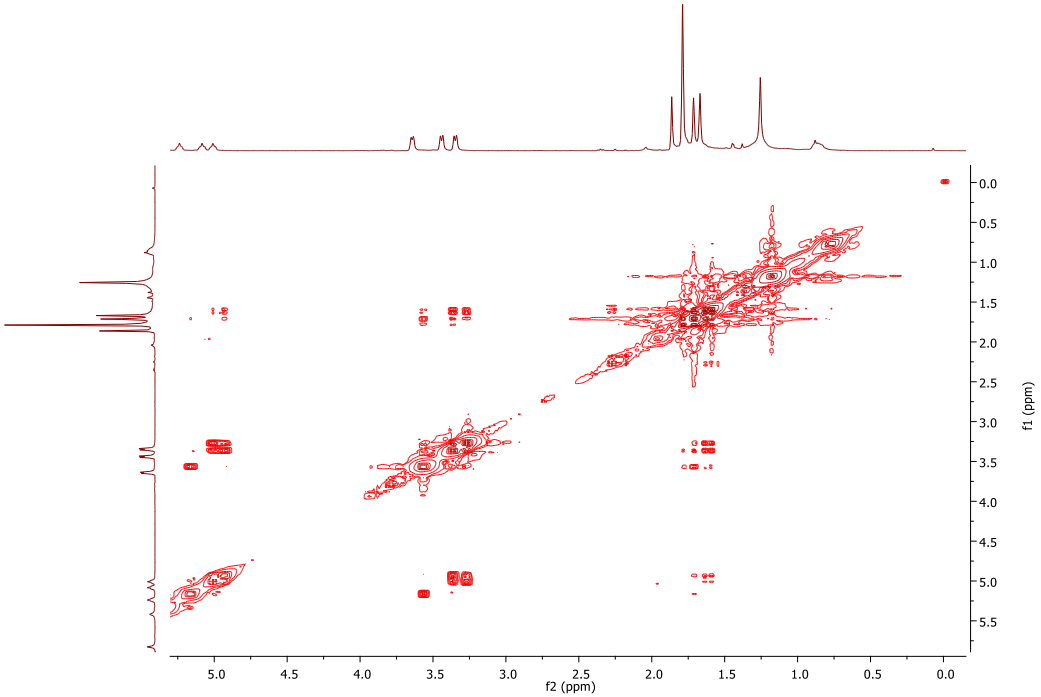


Figure A.2 ^{13}C NMR spectrum of compound **GS1** in $CDCl_3$



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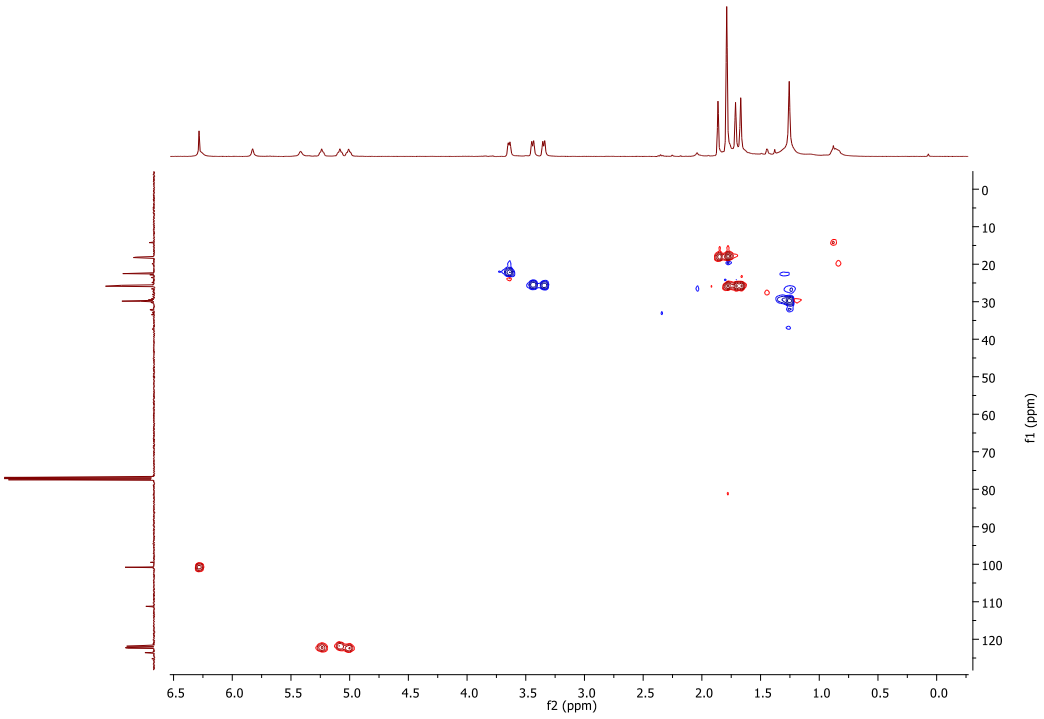

Figure A.4 HSQC spectrum of compound **GS1** in $CDCl_3$

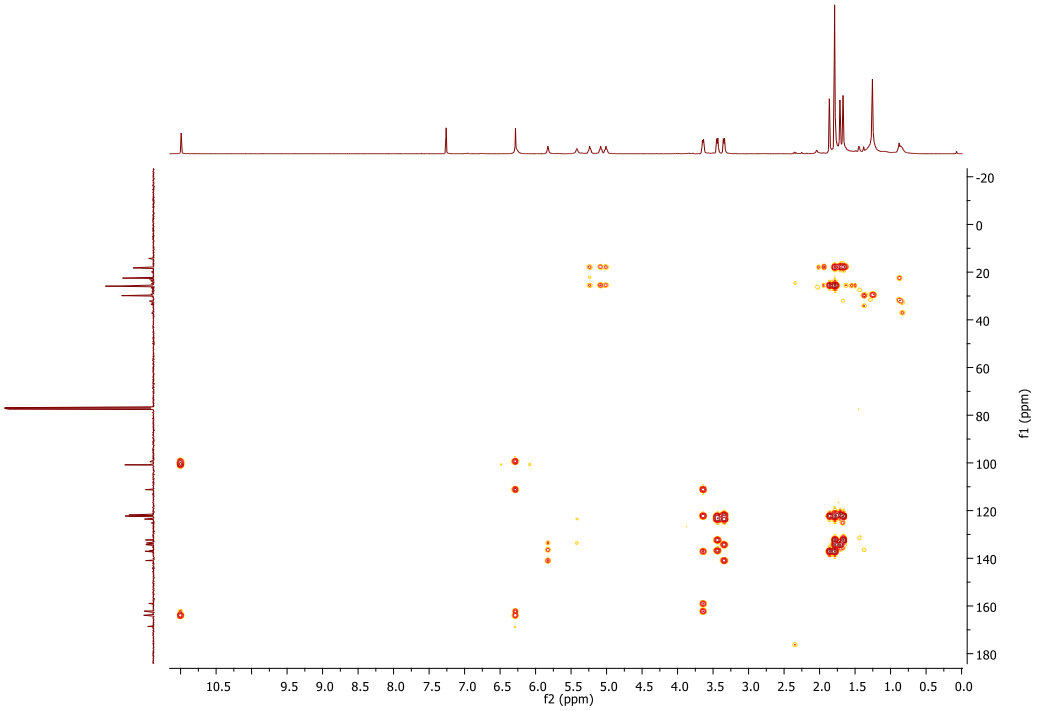

Figure A.5 HMBC spectrum of compound **GS1** in $CDCl_3$

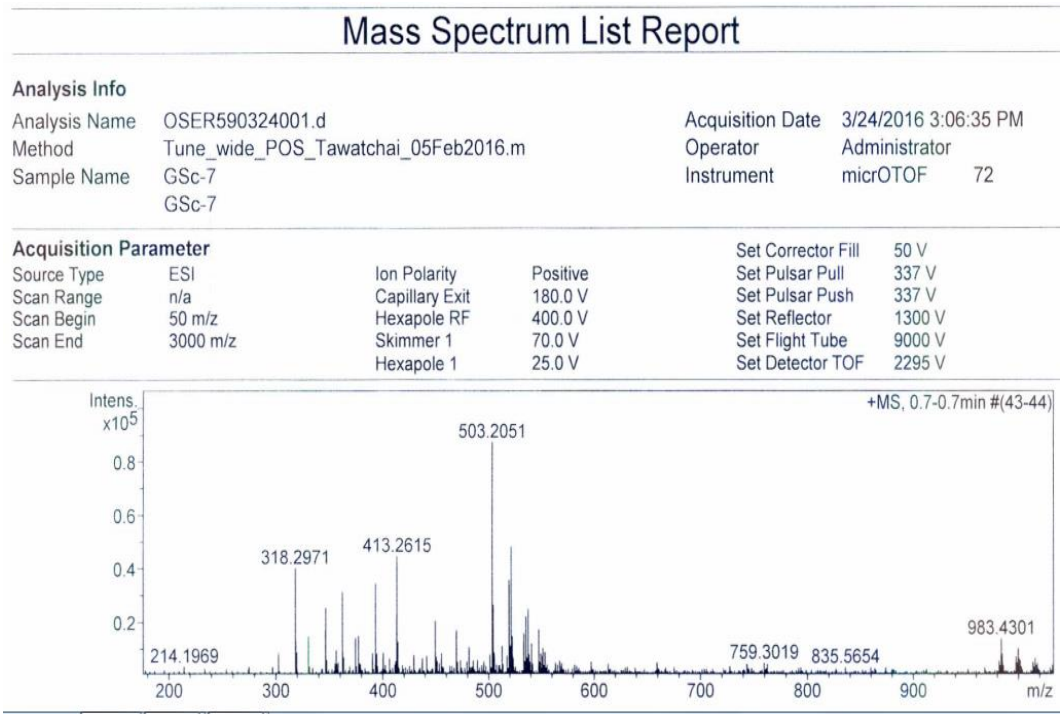

Figure A.6 HR-ESI-MS of compound **GS1**

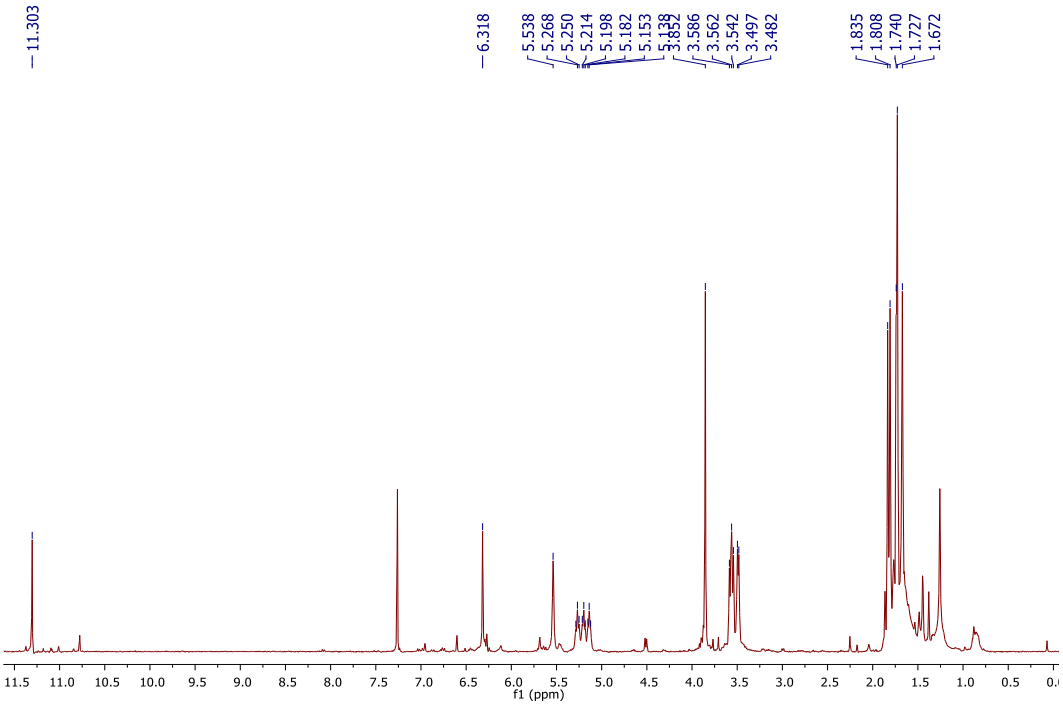

Figure B.1 ^{1}H NMR spectrum of compound **GS2** in $CDCl_3$

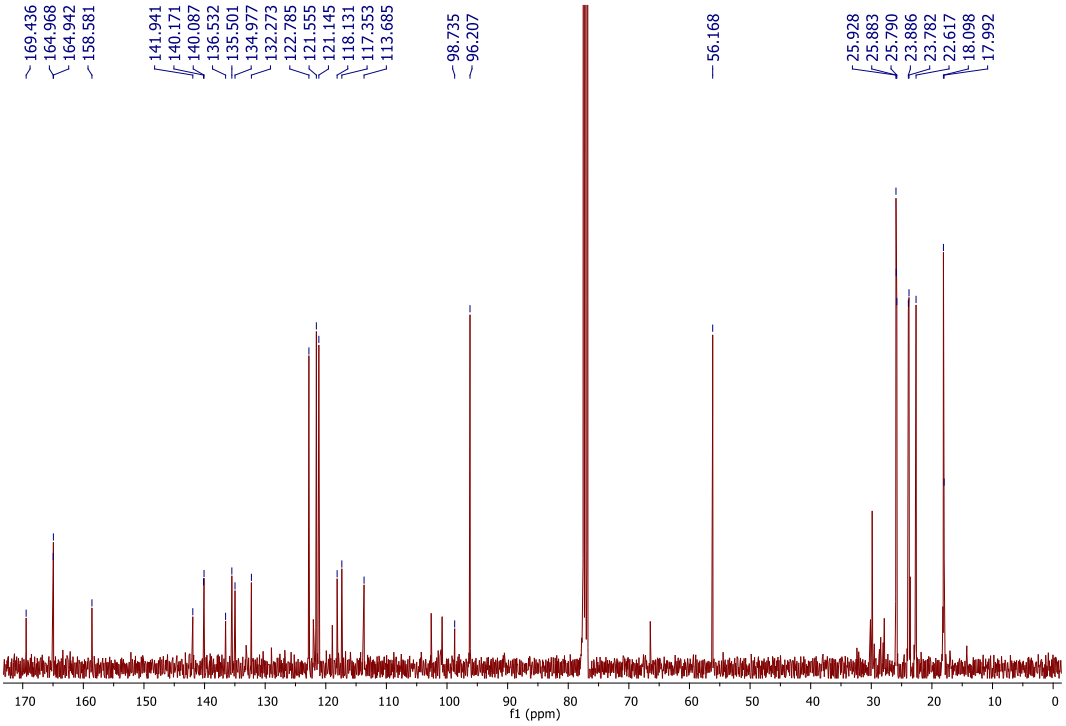

Figure B.2 ^{13}C NMR spectrum of compound **GS2** in $CDCl_3$

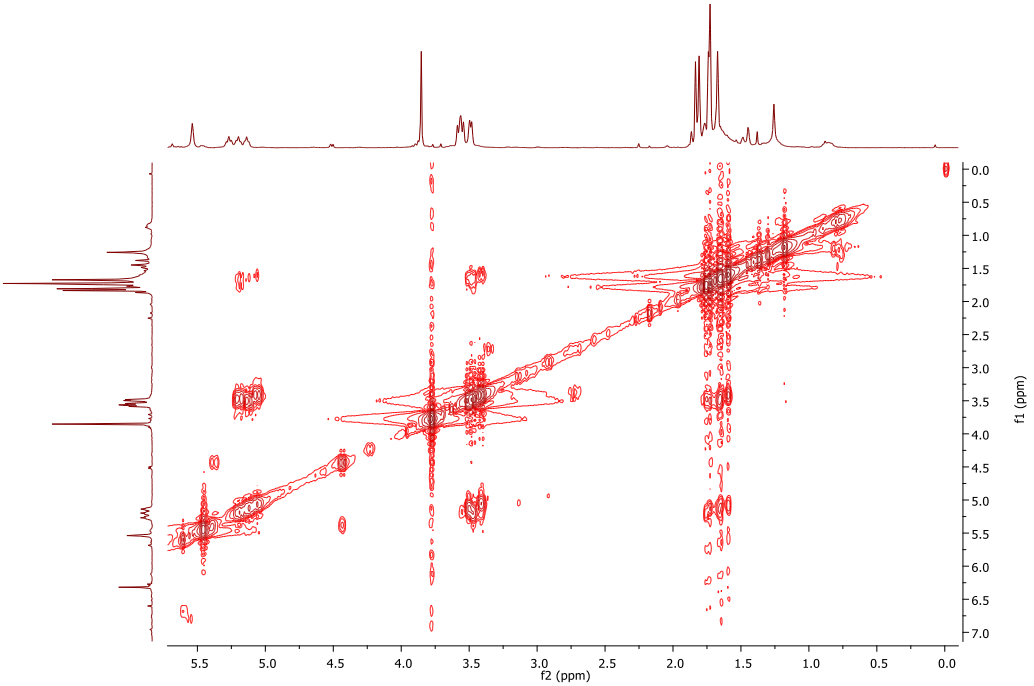

Figure B.3 COSY spectrum of compound **GS2** in $CDCl_3$

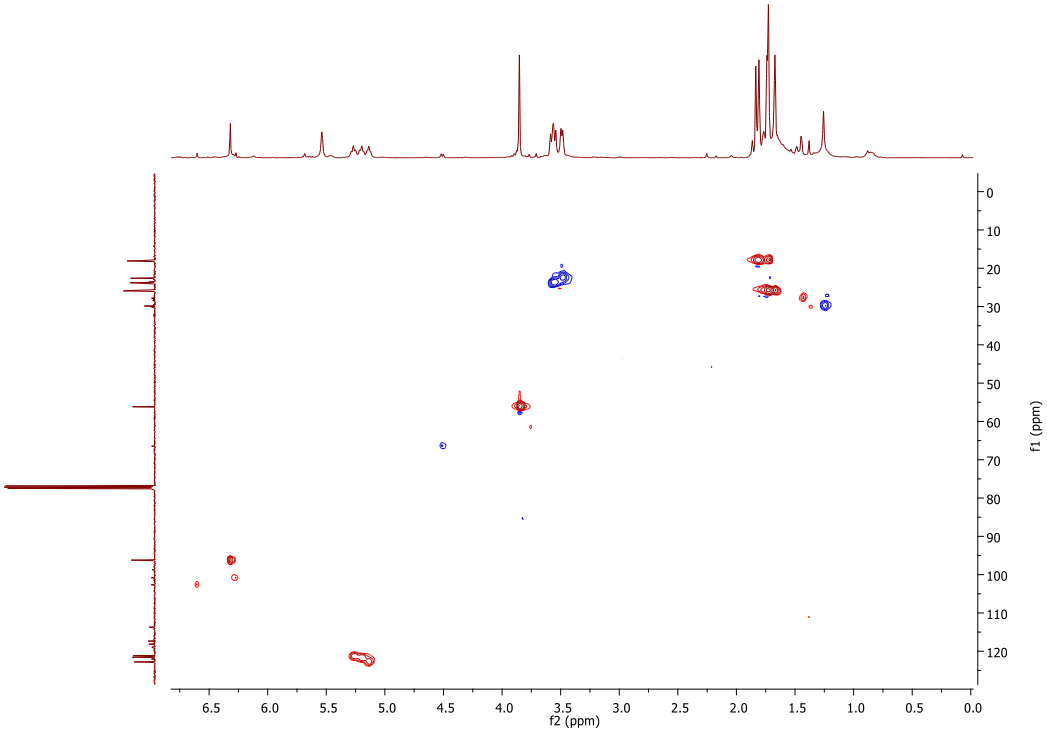

Figure B.4 HSQC spectrum of compound **GS2** in $CDCl_3$

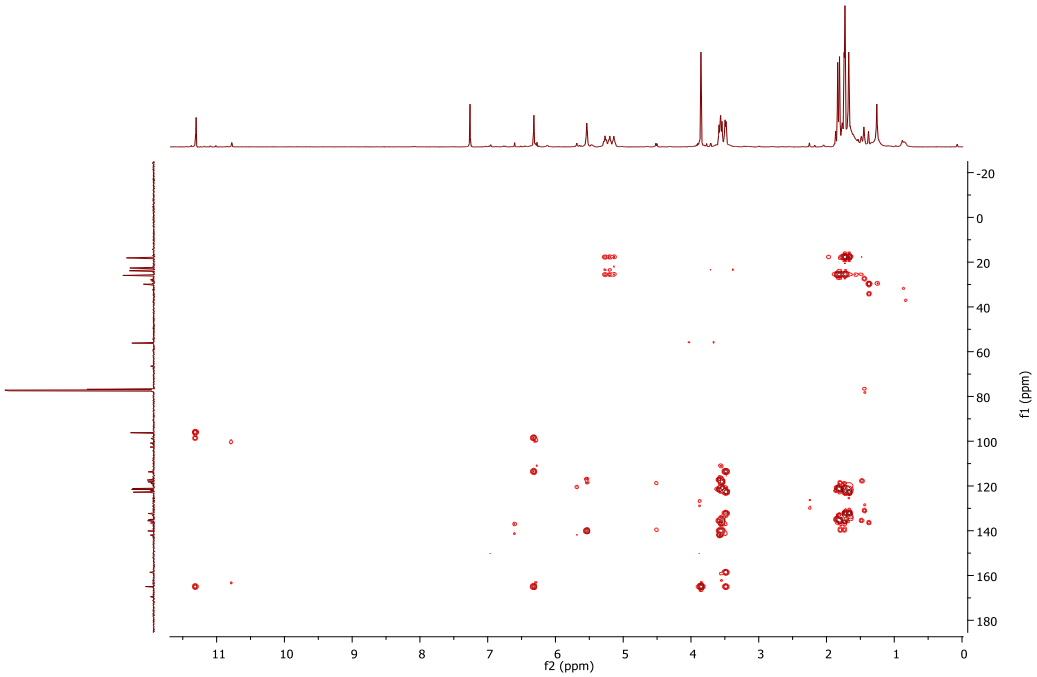

Figure B.5 HMBC spectrum of compound **GS2** in $CDCl_3$

Mass Spectrum List Report

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Scan End	3000 m/z	Skimmer 1	70.0 V	Set Reflector	1300 V
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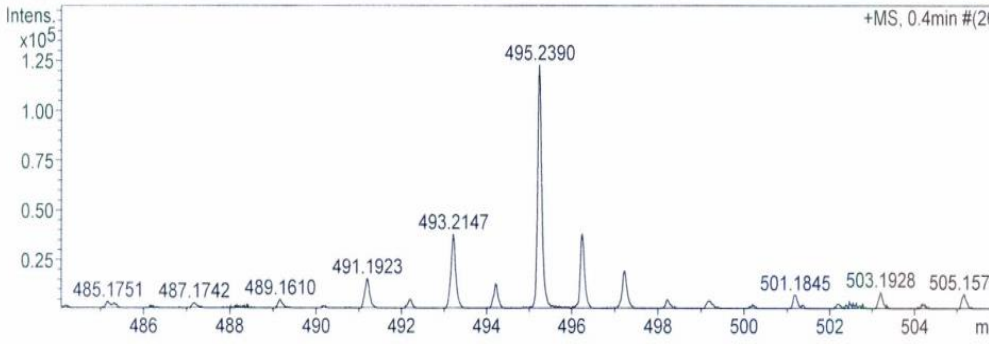

Figure B.6 HR-ESI-MS of compound **GS2**

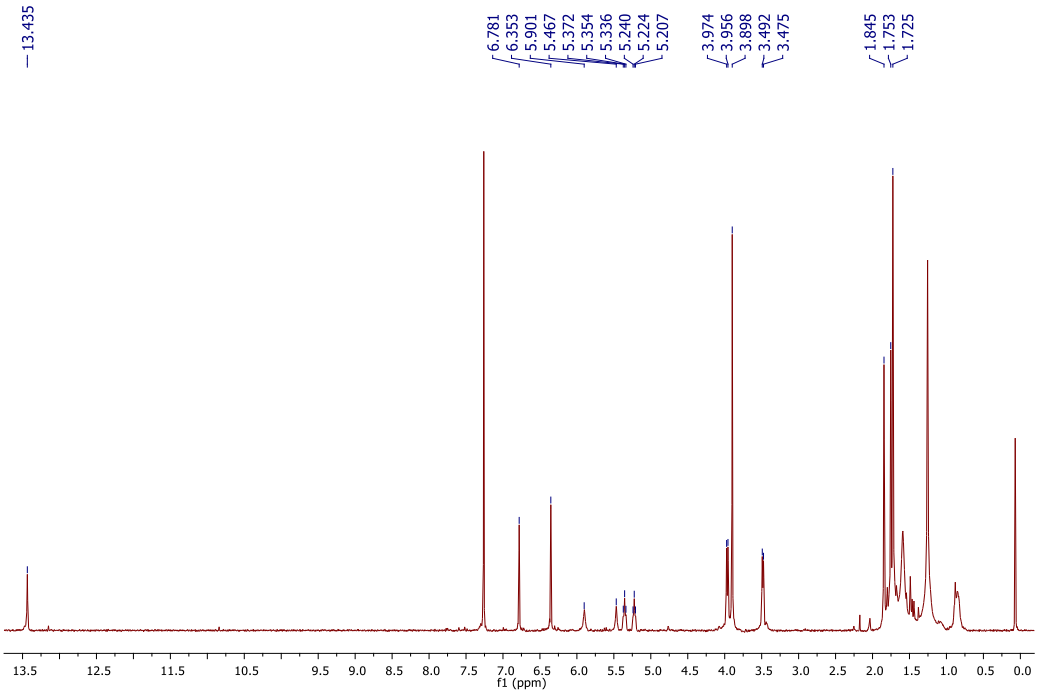

Figure C.1 ^{1}H NMR spectrum of compound **GS3** in $CDCl_3$

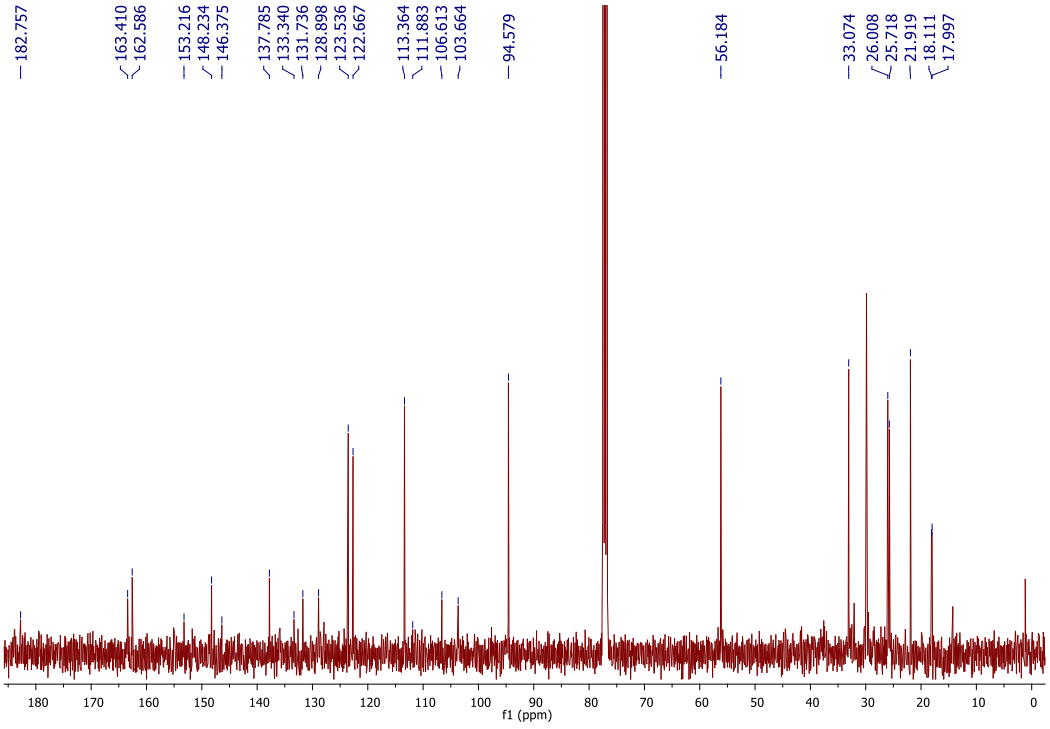

Figure C.2 ^{13}C NMR spectrum of compound **GS3** in $CDCl_3$

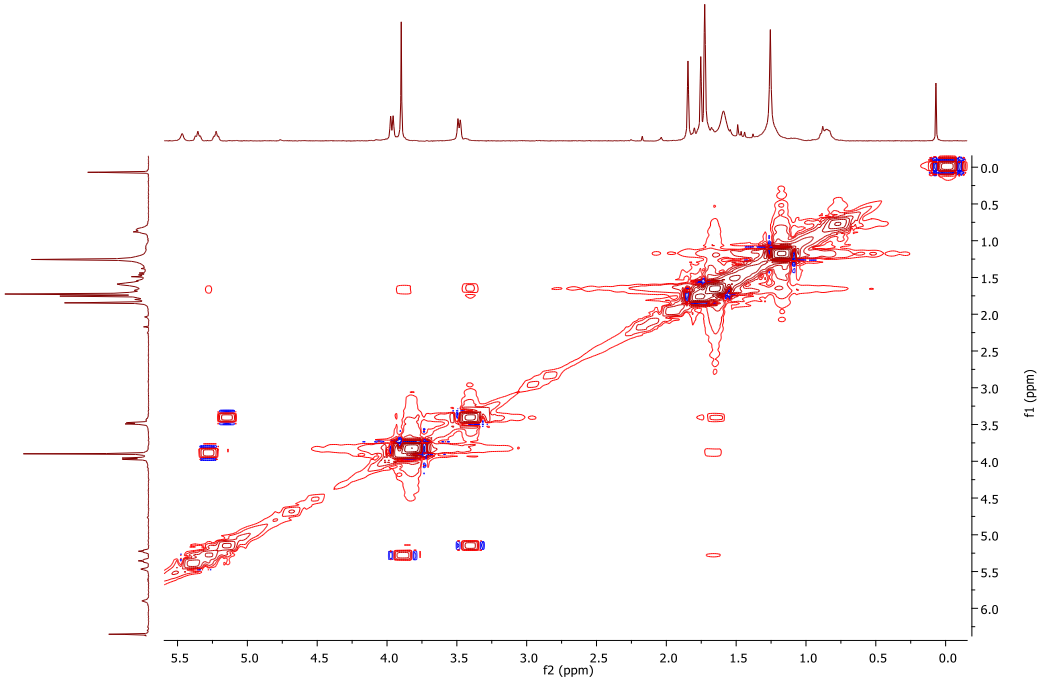

Figure C.3 COSY spectrum of compound **GS3** in $CDCl_3$

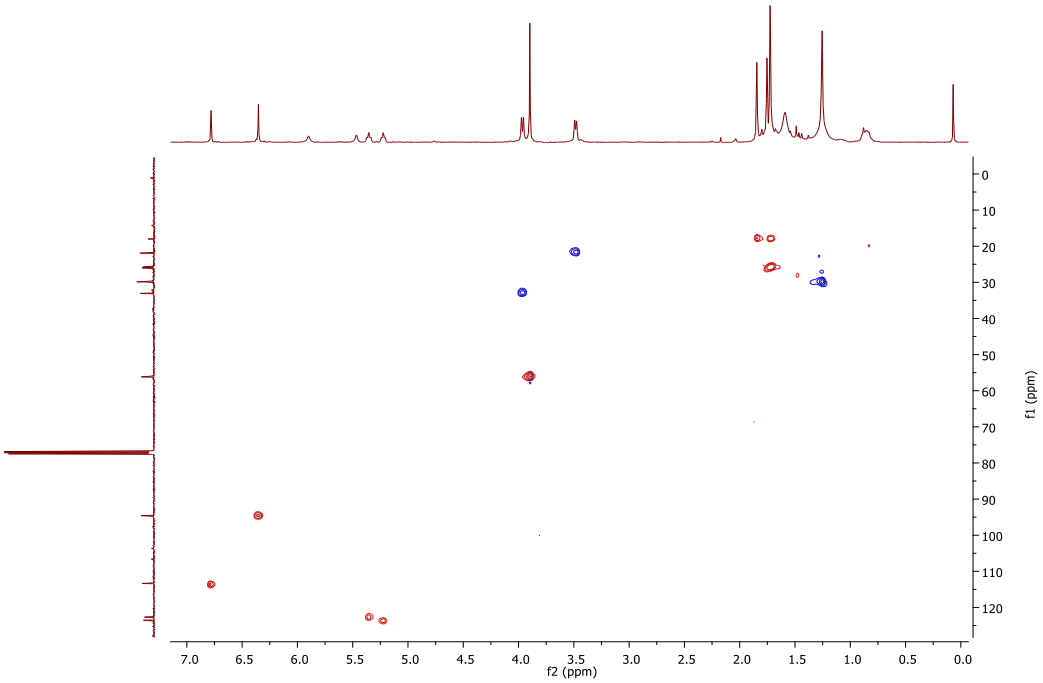

Figure C.4 HSQC spectrum of compound **GS3** in $CDCl_3$

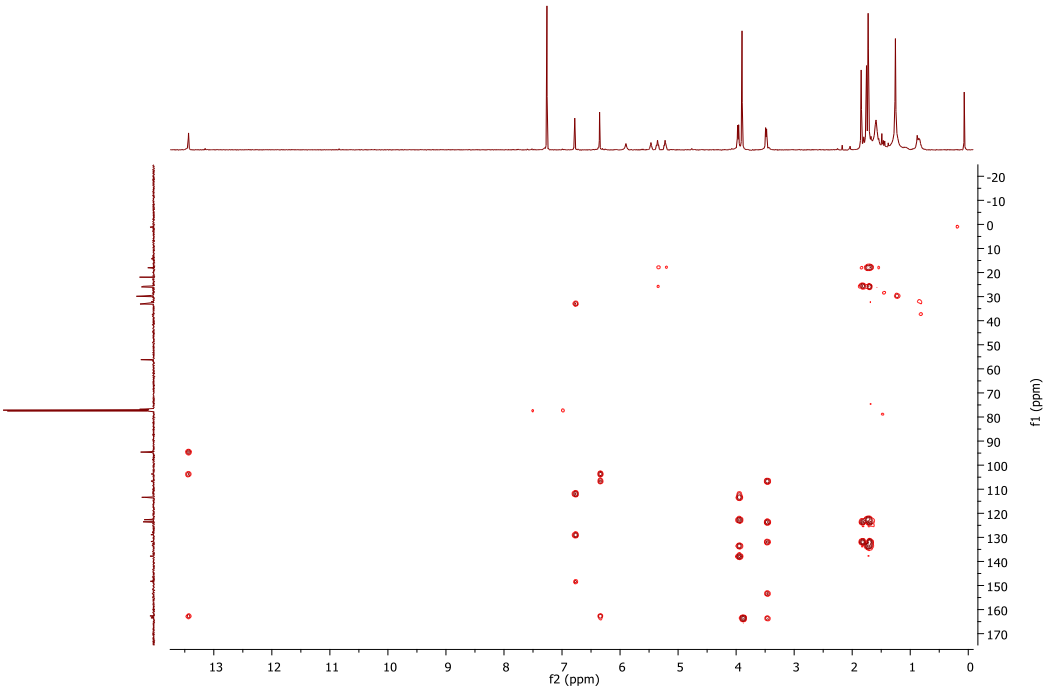

Figure C.5 HMBC spectrum of compound **GS3** in $CDCl_3$

Mass Spectrum List Report

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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

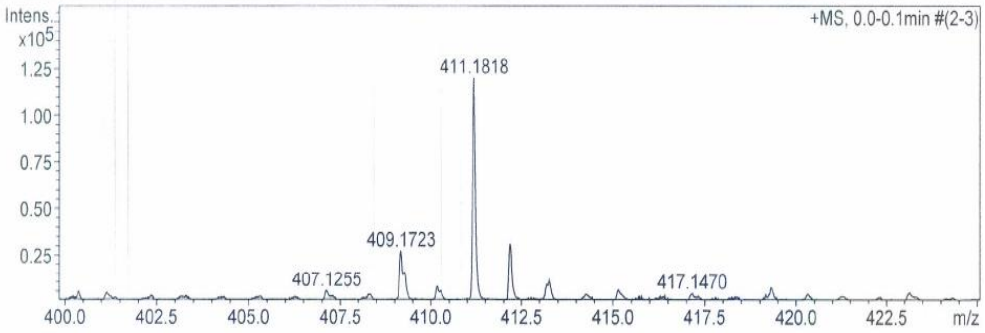

Figure C.6 HR-ESI-MS of compound **GS3**

VITA

Mr. Edwin Risky Sukandar was born on November 15, 1993 in Surabaya, Indonesia. He graduated with Bachelor's degree of Science, major in Chemistry (cumlaude) from Sepuluh Nopember Institute of Technology (ITS), Surabaya, Indonesia in 2014 under supervised Prof. Dr. Taslim Ersam. He then continued his master degree at Department of Chemistry, Chulalongkorn University under supervised Assoc. Prof. Dr. Santi Tip-pyang.

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