

ANTI-*HELICOBACTER PYLORI* OF *GARCINIA XANTHONES* AND
THEIR DERIVATIVES



Presented in Partial Fulfillment of the Requirements for the
Master of Science degree in Chemistry
at Srinakharinwirot University
MAY 2014

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The isolated xanthones obtained from *Garcinia fusca* were evaluated for antibacterial activity against *Helicobacter pylori*. The results showed that α -mangostin (**8**) and β -mangostin (**9**) exhibited moderate activity against *H. pylori* ATCC 43504 with MICs of 38.0 and 36.6 μ M, respectively. Cowaxanthone (**14**) was the most activity (MIC 4.6 μ M) against *H. pylori* DMST 20165 and more potent activity than that of the positive control drug, metronidazole (MIC 11.1 μ M). Isojacareubin (**26**) demonstrated the highest activity against *H. pylori* HP40 at MIC 23.9 μ M and was approximately two times more potent than that of amoxicillin (MIC 42.7 μ M), but much lower activity when compared with clarithromycin (MIC 0.6 μ M).

As the moderate activity against *H. pylori* of α -mangostin, two series of nitrogen analogues of α -mangostin were therefore synthesized to enhance their anti-*H. pylori* activity. The results showed that the alkylamino derivatives bearing 2-4 carbon linkers (**89**, **36**, and **37**) at C-3 and C-6 on the xanthone nucleus were more potent activity than that of the parent compound **8** and that of the longer alkyl chains derivatives. The ammonium iodide derivatives of alkylamino α -mangostins, **94-101**, exhibited the highly increasing anti-*H. pylori* activity with MIC ranges of 0.9-7.1 μ M. Compound with the highest anti-*H. pylori* potency (MIC 0.9 μ M) was 5-fold and 40-fold more active than that of the positive control drugs, amoxicillin and claritromycin, respectively.

การยับยั้งเชื้อ เฮลิโคแบคเตอร์ ไพโลรี ของแซนโทนจากพืช การ์ซิเนีย และ
สารอนุพันธ์



บทคัดย่อ
ของ
วันเฉลิม อุปไมย์

เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา
ตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาเคมี
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ได้นำสารแซนโทนจากต้นมะดันป่า (*Garcinia fusca*) มาทดสอบฤทธิ์การยับยั้งเชื้อแบคทีเรีย *Helicobacter pylori* จากผลการทดสอบพบว่า α -mangostin (8) และ β -mangostin (9) แสดงฤทธิ์ในการยับยั้งเชื้อ *H. pylori* สายพันธุ์ ATCC 43504 ในระดับปานกลาง มีค่า MIC เท่ากับ 38.0 และ 36.6 μ M ตามลำดับ cowaxanthone (14) (MIC 4.6 μ M) มีฤทธิ์ในการยับยั้งเชื้อ *H. pylori* สายพันธุ์ DMST 20165 ได้ดีที่สุด และดีกว่ายา metronidazole (MIC 11.1 μ M) และในสายพันธุ์ HP40 พบว่า isojacareubin (26) มีฤทธิ์ในการยับยั้งเชื้อสูงที่สุด โดยมีค่า MIC เท่ากับ 23.9 μ M ซึ่งดีกว่ายา amoxicillin (MIC 42.7 μ M) เกือบถึง 2 เท่า แต่ยังไม่ดีเทียบเท่า ยา claritromycin (MIC 0.6 μ M)

จากฤทธิ์ในการยับยั้งเชื้อ *H. pylori* ในระดับปานกลาง ของ α -mangostin ดังนั้นจึงได้ทำการสังเคราะห์ สารไนโตรเจนอนาลอก 2 กลุ่มของ α -mangostin เพื่อเพิ่มฤทธิ์ในการยับยั้งเชื้อ *H. pylori* สายพันธุ์ ATCC 43504 จากผลการทดสอบพบว่าอนุพันธ์อัลคิลอะมิโน ของ α -mangostin ที่ความยาวของสายโซ่คาร์บอน 2 ถึง 4 คาร์บอนที่ตำแหน่ง C-3 และ C-6 ในนิวเคลียสแซนโทน สามารถเพิ่มฤทธิ์ในการยับยั้ง *H. pylori* ได้ โดยมีค่า MIC ในช่วง 5.8-6.4 μ M เมื่อเปลี่ยนอนุพันธ์อัลคิลอะมิโน เป็นอนุพันธ์ของเกลือ α -mangostin แอมโมเนียมไอโอไดด์ พบว่าสารที่สังเคราะห์ได้แสดงฤทธิ์ที่สูงมาก ในช่วง MIC 0.9-7.1 μ M ซึ่งสารที่แสดงฤทธิ์ในการยับยั้งเชื้อได้ดีที่สุดนั้น (MIC 0.9 μ M) มีฤทธิ์ดีกว่ายา amoxicillin และ claritromycin ถึง 5 เท่า และ 40 เท่า ตามลำดับ

The thesis titled
“Anti-*Helicobacter pylori* of *Garcinia xanthones* and their derivatives.”
by
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has been approved by the Graduate School as partial fulfillment of the requirements for the
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CHAPTER 1

INTRODUCTION

1. *Helicobacter pylori*

Helicobacter pylori were isolated in 1983 by Warren and Marshal (Marshall and Warren 1984: 1311-5). *H. pylori* are the Gram-negative and microaerophilic bacteria, about 3 micrometres long with a diameter of about 0.5 micrometres, helix-shaped (classified as a curved rod, not spirochaete), and have 4-6 lophotrichous flagella (Figure 1). They produce oxidase, catalase, and urease and contain a hydrogenase (Mehmood; et al. 2010: 1337-51). Unlike other viruses and bacteria, *H. pylori* can colonize in the highly acidic environment of the stomach by metabolizing urea to ammonia via urease, which generates a neutral environment enveloping the bacteria (Wroblewski; et al. 2010: 713-39). Like other typical Gram-negative bacteria, the outer membrane of the bacteria consists of phospholipids and lipopolysaccharide. They can convert from spiral to coccoid form for their survival and be factors in the epidemiology of the bacteria. (Mehmood; et al. 2010: 1337-51).

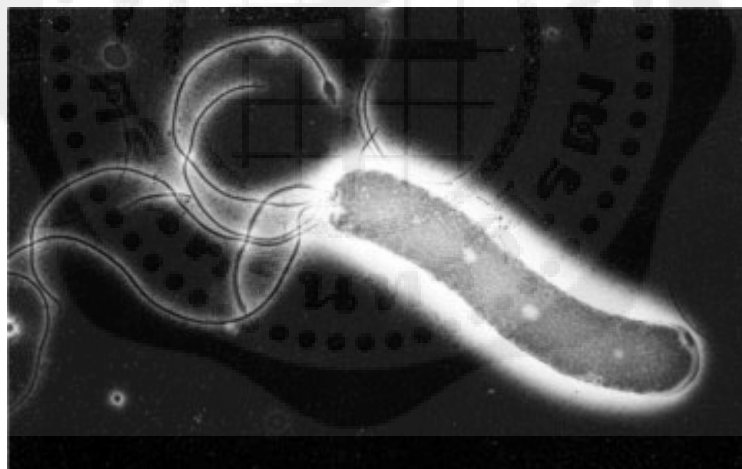


Figure 1 *H. pylori* (Manbir-online. 2014: Online)

Half of the world's population is infected by *H. pylori*. Transmission of *H. pylori* is person-to-person passage that could occur directly from the infected person or indirectly from contaminated water or food. This infection is causes of gastrointestinal inflammations including chronic gastritis, peptic ulcer disease, gastric cancer, and gastric MALT lymphoma (Go 2002: 3-15). Colonization of *H. pylori* contributes to the damage in the gastric epithelium cells because of their virulence factors. The infection by *H. pylori* requires a complex

interaction of both bacterial and host factors (Mehmood; et al. 2010: 1337-51). The virulence factors of *H. pylori* are cytotoxin associated protein (CagA), vacuolating cytotoxin (VacA), urease, lipopolysaccharide, peptidoglycan and several adhesins (Figure 2). Urease is an important enzyme of *H. pylori*, which hydrolyses urea into NH_3 and CO_2 , therefore the acidic environment in stomach is neutralized and the epithelium cells are damaged. This enzyme is an essential role of the *H. pylori* colonization in the stomach. Moreover, lipopolysaccharide, peptidoglycan and adhesions are also the colonization factor and proinflammatory response. CagA and VacA induce the inflammatory gastric epithelial cells (Morales-Guerrero; et al. 2012: 203-22). *H. pylori* damage the mucosal defense system by reducing the thickness of the mucus gel layer, decreasing mucosal blood flow, and interacting with the gastric epithelium throughout all stages of the infection. Inducing inflammation by the virulence factors of *H. pylori* can increase parietal-cell mass and, therefore, the capacity to secrete gastric acid is also increase. The chronic *H. pylori* infection is not only cause of gastric ulcer but also cause of duodenal ulcer by the acid and pepsin load, and gastric metaplasia in the duodenal cap (Yuan; et al. 2006: 80-9).

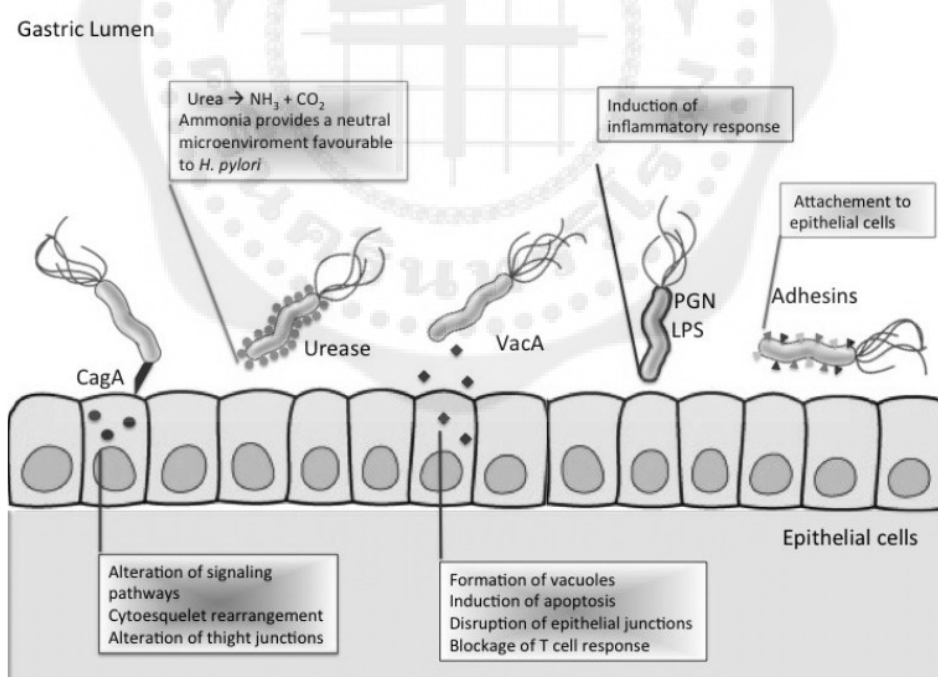


Figure 2 *H. pylori* virulence factors activities (Morales-Guerrero; et al. 2012: 203-22)

H. pylori led to inflammation, associated gastric acid secretion and evolved to gastric cancer. Eradication of *H. pylori* infection is now the mainstay of treatment for gastrointestinal ailments. Combination of antisecretory drugs or proton pump inhibitors (PPI) and antimicrobial agents is an excellent *H. pylori* elimination. The first-line treatment of *H. pylori* infection should be triple therapy with a PPI twice daily plus clarithromycin 500 mg twice daily and either amoxicillin 1,000 mg twice daily (PPI-CA) or metronidazole 500 mg twice daily (PPI-CM) for 7-14 days. In contrast, the poor compliance and bacterial resistance can lead to treatment failure. Moreover, metronidazole is resisted more than amoxicillin, therefore, that is a choice for determination of antibiotics used in the treatments (Yuan; et al. 2006: 80-9).

Described above, *H. pylori* are the major cause of gastritis, peptic ulcer disease and that can evolve to the gastric cancer. Eradication of *H. pylori* shows the great treatment for the infection. However, there are many reports about the drugs resistance of *H. pylori* that also effects on the patients by using more drugs and more times for the treatment. The poor compliance of the patients becomes the complicate treatments. Therefore, searching the new drugs for eradication of *H. pylori* is an important role of the therapy for these gastrointestinal ailments.

2. Natural occurring constituents for the treatment of *H. pylori* infection

In most developing countries, the plant-based medicines have been used throughout history for the treatment of gastrointestinal ailments. Using the treatments for eradication of *H. pylori* infection is very interesting. Recently, Lawal et al. reviewed some of the natural compounds that had the potent activity against *H. pylori* in the book of Bioactive natural products opportunities and challenges in medicinal chemistry (Lawal; et al. 2012: 475-97). Many plant extracts were reported for anti-*H. pylori* activity that well known as the ethnomedicinal plants for treatment of gastrointestinal patients. They classified the active natural compounds against *H. pylori* by chemical constituents, including terpenes, flavonoids, isothiocyanates, phenolics, stilbenes, alkaloids, quinolones, steroids and carotenoids. The examples from this review, a methanol extract of the dried powdered turmeric rhizome from *Curcuma longa* L. and their phenolic constituent, curcumin (**1**) (Figure 3), inhibited the growth of bacterial against 19 *H. pylori* strains with minimum inhibitory concentration (MIC) ranges of 6.25-50 $\mu\text{g/mL}$. Resveratrol (**2**), a phenolic compound in red wine, had an MIC of 25 $\mu\text{g/mL}$.

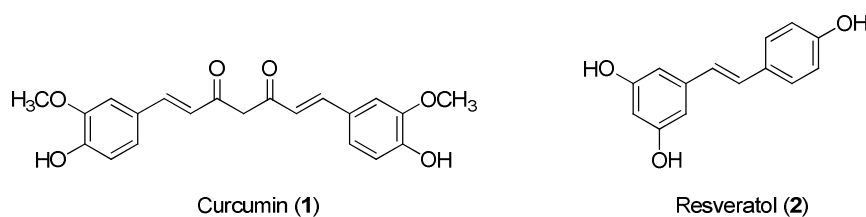


Figure 3 Structures of curcumin and resveratrol

against *H. pylori*, while the red wine extracts had MIC ranges of 25-50 $\mu\text{g/mL}$ (Mahady; et al. 2003: 1440-1). The methanol extracts of the two native American plants, *Sanguinaria canadensis* and *Hydrastis canadensis*, were active against *H. pylori*. Three alkaloids from *S. canadensis*, sanguinarine (3), chelerythrine (4) and protopine (5) (Figure 4) and two alkaloids from *H. canadensis*, berberine (6) and β -hydrastine (7) (Figure 4), were isolated from the active fractions that had MIC ranges of 12.5-100 $\mu\text{g/mL}$ (Mahady; et al. 2003: 217–21).

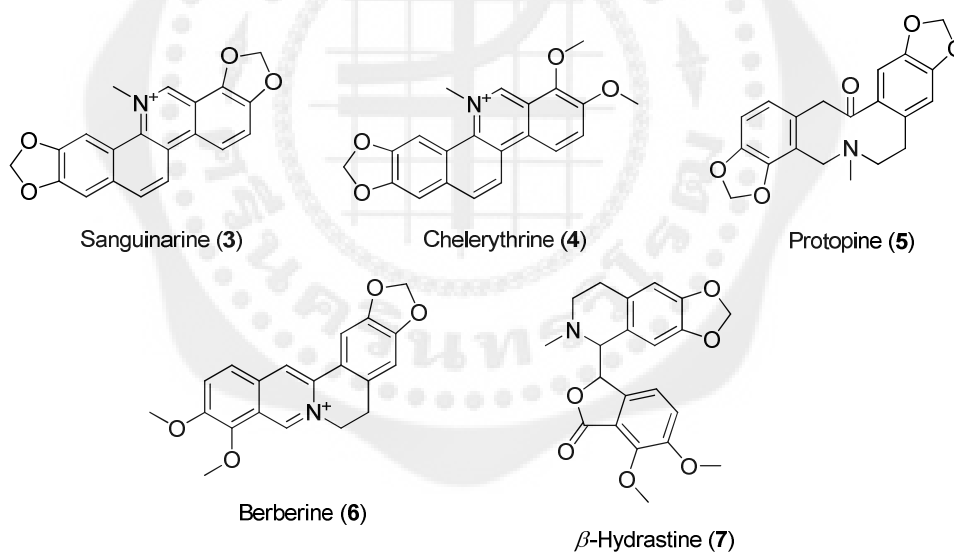


Figure 4 Structure of alkaloids with anti-*H. pylori* activity

Garcinia plants (Family: Clusiaceae) are the plant-based medicines of Southeast Asia. Some *Garcinia* plants are used for the treatment of diarrhea. In addition, the plants have a broad spectrum of biological profiles such as antioxidant, antifungal, antibacterial, anti-HIV, cytotoxic, antiinflammation, cancer preventive, and antihistamine activities (Obolskiy; et al. 2009: 1047-65). In 1996, Hasegawa et al. registered a patent in Japan on the antibacterial activity against *H. pylori* of xanthenes from *G. mangostana*. They found that

the isolated compounds demonstrated anti-*H. pylori* activities with the MIC in the ranges of 1.56-3.13 $\mu\text{g/mL}$ and α -mangostin (**8**) (Figure 5) exhibited the most potent activity (Hasegawa et al. 1996: JP8231896). Moreover, they synthesized the derivatives of mangostin which also showed anti-*H. pylori* activity (Hasegawa et al. 1996: JP8217672). Preliminary studies of our research group, α -mangostin was evaluated for its anti-*H. pylori* activity and found that it exhibited moderate activity (19.0-38.0 μM). The result was contrast with the reported value (1.56 $\mu\text{g/mL}$ or 3.8 μM) (Hasegawa et al. 1996: JP8231896). For the higher activity against *H. pylori*, therefore, we have been searched for natural and some synthetic xanthenes.

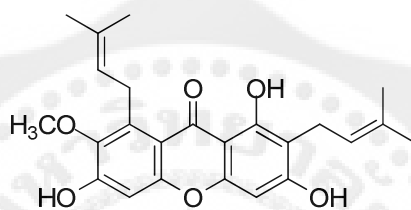


Figure 5 Structure of α -mangostin (**8**)

3. Objectives of the research

Due to the *Garcinia* plants display a broad spectrum of biological profiles, especially antibacterial activity, therefore, the aims of this study were

- To evaluate the in hand natural oxygenated xanthenes for antibacterial activity against *H. pylori*.
- To chemical modify of α -mangostin and to evaluate the anti-*H. pylori* activity of the products obtained.

CHAPTER 2

REVIEW OF THE LITERATURE

1. *Garcinia* plants

Garcinia plants (Family: Clusiaceae) have been used as Thai traditional medicine. For example, the leaves, fruits and roots from *G. cowa* and *G. schomburgkiana* have been used for aperient, expectorant, febrifuge and the treatment of postpartum uterine bleeding and uterine inflammation. The fruit juice from *G. dulcis* has been used for expectorant and scurvy and their roots has been used as antiaphthous. The powder of dried fruit hull of *G. mangostana* have been use for the treatment of skin infections, healing wounds, suppurations and chronic ulcers, treatments of dysentery and the reliefs of diarrhea. The latex of *G. hanburyi* has been used for laxative and cerebral hemorrhage. (วุฒิชัย วุฒิธรรมเวช และคณะ. 2542: 475, 578, 582, 590, 598). Moreover, the crude extracts of some *Garcinia* plants are ingredient of the commodity in Thai market place, i.e. dietary supplement products, beauty treatment products, soaps, dentifrices, juices, etc.

Mangosteen (*Garcinia mangostana* L.) is a plant in this genus. It is interesting because it is one of the best tasting tropical fruits that become to praise as “the queen of fruits”. For hundred years, the parts of *G. mangostana* have been used as medicines for many treatments in Southeast Asia. In India, Thailand, China and other Asian countries, the dried and powdered fruit hull are used as antimicrobial agents and for the antiparasitic treatments in dysentery as well as externally for healing wounds, suppurations and chronic ulcers. *G. mangostana* has been shown to contain high amount of xanthenes, a class of polyphenolic compounds, which were shown to have broad spectrum of biological profiles such as antioxidant, antiinflammatory, hepatoprotective, cancer preventive, antimalarial and antibacterial activities, based on their diverse structures (Obolskiy; et al. 2009: 1047-65). α -, β - and γ -mangostins (**8-10**) are three common xanthenes of *G. mangostana* and other *Garcinia* plants (Figure 6).

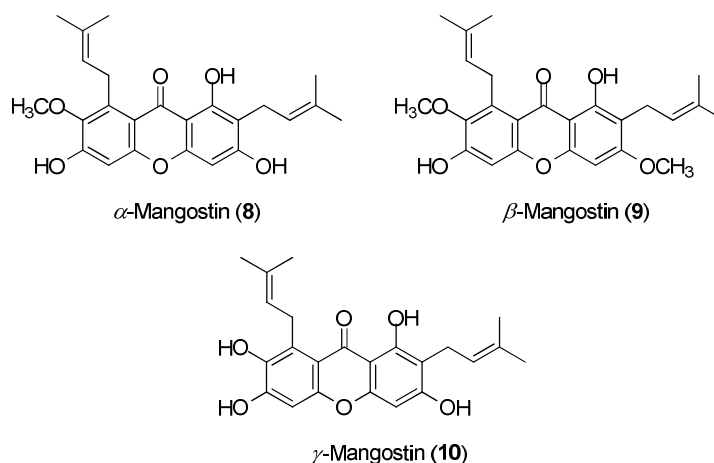


Figure 6 Structure of the major prenylated xanthenes of *G. mangostana*

Our group research has been interested in searching for bioactive phytochemicals from *Garcinia* plants including *G. fusca* Pierre or “Madan-paa” in Thai. *G. fusca* is a native endemic tree grown in the Northeastern of Thailand and many Asian countries. Its edible acidic fruit and leaf are commonly used as ingredient in traditional food. *G. fusca* is also used as a spice in India and as flavoring in soup in Malaysia (Bioversityinternational. 2014: Online). The root, stem, leaf and fruit are folk medicine for treatment of coughs and indigestion, improvement of blood circulation, expectorant, laxative, and the relief of fever (วุฒิ วุฒิธรรมเวช และคณะ. 2542: 578). In 2003, Ito et al. isolated some important xanthenes from the stem bark of *G. fusca*. They are eight known xanthenes, α -mangostin (8), β -mangostin (9), cowanin (11), cowanol (12), norcowanin (13), cowaxanthone (14), rubraxanthone (15), and 7-O-methylgarcinone E (16), and eight new xanthenes of fuscaxanthenes A - H (17 - 24) (Ito; et al. 2003: 200-5), the structures of which are shown in Figure 7. Recently, Nontakham and Suksamrarn isolated nine known xanthenes (8, 9, 11, 12, 14, 15, 23, 25 and 26), two known biflavonoids (27 and 28) and one sterol (29) from the root of *G. fusca* (Figure 7). Xanthenes 25 and 26, in addition to the two known biflavonoids, morelloflavone (27) and vokensiflavone (28), were reported for the first time from this plant. Moreover, the chemical constituents obtained from the fresh green fruit of *G. fusca* were also studied which led to the isolation of six known xanthenes 8, 9, 11, 12, 14 and 17 (Nontakham. 2011: Dissertation).

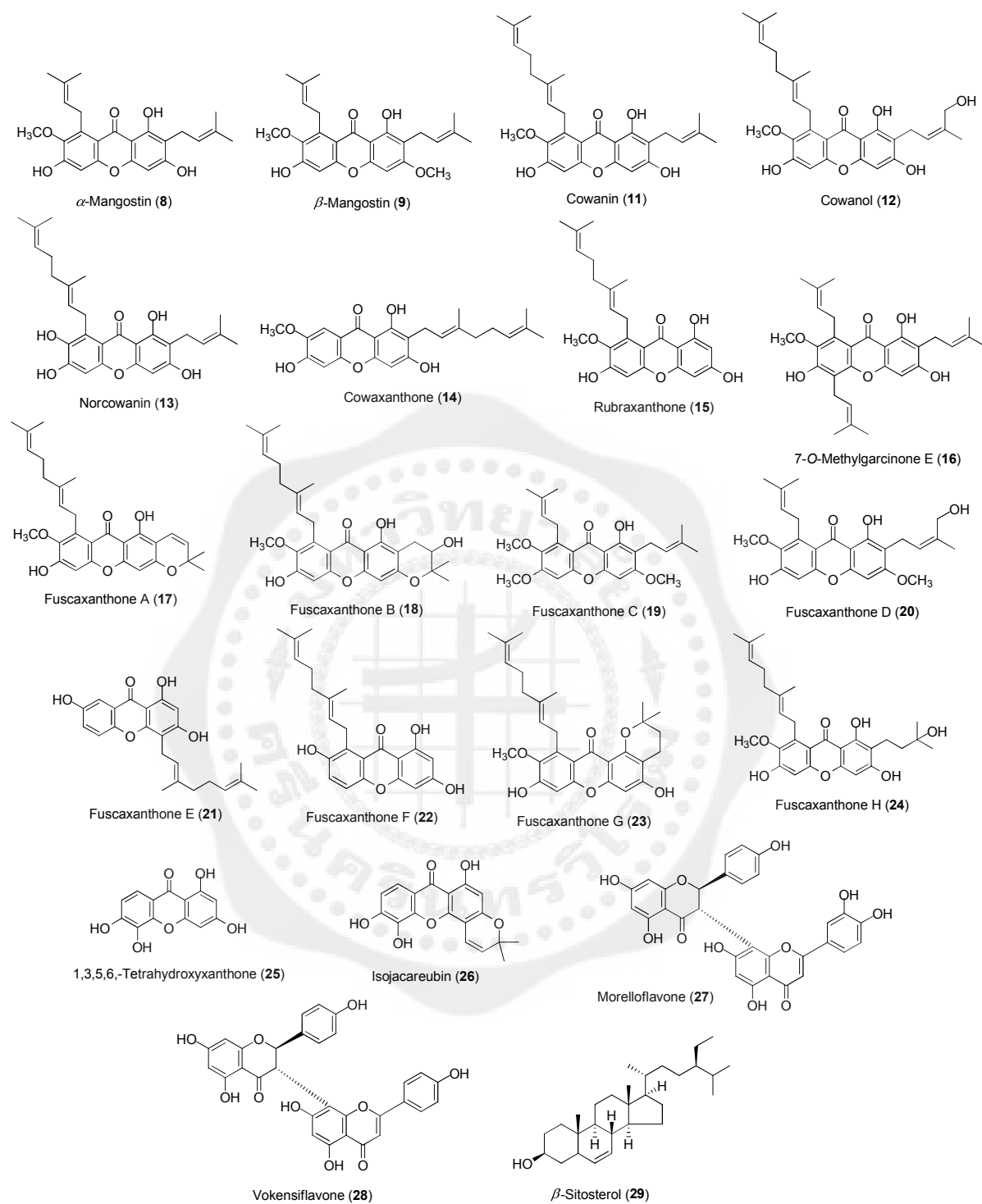


Figure 7 Chemical structures of isolated compound from *G. fusca*

2. Biological activities of the oxygenated xanthenes from *Garcinia fusca*

In 2003, Ito et al. isolated the oxygenated xanthenes from *G. Fusca*. Eight xanthenes, **8**, **9** and **11** - **16**, were evaluated for their tumor-promoting inhibitory activity against Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in Raji cells. They found that compound **16**, having three prenyl groups at C-2, C-5, and C-8 of the xanthone nucleus, exhibited the most potent inhibitory activity with the half maximal inhibitory concentration (IC_{50}) value at 210 mol ratio/32 μ mol TPA. The prenylated xanthenes, α - and β -mangostins (**8** and **9**), showed significant inhibitory activity (IC_{50} 270 and 220 mol ratio/32 μ mol TPA, respectively). Other compounds demonstrated weaker inhibitory activity, however, the tested compounds were lower than β -carotene, a vitamin A precursor commonly used in cancer prevention studies (Table 1). The authors gave an opinion about structure-activity relationship, that the relative location of a hydroxyl group and a hydrophobic prenyl moiety on the xanthone nucleus might be important factors in producing the observed chemopreventive effect against chemical induced carcinogenesis (Ito; et al. 2002: 200-5).

Table 1 Inhibitory effects of xanthenes on TPA-Induced EBV-EA activation

Compounds	IC_{50}^a (mol ratio/32 μ mol TPA)	Compounds	IC_{50}^a (mol ratio/32 μ mol TPA)
α -mangostin (8)	220	norcowanin (13)	315
β -mangostin (9)	270	cowaxanthone (14)	398
cowanin (11)	320	rubraxanthone (15)	340
cowanol (12)	310	7-O-methylgarcinone E (16)	210
β -carotene ^b	400		

a) IC_{50} represents the mol ratio to TPA that inhibits 50% of positive control (100%) activated with 32 μ mol of TPA. b) Positive control substance.

3. Biological activities of mangostin analogues on antimicrobial activities

3.1 *Mycobacterium tuberculosis*

In 2003, Suksamrarn et al. reported their preliminary study on antituberculosis activity of prenylated xanthenes from mangosteen pericarp. Compounds **8**, **9** and garcinone B (**30**) (Figure 8) exhibited strong potent inhibitory effect against *M. tuberculosis* H₃₇Ra, with MIC of 6.25 $\mu\text{g/mL}$ (Suksamrarn; et al. 2003: 857-9). Subsequently, they prepared a new series of mangostin analogs. These compounds were synthesized from the lead natural xanthone of mangosteen, α -mangostin (**8**), which contain alkyl ether and ester analogs and evaluated antituberculosis activity. The result showed that the monoalkyl analogs of tetrahydro α -mangostin (**31**) displayed increased antimycobacterial activity against *M. tuberculosis* strain H₃₇Ra as compared with the parent compound. Among tested compounds, 6-O-methyl tetrahydro α -mangostin (**32**), 6-O-ethyl tetrahydro α -mangostin (**33**), 6-O-allyl tetrahydro α -mangostin (**34**) and 6-O-propyl tetrahydro α -mangostin (**35**) (Figure 8) exhibited the most potent antimycobacterial activity, the MICs were shown in Table 2. Moreover, these active compounds were also active against virulent *M. tuberculosis* H₃₇Rv and ten multidrug-resistant (MDR) clinical strains with MICs of 0.78–1.56 $\mu\text{g/mL}$ (Table 2). The analog **32** still exhibited the most activity against MDR *M. tuberculosis* (Sudta; et al. 2013: 194-203).

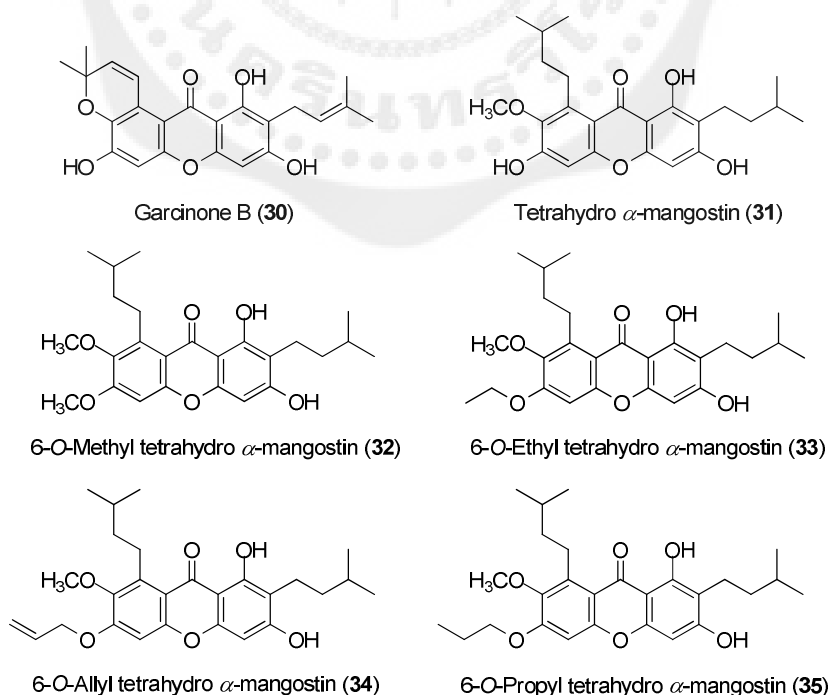


Figure 8 The active compounds against *Mycobacterium tuberculosis*

Table 2 Antimycobacterial activities against *Mycobacterium tuberculosis* H₃₇Ra, virulent and MDR strains of α -mangostin analogs.

<i>M. tuberculosis</i> strains	MIC (μ g/mL)					
	8	31	32	33	34	35
H ₃₇ Ra	6.25	1.56	0.78	1.56	0.78	0.78
H ₃₇ Rv	-	1.56	0.78	1.56	3.12	3.12
M3 ^a	-	1.56	0.78	1.56	3.12	3.12
M4 ^b	-	1.56	1.56	1.56	3.12	3.12
M5 ^a	-	1.56	1.56	1.56	3.12	3.12
M6 ^c	-	1.56	1.56	1.56	3.12	3.12
M8 ^b	-	3.12	0.78	3.12	1.56	3.12
M11 ^c	-	1.56	3.12	1.56	3.12	6.25
M16 ^d	-	3.12	3.12	3.12	3.12	6.25
M21 ^b	-	3.12	1.56	3.12	3.12	6.25
M22 ^c	-	3.12	1.56	3.12	3.12	6.25
M27 ^c	-	3.12	1.56	1.56	3.12	3.12

Drugs: Isoniazid (INH), Rifampin (RMP), Ethambutol (EMB), Streptomycin (SM). a) resisted INH, RMP and SM, b) resisted INH, RMP, EMB and SM, c) resisted INH and RMP, d) resisted INH, RMP and EMB.

3.2 Methicillin resistant *Staphylococcus aureus*

In 1996, linuma et al. studied the antibacterial against methicillin-resistant *S. aureus* (MRSA) of several xanthenes from mangosteen pericarp. α -Mangostin (**8**) exhibited anti-MRSA activity within the MIC in the ranges of 1.57-12.5 μ g/mL, which was more active compound than positive control, vancomycin (MIC = 3.13-6.25 μ g/mL). Moreover, **8** in the presence of vancomycin exhibited increasing anti-MRSA activity that was not found in the case of using other tested xanthenes (linuma; et al. 1996: 861-5). In 2005, Sakagami et al. investigated the antibacterial activity of compound **8** against vancomycin resistant enterococci (VRE) and MRSA. α -Mangostin was found to be active against nine strains of MRSA and five strains of VRE with MIC values of 6.25-12.5 μ g/mL and 6.25 μ g/mL, respectively. Moreover, the study of the synergistic effect between compound **8** and commercially available antibiotics against five strains of VRE and three strains of

vancomycin-sensitive enterococci (VSE), that resulted partial synergisms (Sakagami; et al. 2005: 203-208).

Recently, Koh et al. investigated the antibacterial properties of α -mangostin against Gram-positive bacteria. Among isolated compounds from the fruit hull of mangsteen, α -mangostin was identified as the most potent against Gram-positive bacteria (MIC = 0.78–1.56 $\mu\text{g/mL}$) which included two MRSA isolates. Compound **8** also exhibited rapid *in vitro* bactericidal activity (3-log reduction within 5 min). In a multistep (20 passage) resistance selection study using a MRSA isolated from the eye, they found that no resistance against α -mangostin. In biophysical studies showed that α -mangostin rapidly disrupted the integrity of the cytoplasmic membrane leading to loss of intracellular components (Figure 9) (Koh; et al. 2013: 834-44).

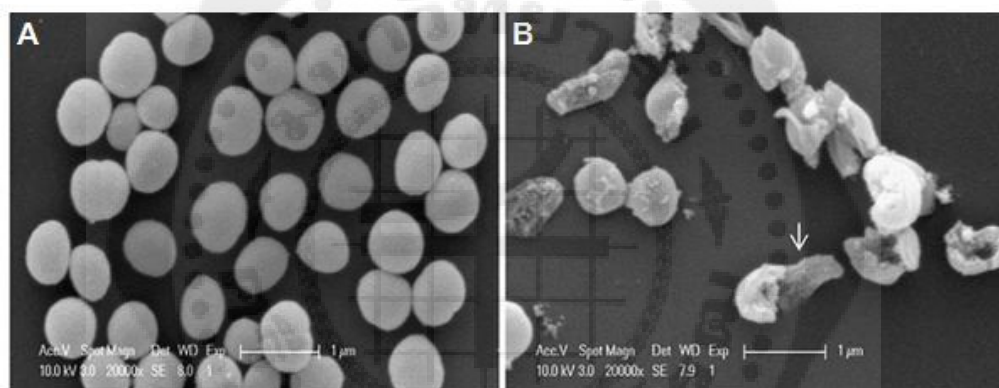


Figure 9 Scanning electron microscopy at magnifications 20,000 $\times$ showed that α -mangostin induced cell lysis and membrane disruption. (A): control without treatment. (B): *S. aureus* incubated with 10 $\mu\text{g/mL}$ α -mangostin after 30 min. White arrow indicated leakage of intracellular components

The same research group designed the new derivatives of α -mangostin for bacterial membrane targeting. They modified the free C3 and C6 hydroxyl groups of α -mangostin by alkylamino groups, the synthesis were shown in Figure 10. Compounds **36-41** exhibited excellent antimicrobial activity against clinical isolates MRSA (DM 21455) and *S. aureus* (ATCC 29213) with MIC of 0.39-1.56 $\mu\text{g/mL}$ (Table 3). In contrast, compounds **42-44** were not active against MRSA or *S. aureus* at high concentration (>200 $\mu\text{g/mL}$, Table 3). A rabbit red blood cell hemolysis assay was used as an indicator of mammalian cell selectivity. Of

the compounds synthesized, compound **37** exhibited the best selectivity with selectivity index (HC_{50}/MIC) of 50.3.

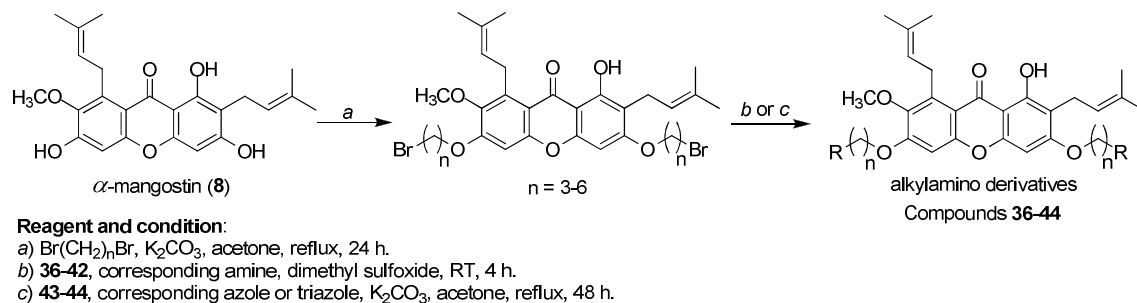


Figure 10 The synthesis of alkylamino derivatives of α -mangostin.

Table 3 Antibacterial activity (MIC), hemolytic properties (HC), and selectivity of α -mangostin derivatives.

compound	n	R	MIC ($\mu g/mL$)		HC_{50} ($\mu g/mL$)	Selectivity Index (HC_{50}/MIC)	
			MRSA	<i>S. aureus</i>		MRSA	<i>S. aureus</i>
8	-	H	1.56	1.56	9	5.77	5.77
36	3		0.39	0.78	16.3 ± 2	41.7	20.9
37	4		0.39	0.39	19.6 ± 3	50.3	50.3
38	5		0.78	1.56	14.0 ± 2	18.0	9.0
39	6		1.56	1.56	26.5 ± 2	17.0	17.0
40	4		1.56	1.56	25.0 ± 3	16.0	16.0
41	4		1.56	0.78	19.3 ± 3	12.3	24.7
42	4		>200	>200	>200	-	-
43	4		>200	>200	>200	-	-
44	4		>200	>200	>200	-	-

Compound **37** was screened for antibacterial activities against Gram-positive bacteria, four ATCC strains of *S. aureus*, four clinical isolates of MRSA, one ATCC strain of MRSA, one vancomycin intermediate-resistant *S. aureus* (VISA), two *Streptococcus faecium* ATCC strains and one *Enterococcus faecalis* ATCC strain, and Gram-negative bacteria, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia* ATCC 10031 (Table 4). The result showed that compound **37** exhibited excellent antimicrobial properties against all tested Gram-positive bacteria with the MIC in the ranges of 0.09-1.56 $\mu\text{g/mL}$. In contrast, compound **37** had moderate antimicrobial activity against two strains of Gram-negative bacteria. Compound **37** killed bacteria rapidly, showed no evidence of resistance or cross-resistance in laboratory assays and also damaged the bacterial membrane leading to loss of intracellular components same as that of **8** (Zou; et al. 2013: 2359-73).

Table 4 MIC values of compound **37** against Gram-positive bacteria and Gram-negative bacteria

Bacterial strains	MIC ($\mu\text{g/mL}$)
Gram-positive bacteria	
<i>Staphylococcus aureus</i> ATCC 29213	0.39
<i>Staphylococcus aureus</i> ATCC 6538	1.56
<i>Staphylococcus aureus</i> ATCC 6538P	0.78
<i>Staphylococcus aureus</i> ATCC 29737	1.56
MRSA DM 09808R; source: eye	1.56
MRSA DM 21595; source: wound	0.39
MRSA DR 42412; source: sputum	0.39
MRSA DR 68004; source: blood	1.56
MRSA ATCC 700669	0.09
VISA 10:DB 6506	0.78
<i>Streptococcus faecium</i> ATCC 10541	0.19
<i>Streptococcus epidermidis</i> ATCC 12228	0.78
<i>Enterococcus faecalis</i> ATCC 29212	0.78
Gram-negative bacteria	
<i>Pseudomonas aeruginosa</i> ATCC 27853	50
<i>Klebsiella pneumoniae</i> ATCC 10031	40

3.3 *Helicobacter pylori*

In 1996, Hasegawa et al. registered two Japanese patents on the antibacterial activity against *H. pylori* of xanthenes from *G. mangostana*. They found that the isolated compounds demonstrated anti-*H. pylori* activity with the MIC in the ranges of 1.56-3.13 $\mu\text{g/mL}$. Among compounds, α -mangostin (**8**) exhibited the most potent activity (Hasegawa et al. 1996: JP8231896). Furthermore, a series of mangostin derivatives such as alkyl, acyl and their pyrano compounds, were synthesized by the same group. 1-Isomangostin (**45**), 3,6-O-dimethyl 1-isomangostin (**46**) and 3,6-O-dimethylcarboxy α -mangostin (**47**) (Figure 11) also showed the anti-*H. pylori* activity with MIC of 3.13 $\mu\text{g/mL}$ (Hasegawa et al. 1996: JP8217672).

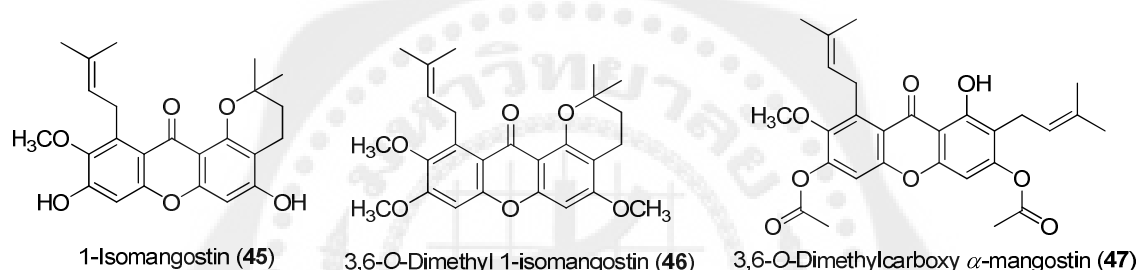


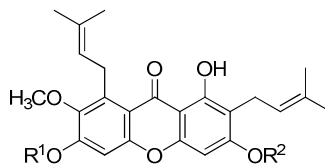
Figure 11 Chemical structures of mangostin derivatives (**45-47**)

Recently, Sidahmed et al. studied the anti-ulcerogenic properties of α -mangostin (**8**) from *Cratoxylum arborescens* (Vahl) Blume. α -Mangostin was evaluated the antibacterial activities against two *H. pylori* strains, the result showed that compound **8** represents MIC of 24 $\mu\text{g/mL}$ against *H. pylori* (ATCC 43504) and 124 $\mu\text{g/mL}$ against *H. pylori* (ATCC 700824). Moreover, they showed the antiulcerogenic mechanism study in a rat model of ulcer. Rats were orally pretreated with α -mangostin and subsequently exposed to acute gastric lesions induced by ethanol. The result reported that compound **8** demonstrated no toxic parameters in comparison to the control group. The compound (doses of 10 and 30 mg/kg) inhibited significantly ethanol induced gastric lesions by 66.04% and 74.39 %, respectively. Moreover, α -mangostin induces the expression of Hsp70, restores GSH levels, decreases lipid peroxidation, and inhibits COX-2 activity. (Sidahmed; et al. 2013: doi: 10.1155/2013/450840).

3.4 *Plasmodium falciparum*

In 2006, Mahabusarakam et al. synthesized a series of α -mangostin (**8**) derivatives for antiparasmodial activity against *Plasmodium falciparum* strain k1 (chloroquine- and pyrimethamine-resistant). The mangostin analogues were produced by acetylation and alkylation reaction. These compounds included one ether derivative (**48**), one ester derivative (**49**), four alcohol derivatives (**50-53**), each two derivatives of amide (**54** and **55**), epoxide (**56** and **57**) and nitrile (**58** and **59**), nine amine derivatives (**60-68**), and five ammonium salt derivatives (**69-73**) (Table 5). Which were evaluated the antiparasmodial activity, the IC_{50} are showed in Table 5. The results showed that among them, the amine derivatives (**60-68**) of α -mangostin were the greatest antiparasmodial activity. In contrast, another compounds exhibited moderate to weak activities. Increasing solubility of amine with ammonium salt formation (**69-73**) did not significantly enhance antiparasmodial activity (Mahabusarakam; et al. 2006: 912-6).

Table 5 Antiplasmodial activity of α -mangostin and its derivatives against *P. falciparum* strain k1.



Compound	Substituent		IC ₅₀ (μ M), Mean $\pm$ SD
	R ¹	R ²	
8	H	H	17.00 $\pm$ 1.00
48	CH ₃	CH ₃	> 20.00
49	H		> 20.00
50		H	5.30 $\pm$ 0.30
51	H		7.40 $\pm$ 0.30
52		H	16.00 $\pm$ 1.00
53			> 18.00
54	H		4.50 $\pm$ 0.30
55		H	8.3 $\pm$ 0.4
56			6.10 $\pm$ 0.30
57		H	13.00 $\pm$ 1.00
58			18.00 $\pm$ 1.00
59		H	21.00 $\pm$ 2.00
60		H	0.30 $\pm$ 0.02

Table 5 (Continued)

Compound	Substituent		IC ₅₀ (μM), Mean ± SD
	R ¹	R ²	
61			1.50 ± 0.10
62		H	0.60 ± 0.03
63		H	0.10 ± 0.01
64		H	0.05 ± 0.005
65		H	0.60 ± 0.03
66			0.60 ± 0.03
67		H	0.60 ± 0.03
68			0.70 ± 0.03
69		H	0.75 ± 0.03
70			0.50 ± 0.02
71		H	2.5 ± 0.1
72		H	0.75 ± 0.03
73		H	4.8 ± 0.3

CHAPTER 3

EXPERIMENTAL

1. Chemistry

1.1 Materials and methods

1.1.1 Solvents and chemicals

The commercial grade solvents were distilled before used. All of organic solvents for putting reaction were analytical grad. Chemicals reagents were analytical grad which were purchased from Sigma Chemical Co., Aldrich Chemical Co., Fluka Chemie AG, and Acros Chemical Co and were used without further purification.

1.1.2 Chromatographic technique

Flash column chromatographic separations were carried out using silica gel (SiliCycle, SILIAFLASH G60, 70-230 mesh). Purity of the samples was checked on precoated TLC plates (silica gel).

1.1.2.1 Column chromatography

Column chromatography was performed on a glass column packed with silica gel (SiliCycle, SILIAFLASH G60, 70-230 mesh) or aluminium oxide 90 active neutral (E. Merck, 70-230 mesh). The extracts were chromatographed after being absorbed onto a small amount of the packing material or applied to the top of the column by a pasture pipette.

1.1.2.2 Centrifugal thin-layer chromatography (Chromatotron)

Centrifugal thin-layer chromatography or chromatotron was performed in a thin layer of sorbent (silica gel, E. Merck, type 60, containing gypsum) on a glass rotor. The motor drives the rotor at a constant speed by a shaft passing through a hold in the center of the main vessel. Solutions of sample are delivered to the rotor via the inlet. Concentric bands of separated substance are eluted by solvents that leave the edge of the rotor together with solvent. Separated bands are detected by UV light

1.1.2.2 Detection of compounds on TLC technique

Chromatography was performed on precoated silica gel GF254 preparative TLC plates (20 x 20, 0.5 mm thick, E. Merck). TLC plates were viewed under UV light at 254 nm for fluorescence quenching spots and at 356 nm for fluorescent spots. The TLC plates were stained with an anisaldehyde solution mixed with 2.5% anisaldehyde, 3.4% sulphuric acid, 1.0% glacial acetic acid and absolute ethanol. The plates were

subsequently heated with a hot plate at 100-120 °C for 1-2 minutes in order to ensure the optimum development of color.

1.1.3 Physical constants

All melting points were determined in glass capillaries using a Stuart melting point apparatus and are uncorrected.

1.1.4 Spectroscopic techniques

1.1.4.1 Infrared spectrometry

Infrared (IR) spectra were taken as KBr pellets and/or as a solution in dichloromethane on a Perkin Elmer FT-IR spectrum BX.

1.1.4.2 Nuclear magnetic resonance spectrometry (NMR)

The ^1H -NMR spectra, ^{13}C -NMR (75 MHz) and 2D-NMR Spectra were recorded on Bruker Avance 300 FT-NMR spectrometer. Samples were run either in deuteriochloroform (CDCl_3), deuteriomethanol (CD_3OD) or acetone- d_6 . The chemical shifts (δ) were in ppm. The coupling constants were measured in Hz and the following abbreviations are adopted: s (singlet); d (doublet); t (triplet); m (multiplet); dd (doublet of doublets); dt (doublet of triplets). The complete assignment of all proton and carbon signals was completed via 1D proton and carbon, distortionless enhancement by polarization transfer (DEPT), correlation spectroscopy (COSY), heteronuclear multiple quantum correlation experiment (HMQC), heteronuclear multiple bond coherence (HMBC) NMR experiments and nuclear Overhauser effect spectroscopy (NOESY).

1.1.4.3 Mass spectrometry

Electrospray ionization (ESI) mass spectra (ESI MS) were obtained with a Thermo Finnigan LC-Q mass spectrometer (Department of chemistry, Faculty of Science, Rhamkhamhaeng University).

1.1.5 Other techniques

1.1.5.1 Hydrogenation reactor

Catalytic hydrogenation reaction was performed by Parr Shaker Hydrogenator (Parr Instrument Company). Parr hydrogenator are sealed in a reaction bottle with a catalyst and connected to a hydrogen reservoir. Air is removed either by evacuating the bottle or by flushing with hydrogen. Pressure is then applied from the reservoir and the bottle is shaken vigorously to initiate the reaction. After the reaction reaches the desired point, the shaker is stopped, the bottle vented and the product and catalyst are recovered.

1.2 Isolation of oxygenated xanthenes from *Garcinia fusca*

Natural oxygenated xanthenes (**8**, **9**, **11**, **12**, **14**, **17**, **23**, **25**, **26** and **74**) were isolated from the roots of *G. fusca* by Miss Jannarin Nontakham (Nontakham. 2011: Dissertation). These oxygenated xanthenes were screened for antibacterial activity against three strains of *Helicobacter pylori*.

1.3 Chemical modification of α -mangostin

1.3.1 General synthesis of 3,6-di-O-bromoalkyl α -mangostin derivatives (**79-86**)

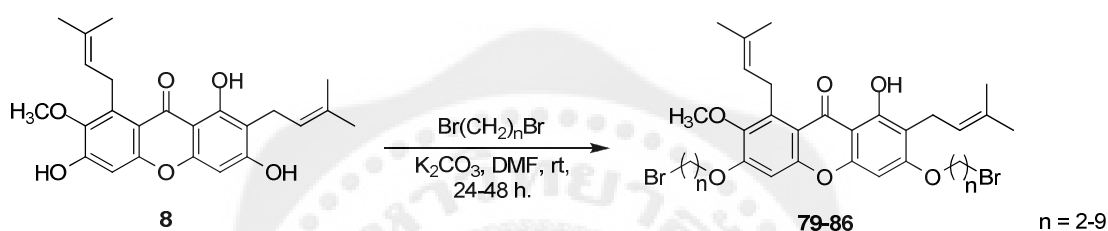


Figure 12 Synthesis of 3,6-di-O-bromoalkyl α -mangostin derivatives (**79-86**)

The starting compound, α -mangostin (**8**), was isolated from the dried fruit hull of *G. mangostana* as previously described (Suksamrarn et al 2003). Thus the EtOAc extract of the fruit hull (70.0 g) was isolated by quick column chromatography. The eluting solvent system was gradually increased as follows: hexane, hexane-acetone, acetone and MeOH. Each 100 mL-fraction was collected and was spotted on TLC plate (using 30% acetone-hexane as solvent system) and viewed under UV light followed by with anisaldehyde reagent. These fractions were appropriately combined. The pure compound **8** (17.6 g, 25% based on crude extract) was obtained by crystallization from 50% CH_2Cl_2 -hexane of the combined fractions with major α -mangostin content.

α -Mangostin (**8**) (1 eq.) and K_2CO_3 (2.5eq.) were dissolved in DMF (3-5 mL) and then individual dibromoalkane (2.5 eq.) was added. The mixture was stirred at room temperature for 24-48 h. After the reaction complete, the mixture was extracted with CH_2Cl_2 (3x10 mL). The organic phase was washed with water (3x10 mL) and dried over anhydrous Na_2SO_4 . Removal of solvent, the residue was purified over column chromatography (silica gel, using hexane to 90% CH_2Cl_2 -hexane as eluting system) to afford the dibromoalkyl derivatives **79-86**, in 13-60 % yield.

3,6-Di-O-(2-bromoethyl) α -mangostin (**79**) (sss5455)

Yellow solid, 10.6 mg, 27 % yield, m.p. 139-141°C

R_f: 0.42 (30% acetone-hexane)

IR (KBr) ν_{max} : 2960, 2926, 1644, 1598, 1466, 1430, 1374, 1273, 1205, 1177, 1121, 829, 796, 784, 574 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.45 (s, 1H, 1-OH), 6.67 (s, 1H, H-5), 6.24 (s, 1H, H-4), 5.22 (br. t, 2H, J=6.4 Hz, H-17, H-12), 4.39 (t, 2H, J=5.8 Hz, H-1"), 4.35 (t, 2H, J=6.1 Hz, H-1'), 4.11 (d, 2H, J=6.4 Hz, H-16), 3.84 (s, 3H, 7-OCH₃), 3.74 (t, 2H, J=5.8 Hz, H-2"), 3.68 (t, 2H, J=6.1 Hz, H-2'), 3.36 (d, 2H, J=7.1 Hz, H-11), 1.83 (s, 3H, H-20), 1.79 (s, 3H, H-15), 1.66 (s, 6H, H-14, H-19)

3,6-Di-O-(3-bromopropyl) α -mangostin (**80**) (sss4991)

Yellow solid, 260.8 mg, 34 % yield, m.p. 132-135°C

R_f: 0.51 (30% acetone-hexane)

IR (KBr) ν_{max} : 2917, 1649, 1598, 1467, 1426, 1374, 1278, 1204, 1175, 1113, 826, 802, 776, 583 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.47 (s, 1H, 1-OH), 6.74 (s, 1H, H-5), 6.30 (s, 1H, H-4), 5.22 (br. t, 2H, H-17, H-12), 4.22 (t, 2H, J=5.9 Hz, H-1"), 4.71 (t, 2H, J=5.8 Hz, H-1'), 4.10 (d, 2H, J=6.5 Hz, H-16), 3.77 (s, 3H, 7-OCH₃), 3.64 (t, 2H, J=6.2 Hz, H-3"), 3.61 (t, 2H, J=6.3 Hz, H-3'), 3.33 (d, 2H, J=6.9 Hz, H-11), 2.41 (q, 2H, J=6.0 Hz, H-2"), 2.36 (q, 2H, J=6.0 Hz, H-2'), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.66 (s, 6H, H-19, H-14)

3,6-Di-O-(4-bromobutyl) α -mangostin (**81**) (sss5009)

Yellow solid, 505 mg, 60 % yield, m.p. 95-98°C

R_f: 0.64 (30% acetone-hexane)

IR (KBr) ν_{max} : 2924, 1644, 1598, 1469, 1376, 1280, 1206, 1113, 856, 821, 787, 581 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.47 (s, 1H, 1-OH), 6.70 (s, 1H, H-5), 6.27 (s, 1H, H-4), 5.20 (br. t, 2H, J=6.5 Hz, H-17, H-12), 4.11 (d, 2H, J=5.7 Hz, H-16), 4.08 (t, 2H, J=6.3 Hz, H-1"), 4.06 (t, 2H, J=6.0 Hz, H-1'), 3.78 (s, 3H, OCH₃), 3.50 (t, 2H, J=6.1 Hz, H-4"), 3.48 (t, 2H, J=6.5 Hz, H-4'), 3.34 (d, 2H, J=6.7 Hz, H-11), 2.04 (m, 8H, H-2', H-2", H-3', H-3"), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.66 (s, 6H, H-19, H-14)

3,6-Di-O-(5-bromopentyl) α -mangostin (**82**) (sss5400)

Yellow solid, 296 mg, 34 % yield, m.p. 72-75°C

R_f: 0.59 (30% acetone-hexane)

IR (KBr) ν_{max} : 2954, 1708, 1601, 1465, 1395, 1286, 1202, 1147, 818, 787, 708 cm⁻¹

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.47 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.26 (s, 1H, H-4), 5.21 (br. t, 2H, $J=6.2$ Hz, H-17, H-12), 4.11 (d, 2H, $J=6.9$ Hz, H-16), 4.07 (t, 2H, $J=6.4$ Hz, H-1"), 4.03 (t, 2H, $J=6.2$ Hz, H-1'), 3.78 (s, 3H, OCH_3), 3.44 (t, 2H, $J=6.6$ Hz, H-5"), 3.43 (t, 2H, $J=6.6$ Hz, H-5'), 3.34 (d, 2H, $J=6.9$ Hz, H-11), 1.91 (m, 8H, H-2', H-2'', H-4', H-4''), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.68 (m, 4H, H-3', H-3''), 1.65 (s, 6H, H-19, H-14)

3,6-Di-O-(6-bromohexyl) α -mangostin (**83**) (sss5602)

Yellow sticky solid, 97 mg, 51 % yield

R_f : 0.70 (30% acetone-hexane)

IR (KBr) ν_{max} : 2936, 2854, 1646, 1597, 1463, 1374, 1278, 1203, 1115, 824, 794, 583 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.47 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.26 (s, 1H, H-4), 5.22 (br. t, 2H, $J=6.2$ Hz, H-17, H-12), 4.11 (d, 2H, $J=6.5$ Hz, H-16), 4.06 (t, 2H, $J=6.3$ Hz, H-1"), 4.01 (t, 2H, $J=6.2$ Hz, H-1'), 3.77 (s, 3H, OCH_3), 3.42 (t, 4H, $J=6.7$ Hz, H-6'', H-6'), 3.34 (d, 2H, $J=7.1$ Hz, H-11), 1.89 (m, 8H, H-2', H-2'', H-5', H-5''), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.65 (s, 6H, H-19, H-14), 1.52 (m, 8H, H-3', H-3'', H-4', H-4'')

3,6-Di-O-(7-bromoheptyl) α -mangostin (**84**) (sss6044)

Yellow oil, 378 mg, 40 % yield

R_f : 0.66 (30% acetone-hexane)

IR (film) ν_{max} : 2933, 2854, 1651, 1595, 1463, 1374, 1277, 1202, 1115, 824, 790 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.68 (s, 1H, H-5), 6.26 (s, 1H, H-4), 5.22 (br. t, 2H, $J=5.9$ Hz, H-17, H-12), 4.11 (d, 2H, $J=6.5$ Hz, H-16), 4.05 (t, 2H, $J=6.5$ Hz, H-1"), 4.01 (t, 2H, $J=6.1$ Hz, H-1'), 3.78 (s, 3H, OCH_3), 3.40 (t, 4H, $J=6.7$ Hz, H-7'', H-7'), 3.34 (d, 2H, $J=6.8$ Hz, H-11), 1.86 (m, 8H, H-2', H-2'', H-6', H-6''), 1.83 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.65 (s, 6H, H-19, H-14), 1.45 (m, 12H, H-3', H-3'', H-4', H-4'', H-5', H-5'')

3,6-Di-O-(8-bromooctyl) α -mangostin (**85**) (sss6048)

Yellow oil, 375 mg, 38 % yield

R_f : 0.76 (30% acetone-hexane)

IR (KBr) ν_{max} : 2931, 2856, 1645, 1596, 1463, 1374, 1277, 1202, 1115, 824, 795 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.26 (s, 1H, H-4), 5.22 (br. t, 2H, $J=6.7$ Hz, H-17, H-12), 4.11 (d, 2H, $J=6.4$ Hz, H-16), 4.05 (t, 2H, $J=6.4$ Hz, H-1"), 4.00 (t, 2H, $J=6.2$ Hz, H-1'), 3.78 (s, 3H, OCH_3), 3.39 (t, 4H, $J=6.8$ Hz, H-8'', H-8'), 3.34 (d, 2H, $J=7.0$ Hz, H-11), 1.85 (m, 8H, H-2', H-2'', H-7', H-7''), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.65 (s, 6H, H-19, H-14), 1.44 (m, 12H, H-3', H-3'', H-4', H-4'', H-5', H-5'', H-6', H-6'')

3,6-Di-O-(9-bromononyl) α -mangostin (**86**) (sss6051)

Yellow oil, 136 mg, 13 % yield

R_f : 0.66 (30% acetone-hexane)

IR (KBr) $\nu_{\max}$: 2928, 2855, 1645, 1595, 1463, 1374, 1277, 1202, 1114, 824, 785 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.26 (s, 1H, H-4), (br. t, 2H, $J=6.6$ Hz, H-17, H-12), 4.11 (d, 2H, $J=6.4$ Hz, H-16), 4.04 (t, 2H, $J=6.3$ Hz, H-1''), 4.00 (t, 2H, $J=6.3$ Hz, H-1'), 3.78 (s, 3H, OCH_3), 3.39 (t, 4H, $J=6.8$ Hz, H-9'', H-9'), 3.34 (d, 2H, $J=7.2$ Hz, H-11), 1.86 (m, 8H, H-2', H-2'', H-8', H-8''), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.65 (s, 6H, H-19, H-14), 1.41 (m, 20H, H-3', H-3'', H-4', H-4'', H-5', H-5'', H-6', H-6'', H-7', H-7'')

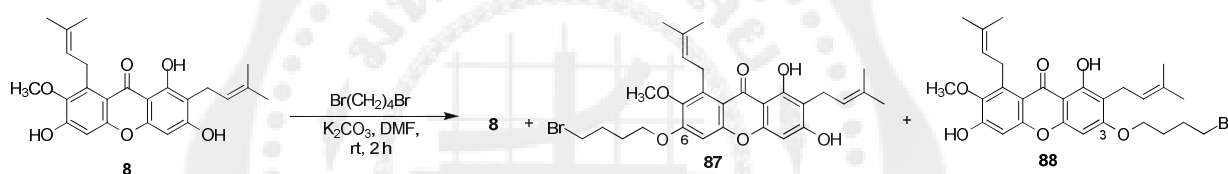
1.3.2 Synthesis of mono-O-bromoalkyl α -mangostin derivatives (**87** and **88**)

Figure 13 Synthesis of mono-O-bromoalkyl α -mangostin derivatives

α -Mangostin (**8**) (225 mg, 0.55 mmol) and K_2CO_3 (75 mg, 0.55 mmol) were dissolved in DMF (about 5 ml) and then added 1,4-dibromobutane (0.08 mL, 0.55 mmol). The mixture was stirred at room temperature for 2 h. After that, the mixture was extracted with CH_2Cl_2 (3x10 mL). The organic phase was washed with water (3x10 mL) and dried over anhydrous Na_2SO_4 . After removal of solvent, the residue was purified over column chromatography (silica gel, using hexane to 20% acetone-hexane) to afford two fractions for mixtures of **87** and **88**. Fraction **A**, 70.6 mg, 23 % yield, was in the ratio of **87:88** about 11:1 and fraction **B**, 7.1 mg, 2 % yield, was in the ratio of **87:88** about 1:14. Further purification of fraction **A** by centrifugal thin-layer chromatography (chromatotron) using 20-50 % CH_2Cl_2 -hexane that furnished 6-O-bromobutyl α -mangostin (**87**) as yellow solid. However, compound **87** was easily decomposed at room temperature.

Fraction **A**, mixture of 6-O-bromobutyl α -mangostin (**87**) and 3-O-bromobutyl α -mangostin (**88**) (11:1) (sss5862)

Yellow solid, 70.6 mg, 23 % yield, m.p. 60-62°C

R_f: 0.50 (30% acetone-hexane)

IR (KBr) $\nu_{\max}$: 3383, 2957, 2925, 1645, 1605, 1464, 1374, 1279, 1201, 1106, 821, 785, 583 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.81 (s, 1H, 1-OH), 6.70 (s, 1H, H-5), 6.26 (s, 1H, H-4), 6.21 (br. s, 1H, 3-OH), 5.27 (br. t, 1H, J= 6.4 Hz, H-17), 5.21 (br. t, 1H, J= 6.9 Hz, H-12), 4.09 (m, 4H, H-1'', H-16), 3.77 (s, 3H, 7-OCH₃), 3.50 (t, 2H, J=6.1 Hz, H-4''), 3.43 (d, 2H, J= 6.9 Hz, H-11), 2.09 (m, 4H, H-2'', H-3''), 1.82 (s, 6H, H-14, H-19), 1.75 (s, 3H, H-20), 1.66 (s, 3H, H-15)

Fraction **B**, mixture of 6-O-bromobutyl α -mangostin (**87**) and 3-O-bromobutyl α -mangostin (**88**) (1:14) (sss5863)

Yellow sticky solid, 7.1 mg, 2 % yield

R_f: 0.50 (30% acetone-hexane)

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.41 (s, 1H, 1-OH), 6.80 (s, 1H, H-5), 6.33 (br. s, 1H, 6-OH), 6.29 (s, 1H, H-4), 5.21 (br. t, 1H, J= 6.9 Hz, H-17, H-12), 4.06 (m, 4H, H-1', H-16), 3.78 (s, 3H, 7-OCH₃), 3.47 (t, 2H, J=6.3 Hz, H-4'), 3.33 (d, 2H, J=6.8 Hz, H-11), 2.03 (m, 4H, H-2', H-3'), 1.80 (s, 3H, H-20), (1.77 (s, 3H, H-15) 1.66 s, 6H, H-14, H-19)

1.3.3 General synthesis of 3,6-di-O-(*N,N*-diethylamino) alkyl- α -mangostin derivatives (**89**, **36-39**, **90-92**)

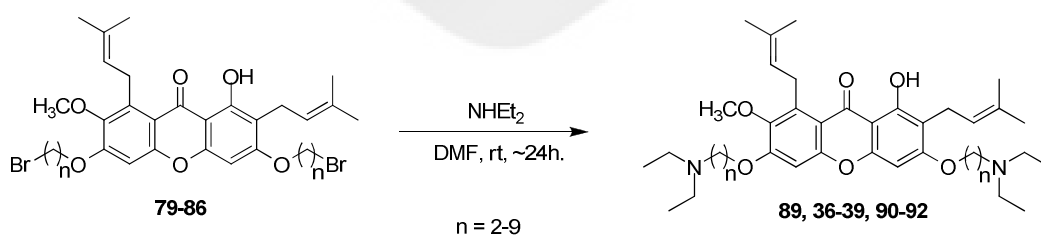


Figure 14 Synthesis of 3,6-di-O-(*N,N*-diethylamino) alkyl- α -mangostin derivatives (**89**, **36-39**, **90-92**)

Each the alkyl halide derivative (1 eq.), compounds **79-86**, was dissolved in DMF and was added *N,N*-diethylamine (2.5 eq.) and stirred at room temperature for about 24 h. Then, the mixture was extracted with EtOAc (3x10 mL). The combination of EtOAc

layer was washed with water and dried over anhydrous Na_2SO_4 . After removal of the solvent, the residue was purified over by column chromatography (aluminium oxide neutral) using 1% MeOH: CH_2Cl_2 as eluent to yield the 3,6-di-O-(*N,N*-diethylamino) alkyl- α -mangostin (**89**, **36-39**, **90-92**), 50-95 % yield.

3,6-Di-O-(2-(*N,N*-diethylamino) ethyl)- α -mangostin (**89**) (sss5431)

Yellow solid, 5.3 mg, 75 % yield, m.p. 49-51°C

R_f : 0.39 (30% acetone-hexane + 3 drops NH_4OH)

IR (KBr) ν_{max} : 2969, 2920, 2807, 1646, 1598, 1464, 1374, 1278, 1204, 1115, 824, 786 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.73 (s, 1H, H-5), 6.30 (s, 1H, H-4), 5.21 (br. t, 1H, $J=6.1$ Hz, H-17), 5.21 (br. t, 1H, $J=6.5$ Hz, H-12), 4.13 (t, 4H, $J=6.2$ Hz, H-1', H-1''), 4.11 (d, 2H, $J=6.1$ Hz, H-16), 3.78 (s, 3H, 7- OCH_3), 3.33 (d, 2H, $J=6.5$ Hz, H-11), 2.95 (t, 4H, $J=6.2$ Hz, H-2', H-2''), 2.65 (q, 8H, $J=7.1$ Hz, 2 x $\text{N}(\text{CH}_2\text{CH}_3)_2$), 1.83 (s, 3H, H-20), 1.78 (s, 3H, H-15), 1.67 (s, 6H, H-14, H-19), 1.07 (t, 2 x 6H, $J=7.1$ Hz, 2 x $\text{N}(\text{CH}_2\text{CH}_3)_2$)

ESIMS (+ve) m/z (% rel. intensity): 609.8 $[\text{M}+\text{H}]^+$ (100)

3,6-Di-O-(3-(*N,N*-diethylamino) propyl)- α -mangostin (**36**) (sss4995)

Yellow oil, 11.6 mg, 50 % yield

R_f : 0.34 (30% acetone-hexane + 3 drops NH_4OH)

IR (film) ν_{max} : 2967, 2920, 2800, 1646, 1596, 1463, 1374, 1277, 1202, 1114, 823, 786 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.47 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.27 (s, 1H, H-4), 5.19 (br. t, 2H, $J=6.1$ Hz, H-17, H-12), 4.07 (bt. t, 4H, $J=5.9$ Hz, H-1'', H-1'), 4.03 (d, 2H, $J=5.9$ Hz, H-16), 3.75 (s, 3H, OCH_3), 3.21 (d, 2H, $J=6.9$ Hz, H-11), 2.62 (t, 4H $J=6.0$ Hz, H-3'', H-3'), 2.53 (q, 8H, $J=7.1$ Hz, 2 x $\text{N}(\text{CH}_2\text{CH}_3)_2$), 1.98 (m, 4H, H-2', H-2''), 1.80 (s, 3H, H-20), 1.74 (s, 3H, H-15), 1.61 (s, 6H, H-19, H-14), 1.02 (t, 2 x 6H, $J=7.1$ Hz, 2 x $\text{N}(\text{CH}_2\text{CH}_3)_2$)

ESIMS (+ve) m/z (% rel. intensity): 637.9 $[\text{M}+\text{H}]^+$ (100)

3,6-Di-O-(4-(*N,N*-diethylamino) butyl)- α -mangostin (**37**) (sss5690)

Yellow oil, 14.5 mg, 52 % yield

R_f : 0.26 (30% acetone-hexane + 3 drops NH_4OH)

IR (film) ν_{max} : 2967, 2920, 2800, 1646, 1597, 1463, 1374, 1277, 1202, 1114, 824, 788 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.70 (s, 1H, H-5), 6.27 (s, 1H, H-4), 5.22 (br. t, 2H, $J=6.7$ Hz, H-17, H-12), 4.11 (d, 2H, $J=6.7$ Hz, H-16), 4.07 (t, H, $J=5.9$ Hz, H-1''), 4.03 (t, 2H, $J=6.1$ Hz, H-1'), 3.78 (s, 3H, OCH_3), 3.34 (d, 2H, $J=6.7$ Hz, H-11), 2.52 (q, 8H, $J=7.1$ Hz, 2 x $\text{N}(\text{CH}_2\text{CH}_3)_2$), 2.47 (t, 4H $J=7.5$ Hz, H-4'', H-4'), 1.86 (m, 4H, H-2', H-2''),

1.82 (s, 3 H, H-20), 1.77 (s, 3H, H-15), 1.65 (s, 6H, H-19, H-14), 1.65 (m, 4H, H-3', H-3''), 1.01 (t, 2 x 6H, J=7.1 Hz, 2 x N(CH₂CH₃)₂)

ESIMS (+ve) m/z (% rel. intensity): 666.0 [M] (100), 667.1 [M+H]⁺ (28)

3,6-Di-O-(5-(*N,N*-diethylamino) pentyl)- α -mangostin (**38**) (sss5406)

Yellow oil, 20.8 mg, 84 % yield

R_f: 0.33 (30% acetone-hexane + 3 drops NH₄OH)

IR (film) $\nu_{\max}$: 2967, 2920, 2800, 1646, 1596, 1463, 1374, 1277, 1202, 1114, 823, 786 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.68 (s, 1H, H-5), 6.26 (s, 1H, H-4), 5.22 (br. t, 2H, J=6.3 Hz, H-17, H-12), 4.10 (d, 2H, J=6.4 Hz, H-16), 4.05 (t, H, J=6.5 Hz, H-1''), 4.01 (t, 2H, J=6.4 Hz, H-1'), 3.77 (s, 3H, OCH₃), 3.33 (d, 2H, J=7.1 Hz, H-11), 2.51 (q, 8H, J=7.0 Hz, 2 x N(CH₂CH₃)₂), 2.43 (t, 4H J=7.5 Hz, H-5'', H-5'), 1.89 (m, 4H, H-2', H-2''), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.65 (s, 6H, H-19, H-14), 1.50 (m, 8H, H-3', H-3'', H-4', H-4''), 1.00 (t, 2 x 6H, J=7.0 Hz, 2 x N(CH₂CH₃)₂)

ESIMS (+ve) m/z (% rel. intensity): 693.6 [M+H]⁺ (100)

3,6-Di-O-(6-(*N,N*-diethylamino) hexyl)- α -mangostin (**39**) (sss5612)

Yellow oil, 14.0 mg, 57% yield

R_f: 0.29 (30% acetone-hexane + 3 drops NH₄OH)

IR (film) $\nu_{\max}$: 2967, 2931, 2800, 1645, 1596, 1463, 1374, 1277, 1202, 1114, 823, 781 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.68 (s, 1H, H-5), 6.26 (s, 1H, H-4), 5.22 (br. t, 2H, J=6.6 Hz, H-17, H-12), 4.10 (d, 2H, J=6.4 Hz, H-16), 4.04 (t, H, J=6.4 Hz, H-1''), 4.00 (t, 2H, J=6.3 Hz, H-1'), 3.77 (s, 3H, OCH₃), 3.33 (d, 2H, J=7.0 Hz, H-11), 2.51 (q, 8H, J=7.0 Hz, 2 x N(CH₂CH₃)₂), 2.41 (t, 4H J=7.5 Hz, H-6'', H-6'), 1.85 (m, 4H, H-2', H-2''), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.65 (s, 6H, H-19, H-14), 1.48 (m, 8H, H-3', H-3'', H-4', H-4''), 1.34 (m, 4H, H-5', H-5''), 1.00 (t, 2 x 6H, J=7.1 Hz, 2 x N(CH₂CH₃)₂)

ESIMS (+ve) m/z (% rel. intensity): 722.3 [M+H]⁺ (100)

3,6-Di-O-(7-(*N,N*-diethylamino) heptyl)- α -mangostin (**90**) (sss6054)

Yellow oil, 24.4 mg, 50 % yield

R_f: 0.14 (30% acetone-hexane + 3 drops NH₄OH)

IR (film) $\nu_{\max}$: 2967, 2931, 2800, 1644, 1597, 1463, 1374, 1277, 1203, 1115, 823, 786 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.27 (s, 1H, H-4), 5.23 (br. t, 2H, J=6.2 Hz, H-17, H-12), 4.11 (d, 2H, J=6.1 Hz, H-16), 4.05 (t, H, J=6.4 Hz, H-1''), 4.01 (t, 2H, J=6.3 Hz, H-1'), 3.78 (s, 3H, OCH₃), 3.34 (d, 2H, J=6.3 Hz, H-11), 2.51 (q, 8H, J=7.1 Hz, 2 x N(CH₂CH₃)₂), 2.40 (t, 4H J=7.5 Hz, H-7'', H-7'), 1.88 (m, 4H, H-2', H-2''),

1.83 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.65 (s, 6H, H-19, H-14), 1.40 (m, 16H, H-3', H-3'', H-4', H-4'', H-5', H-5'', H-6', H-6''), 1.00 (t, 2 x 6H, J=7.1 Hz, 2 x N(CH₂CH₃)₂)

ESIMS (+ve) m/z (% rel. intensity): 750.0 [M+H]⁺ (100)

3,6-Di-O-(8-(*N,N*-diethylamino) octyl)- α -mangostin (**91**) (sss6055)

Yellow sticky oil, 56.1 mg, 95 % yield

R_f: 0.12 (30% acetone-hexane + 3 drops NH₄OH)

IR (KBr) $\nu_{\max}$: 2967, 2929, 2854, 2800, 1636, 1593, 1458, 1374, 1276, 1201, 1116, 865, 822, 792 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.26 (s, 1H, H-4), 5.22 (br. t, 2H, J=6.0 Hz, H-17, H-12), 4.11 (d, 2H, J=6.5 Hz, H-16), 4.04 (t, H, J=6.4 Hz, H-1''), 4.00 (t, 2H, J=6.2 Hz, H-1'), 3.77 (s, 3H, OCH₃), 3.34 (d, 2H, J=6.9 Hz, H-11), 2.50 (q, 8H, J=7.1 Hz, 2 x N(CH₂CH₃)₂), 2.38 (t, 4H J=7.6 Hz, H-8'', H-8'), 1.87 (m, 4H, H-2', H-2''), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.72 (s, 6H, H-19, H-14), 1.40 (m, 20H, H-3', H-3'', H-4', H-4'', H-5', H-5'', H-6', H-6'', H-7', H-7''), 1.00 (t, 2 x 6H, J=7.1 Hz, 2 x N(CH₂CH₃)₂)

ESIMS (+ve) m/z (% rel. intensity): 778.2 [M+H]⁺ (100)

3,6-Di-O-(9-(*N,N*-diethylamino) nonyl)- α -mangostin (**92**) (sss6056)

Yellow oil, 34.5 mg, 75 % yield

R_f: 0.38 (30% acetone-hexane + 3 drops NH₄OH)

IR (film) $\nu_{\max}$: 2967, 2928, 2800, 1640, 1597, 1463, 1369, 1277, 1202, 1114, 823, 786 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.48 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.27 (s, 1H, H-4), 5.22 (br. t, 2H, J=6.5 Hz, H-17, H-12), 4.11 (d, 2H, J=6.1 Hz, H-16), 4.04 (t, H, J=6.4 Hz, H-1''), 4.00 (t, 2H, J=6.3 Hz, H-1'), 3.78 (s, 3H, OCH₃), 3.34 (d, 2H, J=7.3 Hz, H-11), 2.50 (q, 8H, J=7.1 Hz, 2 x N(CH₂CH₃)₂), 2.38 (t, 4H J=7.7 Hz, H-9'', H-9'), 1.86 (m, 4H, H-2', H-2''), 1.82 (s, 3H, H-20), 1.77 (s, 3H, H-15), 1.71 (s, 6H, H-19, H-14), 1.40 (m, 24H, H-3', H-3'', H-4', H-4'', H-5', H-5'', H-6', H-6'', H-7', H-7'', H-8', H-8''), 0.99 (t, 2 x 6H, J=7.1 Hz, 2 x N(CH₂CH₃)₂)

ESIMS (+ve) m/z (% rel. intensity): 806.1 [M+H]⁺ (100)

1.3.4 Synthesis of 6-O-(4-diethylamino butyl) α -mangostin (**93**)

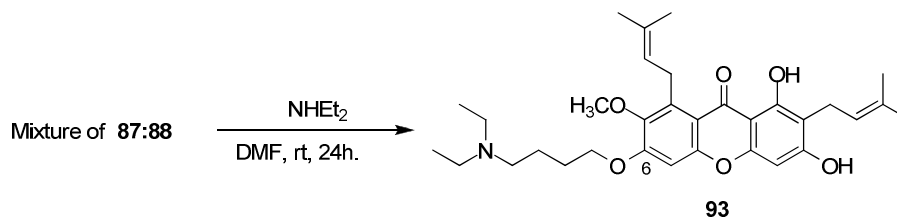


Figure 15 Synthesis of 6-O-(4-diethylamino butyl) α -mangostin (**93**)

The major fraction **A**, a mixture of 6-O-(4-bromobutyl) α -mangostin (**87**) and 3-O-(4-bromobutyl) α -mangostin (**88**) (11:1) (17.8 mg, 0.03 mmol), was reacted with an equal mole equivalent of *N,N*-diethylamine by using the same method as described for **37** to yield **93** in 56 % yield.

6-Mono-O-(4-(*N,N*-diethylamino) butyl)- α -mangostin (**93**) (sss6004)

Yellow sticky oil, 10 mg, 56 % yield

R_f : 0.11 (30% acetone-hexane + 3 drops NH_4OH)

IR (KBr) ν_{max} : 3383, 2957, 2926, 1636, 1586, 1464, 1379, 1278, 1200, 1107, 824, 781 cm^{-1} .

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.56 (s, 1H, 1-OH), 6.56 (s, 1H, H-5), 6.10 (s, 1H, H-4), 5.25 (br. t, 1H, $J=6.0$ Hz, H-17), 5.16 (br. t, 1H, $J=6.7$ Hz, H-12), 4.00 (m, 4H, H-1'', H-16), 3.59 (s, 3H, OCH_3), 3.35 (d, 2H, $J=6.7$ Hz, H-11), 2.72 (q, 4H, $J=7.1$ Hz, $\text{N}(\text{CH}_2\text{CH}_3)_2$), 2.65 (br. t, 2H, $J=5.8$ Hz, H-4''), 1.85 (m, 4H, H-2'', H-3''), 1.79 (s, 6H, H-14, H-19), 1.69 (s, 3H, H-20), 1.65 (s, 3H, H-15), 1.10 (t, 6H, $J=7.1$ Hz, $\text{N}(\text{CH}_2\text{CH}_3)_2$)

ESIMS (+ve) m/z (% rel. intensity): 538.9 $[\text{M}+\text{H}]^+$ (100)

1.3.5 General synthesis of α -mangostin-3,6-Di-O-(*N*-methylalkyl) ammonium iodide derivatives (**94-101**)

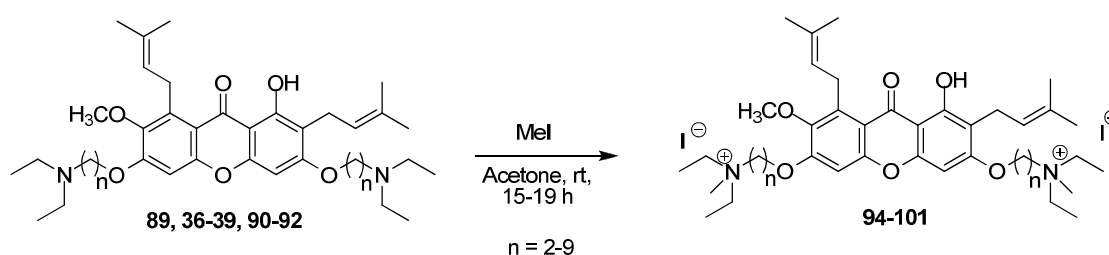


Figure 16 Synthesis of α -mangostin-3,6-di-O-(*N*-methylalkyl) ammonium iodide derivatives (**94-101**)

To a solution of each alkylamino α -mangostin derivative (1 eq.) (**89**, **36-39**, **90-92**) in acetone, excess iodomethane was added (15 eq.). The mixture was stirred at room temperature for 15-19 h. After evaporation to dryness by using a rotary evaporator, the ammonium iodides **94-101** were yielded in 51-99 % yields.

*α -Mangostin-3,6-di-O-(N,N-diethyl)-N-methylethylammonium iodide (**94**)*

(sss5322)

Yellow solid, 5.2 mg, 98 % yield, m.p. 200-202°C

R_f: base line (30% acetone-hexane + 3 drops NH₄OH)

IR (KBr) $\nu_{\max}$: 3447, 2926, 2850, 1647, 1598, 1458, 1276, 1207, 1118, 828, 784 cm⁻¹

¹H-NMR (CDCl₃+CD₃OD, 300 MHz): δ_{H} 13.34 (s, 1H, 1-OH), 6.96 (s, 1H, H-5), 6.51 (s, 1H, H-4), 5.07 (br. t, 1H, J= 6.9 Hz, H-17), 4.95 (br. t, 1H, J= 6.9 Hz, H-12), 4.59 (br. t, 4H, H-1', H-1''), 4.04 (br. t, 4H, H-2', H-2''), 3.94 (d, 2H, J= 6.9 Hz, H-16), 3.68 (s, 3H, OCH₃), 3.58 (q x 4, 8H, J=7.1, 7.3 Hz, 2 x N(CH₂CH₃)₂), 3.32 (br. d, 2H, H-11), 3.24 (s, 3H, 2''-N⁺CH₃), 3.21 (s, 3H, 2'-N⁺CH₃), 1.74 (s, 3H, H-20), 1.69 (s, 3H, H-15), 1.59 (s, 6H, H-14, H-19), 1.41 (t, 2 x 6H, J= 7.1, 7.3 Hz, 2 x N(CH₂CH₃)₂)

ESIMS (+ve) m/z (% rel. intensity): 319.4 [M]²⁺ (100), 765.2 [M+I]⁺ (94)

*α -Mangostin-3,6-di-O-(N,N-diethyl)-N-methylpropylammonium iodide (**95**)*

(sss5322)

Yellow solid, 37.2 mg, 99 % yield, m.p. 192-194°C

R_f: base line (30% acetone-hexane + 3 drops NH₄OH)

IR (KBr) $\nu_{\max}$: 3447, 2929, 1647, 1597, 1458, 1275, 1207, 1112, 842, 766 cm⁻¹

¹H-NMR (CDCl₃+CD₃OD, 300 MHz): δ_{H} 13.36 (s, 1H, 1-OH), 6.73 (s, 1H, H-5), 6.03 (s, 1H, H-4), 5.07 (br. t, 1H, J=6.6 Hz, H-17), 5.01 (br. t, 1H, J=6.4 Hz, H-12), 4.17 (m, 4H, H-1'', H-1'), 3.96 (d, 2H, J=6.6 Hz, H-16), 3.65 (s, 3H, OCH₃), 3.36-3.57 (m, 12H, H-3', H-3'', 2 x N(CH₂CH₃)₂), 3.19 (d, 2H, J=6.4 Hz, H-11), 3.04 (s, 2 x 3H, 2 x N⁺CH₃), 2.26 (m, 4H, H-2'', H-2'), 1.71 (s, 3H, H-20), 1.66 (s, 3H, H-15), 1.55 (s, 6H, H-19, H-14), 1.32 (t, 6H, J=6.8 Hz, 6-N(CH₂CH₃)₂), 1.30 (t, 6H, J=6.8 Hz, 3-N(CH₂CH₃)₂)

ESIMS (+ve) m/z (% rel. intensity): 333.6 [M+H]²⁺ (100), 793.2 [M+I]⁺ (24)

*α -Mangostin-3,6-di-O-(N,N-diethyl)-N-methylbutylammonium iodide (**96**)*

(sss3712)

Yellow solid, 36.1 mg, 51 % yield, m.p. 180-182°C

R_f: base line (30% acetone-hexane + 3 drops NH₄OH)

IR (KBr) $\nu_{\max}$: 3447, 2929, 1653, 1598, 1464, 1278, 1204, 1115, 823, 780 cm⁻¹

$^1\text{H-NMR}$ (acetone- d_6 , 300 MHz): δ_{H} 13.59 (s, 1H, 1-OH), 7.02 (s, 1H, H-5), 6.54 (s, 1H, H-4), 5.22 (m, 2H, H-17, H-12), 4.36 (br.t, 2H, $J=5.8$ Hz, H-1''), 4.28 (br.t, 2H, $J=5.8$ Hz, H-1'), 4.10 (d, 2H, $J=6.5$ Hz, H-16), 3.81 (s, 3H, OCH_3), 3.72 (m, 4H, H-4', H-4''), 3.64 (q, 8H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$), 3.32 (d, 2H, $J=6.8$ Hz, H-11), 3.27 (s, $2 \times 3\text{H}$, $2 \times \text{N}^+\text{CH}_3$), 2.13 (m, 4H, H-3'', H-3'), 2.07 (m, 4H, H-2'', H-2'), 1.81 (s, 3H, H-20), 1.78 (s, 3H, H-15), 1.63 (s, 6H, H-19, H-14), 1.41 (t, 12H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$).

ESIMS (+ve) m/z (% rel. intensity): 347.5 $[\text{M}+\text{H}]^{2+}$ (100), 821.3 $[\text{M}+\text{I}]^+$ (52)

α -Mangostin-3,6-di-O-(N,N-diethyl)-N-methylpentylammonium iodide (97)

(sss5412)

Yellow solid, 24.7 mg, 94 % yield, m.p. 133-135°C

R_f : base line (30% acetone-hexane + 3 drops NH_4OH)

IR (KBr) ν_{max} : 3436, 2926, 2850, 1647, 1598, 1463, 1277, 1203, 1114, 820, 788 cm^{-1}

$^1\text{H-NMR}$ ($\text{CDCl}_3+\text{CD}_3\text{OD}$, 300 MHz): δ_{H} 13.37 (s, 1H, 1-OH), 6.69 (s, 1H, H-5), 6.25 (s, 1H, H-4), 5.07 (m, 2H, H-17, H-12), 4.00 (m, 6H, H-1'', H-1', H-16), 3.65 (s, 3H, OCH_3), 3.30 (q, 8H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$), 3.21 (m, 6H, H-11 H-5', H-5''), 2.94 (s, $2 \times 3\text{H}$, $2 \times \text{N}^+\text{CH}_3$), 1.6-1.9 (m, 8H, H-2'', H-2', H-4'', H-4'), 1.70 (s, 3H, H-20), 1.64 (s, 3H, H-15), 1.53 (s, 6H, H-19, H-14), 1.49 (m, 4H, H-3'', H-3'), 1.24 (t, 12H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$)

ESIMS (+ve) m/z (% rel. intensity): 361.6 $[\text{M}+\text{H}]^{2+}$ (100), 849.3 $[\text{M}+\text{I}]^+$ (38)

α -Mangostin-3,6-di-O-(N,N-diethyl)-N-methylhexylammonium iodide (98)

(sss5616)

Yellow solid, 20.7 mg, 78 % yield, m.p. 123-125°C

R_f : base line (30% acetone-hexane + 3 drops NH_4OH)

IR (KBr) ν_{max} : 3447, 2925, 2854, 1653, 1596, 1458, 1277, 1203, 1116, 856, 776 cm^{-1}

$^1\text{H-NMR}$ ($\text{CDCl}_3+\text{CD}_3\text{OD}$, 300 MHz): δ_{H} 13.43 (s, 1H, 1-OH), 6.72 (s, 1H, H-5), 6.28 (s, 1H, H-4), 5.14 (m, 2H, H-17, H-12), 4.03 (m, 6H, H-1'', H-1', H-16), 3.71 (s, 3H, OCH_3), 3.38 (q, 8H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$), 3.30 (m, 6H, H-11 H-6', H-6''), 3.05 (s, $2 \times 3\text{H}$, $2 \times \text{N}^+\text{CH}_3$), 1.6-1.9 (m, 8H, H-2'', H-2', H-5'', H-5'), 1.76 (s, 3H, H-20), 1.71 (s, 3H, H-15), 1.59 (s, 6H, H-19, H-14), 1.4-1.6 (m, 8H, H-3'', H-3', H-4'', H-4'), 1.30 (t, 12H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$)

ESIMS (+ve) m/z (% rel. intensity): 376.2 $[\text{M}+2\text{H}]^{2+}$ (100), 877.8 $[\text{M}+\text{I}]^+$ (10)

α -Mangostin-3,6-di-O-(N,N-diethyl)-N-methylheptylammonium iodide (99)

(sss6061)

Yellow sticky solid, 12.2 mg, 88 % yield

R_f : base line (30% acetone-hexane + 3 drops NH_4OH)

IR (KBr) $\nu_{\max}$: 3447, 2928, 2850, 1653, 1596, 1458, 1278, 1204, 1114, 823, 781 cm^{-1}

$^1\text{H-NMR}$ ($\text{CDCl}_3 + \text{CD}_3\text{OD}$, 300 MHz): δ_{H} 13.45 (s, 1H, 1-OH), 6.75 (s, 1H, H-5), 6.32 (s, 1H, H-4), 5.18 (m, 2H, H-17, H-12), 4.06 (m, 6H, H-1'', H-1', H-16), 3.74 (s, 3H, OCH_3), 3.47 (q, 8H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$), 3.31 (m, 6H, H-11, H-7', H-7''), 3.14 (s, $2 \times 3\text{H}$, $2 \times \text{N}^+\text{CH}_3$), 2.10 (m, 4H, H-2'', H-2'), 1.84 (m, 4H, H-6', H-6''), 1.80 (s, 3H, H-20), 1.74 (s, 3H, H-15), 1.63 (s, 6H, H-19, H-14), 1.43 (m, 12H, H-3'', H-3', H-4'', H-4' H-5'', H-5'), 1.34 (t, 12H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$)

ESIMS (+ve) m/z (% rel. intensity): 389.1 $[\text{M}+2\text{H}]^{2+}$ (100), 905.8 $[\text{M}+\text{I}]^+$ (16)

α -Mangostin-3,6-di-O-(*N,N*-diethyl)-*N*-methyloctylammonium iodide (**100**)

(sss6062)

Yellow sticky solid, 23.0 mg, 99 % yield, m.p. 118-120°C

R_f : base line (30% acetone-hexane + 3 drops NH_4OH)

IR (KBr) $\nu_{\max}$: 3447, 2928, 2854, 1653, 1597, 1458, 1277, 1203, 1114, 823, 790 cm^{-1}

$^1\text{H-NMR}$ ($\text{CDCl}_3 + \text{CD}_3\text{OD}$, 300 MHz): δ_{H} 13.46 (s, 1H, 1-OH), 6.73 (s, 1H, H-5), 6.31 (s, 1H, H-4), 5.19 (m, 2H, H-17, H-12), 4.04 (m, 6H, H-1'', H-1', H-16), 3.75 (s, 3H, OCH_3), 3.49 (q, 8H, $J=7.1$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$), 3.34 (m, 6H, H-11, H-8', H-8''), 3.16 (s, $2 \times 3\text{H}$, $2 \times \text{N}^+\text{CH}_3$), 1.89 (m, 8H, H-2'', H-2', H-7', H-7''), 1.80 (s, 3H, H-20), 1.75 (s, 3H, H-15), 1.63 (s, 6H, H-19, H-14), 1.35 (m, 28H, H-3'', H-3', H-4'', H-4' H-5'', H-5', H-6', H-6'', $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$)

ESIMS (+ve) m/z (% rel. intensity): 404.2 $[\text{M}+2\text{H}]^{2+}$ (100), 399.7 $[\text{M}+\text{I}]^+$ (38)

α -Mangostin-3,6-di-O-(*N,N*-diethyl)-*N*-methylnonylammonium iodide (**101**)

(sss6063)

Yellow sticky solid, 18.5 mg, 96 % yield

R_f : base line (30% acetone-hexane + 3 drops NH_4OH)

IR (KBr) $\nu_{\max}$: 3446, 2927, 2854, 1653, 1593, 1465, 1278, 1204, 1115, 823, 781 cm^{-1}

$^1\text{H-NMR}$ ($\text{CDCl}_3 + \text{CD}_3\text{OD}$, 300 MHz): δ_{H} 13.46 (s, 1H, 1-OH), 6.72 (s, 1H, H-5), 6.30 (s, 1H, H-4), 5.20 (m, 2H, H-17, H-12), 4.03 (m, 6H, H-1'', H-1', H-16), 3.75 (s, 3H, OCH_3), 3.50 (q, 8H, $J=7.2$ Hz, $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$), 3.32 (m, 6H, H-11, H-9', H-9''), 3.16 (s, $2 \times 3\text{H}$, $2 \times \text{N}^+\text{CH}_3$), 1.88 (m, 8H, H-2'', H-2', H-8', H-8''), 1.80 (s, 3H, H-20), 1.75 (s, 3H, H-15), 1.63 (s, 6H, H-19, H-14), 1.35 (m, 32H, H-3'', H-3', H-4'', H-4' H-5'', H-5', H-6', H-6'', H-7', H-7'', $2 \times \text{N}(\text{CH}_2\text{CH}_3)_2$)

ESIMS (+ve) m/z (% rel. intensity): 418.3 $[\text{M}+2\text{H}]^{2+}$ (100), 961.9 $[\text{M}+\text{I}]^+$ (30)

1.3.6 Synthesis of tetrahydro α -mangostin-3,6-Di-O-(*N,N*-diethyl)-*N*-methylbutyl ammonium iodide (**103**)

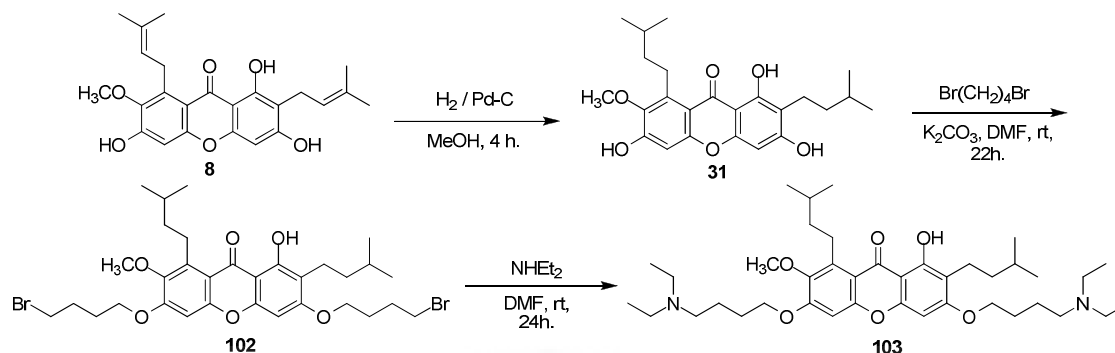


Figure 17 Synthesis of tetrahydro α -mangostin derivatives

The compound **103** was prepared from α -mangostin by using catalytic hydrogenation, alkylation, amine substitution and methylation reaction, respectively. First step, a mixture of α -mangostin (508 mg, 1.23 mmol) and Pd black (10 mg, 0.5 mol %) in MeOH was vigorously stirred under H_2 (40-60 psi) for 4 h with Parr Hydrogenator, and the reaction progress was monitored by TLC. The insoluble material was filtered off through a Celite pad, and was concentrated *in vacuo*. A yellow viscous residue was recrystallized by hexane to obtain the tetrahydro α -mangostin (**31**), 98 % yield. After that, compound **31** (242 mg, 0.58 mmol) was used for prepared 3,6-di-O-(4-bromobutyl) tetrahydro α -mangostin (**102**), by the same method as described for **81** using 1,4 dibromobutane as the reagent. The compound **102** was obtained in 30 % yield. Next step, The compound **102** (55 mg, 0.08 mmol) was reacted with *N,N*-diethylamine using the same method as described for **37** to give the compound **103**, 3,6-di-O-(4-(*N,N*-diethylamino) butyl) tetrahydro α -mangostin, 87 % yield.

Tetrahydro α -mangostin (**31**) (sss5710)

Yellow solid, 503 mg, 98 % yield, m.p. 128-130°C

R_f : 0.42 (30% acetone-hexane)

IR (KBr) $\nu_{\max}$: 3475, 3203, 2953, 1646, 1608, 1467, 1206, 1122, 1067, 980, 820, 793 cm^{-1}

$^1\text{H-NMR}$ (CDCl_3 , 300 MHz): δ_{H} 13.81 (s, 1H, 1-OH), 6.77 (s, 1H, H-5), 6.27 (s, 1H, 6-OH), 6.23 (s, 1H, H-4), 5.49 (s, 1H, 3-OH), 3.81 (s, 3H, OCH_3), 3.31 (br. t, 2H, $J=8.0$ Hz, H-16), 2.63 (br. t, 2H, $J=8.0$ Hz, H-11), 1.5-1.7 (m, 2H, H-18, H-13), 1.3-1.5 (m, 4H, H-12, H-17), 0.98 (d, 6H, $J=6.9$ Hz, H-19, H-20), 0.95 (d, 6H, $J=6.8$ Hz, H-14, H-15)

3,6-Di-O-(4-bromobutyl) tetrahydro α -mangostin (**102**) (sss5653)

Yellow solid, 123 mg, 30 % yield, m.p. 96-98°C

R_f: 0.66 (30% acetone-hexane)

IR (KBr) $\nu_{\max}$: 2957, 2863, 1647, 1597, 1466, 1363, 1286, 1202, 1139, 820, 793 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.62 (s, 1H, 1-OH), 6.68 (s, 1H, H-5), 6.27 (s, 1H, H-4), 4.10 (t, 2H, J=5.6 Hz, H-1''), 4.05 (t, 2H, J=5.6 Hz, H-1'), 3.80 (s, 3H, OCH₃), 3.51 (t, 2H, J=5.8 Hz, H-4''), 3.49 (t, 2H, J=6.3 Hz, H-4'), 3.33 (br. t, 2H, J=8.1 Hz, H-16), 2.63 (br. t, 2H, J=8.1 Hz, H-11), 1.9-2.1 (m, 8H, H-2', H-2'', H-3', H-3''), 1.5-1.7 (m, 2H, H-18, H-13), 1.3-1.5 (m, 4H, H-12, H-17), 0.98 (d, 6H, J=6.6 Hz, H-19, H-20), 0.94 (d, 6H, J=6.5 Hz, H-14, H-15)

3,6-Di-O-(4-(*N,N*-diethylamino) butyl) tetrahydro α -mangostin (**103**) (sss5215)

Yellow oil, 47 mg, 87 % yield

R_f: 0.16 (30% Acetone: Hexane + 3 drops NH₄OH)

IR (KBr) $\nu_{\max}$: 2954, 2870, 2801, 1643, 1598, 1463, 1383, 1286, 1198, 1139, 823, 791 cm⁻¹

¹H-NMR (CDCl₃, 300 MHz): δ_{H} 13.64 (s, 1H, 1-OH), 6.68 (s, 1H, H-5), 6.26 (s, 1H, H-4), 4.08 (t, 2H, J=6.3 Hz, H-1''), 4.02 (t, 2H, J=6.2 Hz, H-1'), 3.80 (s, 3H, OCH₃), 3.32 (br. t, 2H, J=8.0 Hz, H-16), 2.63 (br. t, 2H, J=7.8 Hz, H-11), 2.53 (q, 8H, J=7.1 Hz, 2 x N(CH₂CH₃)₂), 2.50 (t, 4H, J=7.1 Hz, H-4', H-4''), 1.3-1.9 (m, 16H, H-12, H-13, H-17, H-18, H-2', H-2'', H-3', H-3''), 1.01 (t, 12H, J=7.1 Hz, 2 x N(CH₂CH₃)₂), 0.98 (d, 6H, J=7.1 Hz, H-19, H-20), 0.93 (d, 6H, J=6.5 Hz, H-14, H-15)

ESIMS (+ve) m/z (% rel. intensity): 670.0 [M+H]⁺ (100)

2. Bioassays

2.1 Materials

H. pylori were grown on Columbia Blood (CB) agar Base (Hi-Media, India) supplemented with horse serum (GIBCO Invitrogen, England) and sheep blood (Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Thailand). In Minimum inhibitory concentration (MIC) assay, Brain Heart Infusion (BHI) broth (Hi-Media, India) was used for the growth of bacteria. These culture medias were protected from fungal by Amphotericin B (Sigma-Aldrich, USA). Microaerobic condition (5% O₂, 10% CO₂, 85% N₂) was generated by using a micro-aerobic GasPak (Mitsubishi, Japan) in an anaerobic box (Mitsubishi, Japan). Amoxicillin, metronidazole (Government Pharmaceutical Organization, Thailand), and clarithromycin (KLACID[®]) were used as positive control drugs. Test samples

were dissolved in dimethyl sulfoxide (DMSO, Merck). A 0.5 McFarland standard for standardized the inoculum density for a susceptibility test, was prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), with 9.95 mL of 1% sulfuric acid (H_2SO_4) (NCCLS, 2006). Other equipments and instruments were facilitated by Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Thailand.

2.2 Bacterial strains and growth conditions

The clinical isolate of *H. pylori* HP40 was obtained from King Chulalongkorn Memorial Hospital kindly provided by Dr. Thanitta Chatsuwat, Chulalongkorn University, Thailand. *H. pylori* DMST 20165 was obtained from the Department of Medical Science, Ministry of Public Health, Thailand and *H. pylori* ATCC 43504 was used as the reference strain. Bacterial strains were grown on CB agar supplemented with 7 % horse serum, 7% sheep blood and 5 $\mu\text{g/mL}$ Amphotericin B and incubated at 37°C for 2-4 days under microaerobic conditions. To prepare inocula, tested bacteria were prepared in BHI broth supplemented with 10 % horse serum and 5 $\mu\text{g/mL}$ Amphotericin B by adjusting to 0.5 McFarland standard that had 1.5×10^8 colony forming units (CFU)/mL.

2.2 Minimum inhibitory concentration (MIC) assay

A broth microdilution method (Wangchuk; et al. 2011: 730-742) carried out in a 96-well sterilized micro plate was used to determine the MICs of the pure compounds. MICs were determined as the lowest concentration that produces complete growth inhibition of the tested bacteria. Individual test sample was dissolved in DMSO. Serial two fold dilutions of the test sample were mixed with BHI broth in 96-well microtiter plates (Corning, USA) in an initial concentration of 250 $\mu\text{g/mL}$. The suspension of each tested bacteria (1.5×10^8 CFU/mL) was added in each well in equal volume of test sample (50 μL). The microtiter plates were incubated at 37°C for 4 days under microaerobic conditions. MICs were recorded by reading the lowest concentration that inhibited visible growth. The experiments were performed three times in duplicate.

CHAPTER 4

RESULT AND DISCUSSION

1. Natural oxygenated xanthenes from *Garcinia fusca*

Ten natural oxygenated xanthenes (**8**, **9**, **11**, **12**, **14**, **17**, **23**, **25**, **26** and **74**) from the roots of *G. fusca* were isolated and structural elucidated by Miss Jannarin Nontakham (Nontakham. 2011: Dissertation). These were two prenylated xanthenes, α -mangostin (**8**) and β -mangostin (**9**), six geranylated xanthenes, cowanin (**11**), cowanol (**12**), cowaxanthone (**14**), fuscaxanthenes A (**17**) fuscaxanthenes G (**23**) and fuscaxanthone I (**74**), and non-prenylated xanthenes 1,3,5,6,-tetrahydroxyxanthone (**25**) and isojacareubin (**26**) (Nontakham. 2011: Dissertation). These xanthenes were screened for antibacterial activity against three strains of *Helicobacter pylori*.

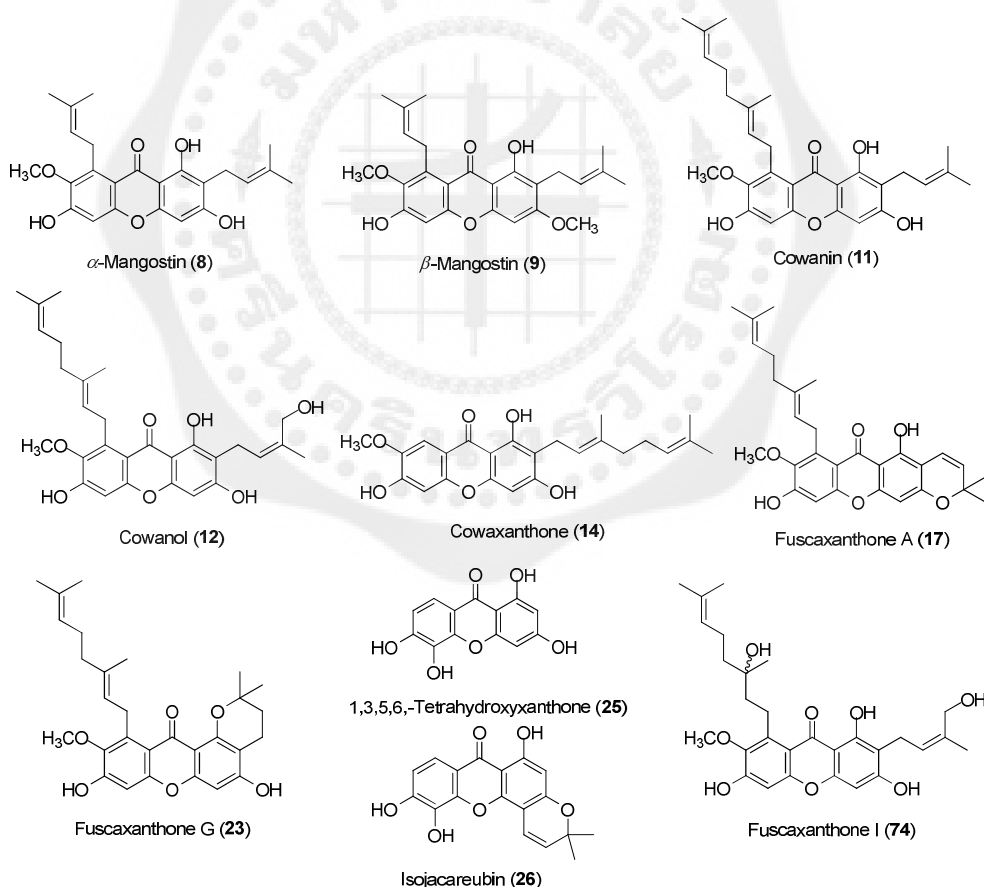


Figure 18 Natural oxygenated xanthenes from the roots of *G. fusca*

2. Chemical modifications of α -mangostin

Described above, α -mangostin (**8**) was shown to have a broad spectrum of biological profiles such as antioxidant, antiinflammatory, hepatoprotective, cancer preventive, antimalarial and antibacterial activities (Obolskiy; et al. 2009: 1047-65). α -Mangostin is well known to inhibit the growth of several Gram-positive bacteria such as *S. aureus*, MRSA, VRE, VSE (Sakagami; et al. 2005: 203-208), *Streptococcus faecium* and *Enterococcus faecalis* (Zou; et al. 2013: 2359-73). α -Mangostin was also reported to exhibit strong antibacterial activity against *H. pylori* (MIC 3.80 μ M), a Gram-negative bacteria, (Hasegawa et al. 1996: JP8231896), however, the result of which was contrasted with our moderate anti *H. pylori* effect (MIC 38.0 μ M) in our screening. To date, the naturally occurring bioactive molecules constitute ideal starting material templates and natural-product like libraries synthesis in particular is of increasing interest. Functionalization at the free C3 and C6 hydroxy groups of α -mangostin to be several alkylamino derivatives were shown to enhance antibacterial activities as compared with the parent compound (Zou; et al. 2013: 2359-73). In this regards, the alkylamino and their ammonium analogues of the free C3 and C6 hydroxy groups were prepared from α -mangostin and their anti gram-negative *H. pylori* activity were evaluated. The alkylamino analogs (**89**, **36-39**, **90-92**) were synthesized via alkylation of α -mangostin (**8**) with a series of dibromoalkanes in the presence of base, followed by reacting of resulting bromoalkylated intermediates (**79-86**) with *N,N*-diethylamine. Subsequently, methylation with methyl iodide furnished the corresponding ammonium iodides (**94-101**) (Figure 19).

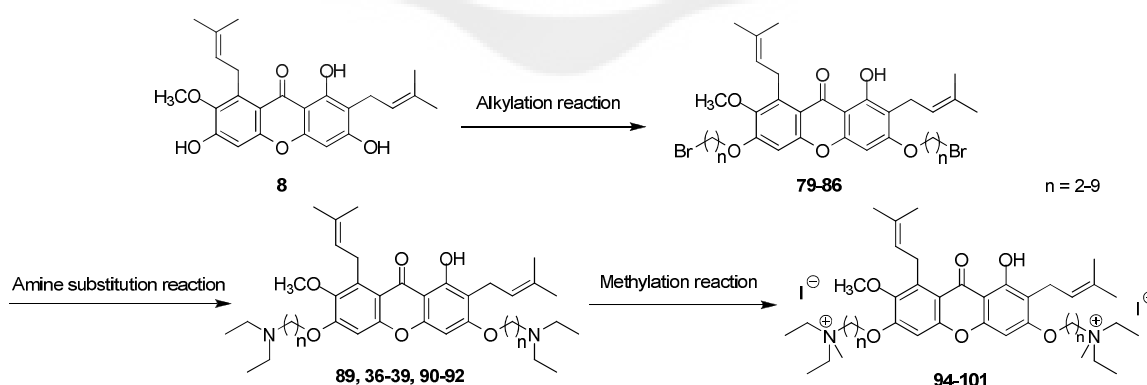


Figure 19 Synthesis of the alkylamino and their ammonium analogues of α -mangostin

2.1 Synthesis of bromoalkyl α -mangostin derivatives (**79-86**)

α -Mangostin was reacted with a series dibromoalkane of different chain length (in 1:2.5 eq.) in the presence of K_2CO_3 in DMF at room temperature for 24-48 h (Figure 12) or in some cases potassium iodide (KI) was added as catalyst. After purification, the products of 3,6-O-dibromoalkyl derivatives (**79-86**) were obtained in 13-60 % yield. The reaction condition and % yields of the dibromoalkylated series were summarized in Table 6. ^1H -NMR spectra of the obtained 3,6-O-dibromoalkyl derivatives, **79-86**, showed similar pattern. The ^1H -NMR data of **79** (Figure 20), taken as an example, displayed two triplets of oxymethylenes at δ_H 4.39 (2H) and 4.35 (2H) and two triplets of methylenes bromide at δ_H 3.74 and 3.68 (each 2H). Other 3,6-O-dibromoalkyl α -mangostin derivatives, the multiplet signals at δ_H 1.4-2.5 for assigned longer aliphatic methylenes were also found.

Table 6 Reaction conditions and % yields for the synthesis of bromoalkyl α -mangostin derivatives

Compound	mmol	Base/Reagent	mmol	Time (h.)	Product	n	% yield
8	0.06	K_2CO_3	0.15	42	79	2	27
		$Br(CH_2)_2Br$	0.15				
8	1.15	K_2CO_3	2.30	20	80	3	34
		$Br(CH_2)_3Br$	2.30				
8	1.22	K_2CO_3	2.44	25	81	4	60
		$Br(CH_2)_4Br$	2.44				
8	1.21	K_2CO_3	3.04	30	82	5	34
		$Br(CH_2)_5Br$	3.04				
8	0.25	K_2CO_3	0.64	22	83	6	51
		$Br(CH_2)_6Br$	0.64				
8	1.21	K_2CO_3	3.04	20	84	7	40
		$Br(CH_2)_7Br$	3.04				
8	1.21	K_2CO_3	3.04	39	85	8	38
		$Br(CH_2)_8Br$	3.04				
8	1.21	K_2CO_3	3.04	48	86	9	13
		$Br(CH_2)_9Br$	3.04				

2.2 Synthesis of 3,6-di-*O*-alkylamino α -mangostin derivatives (89, 36-39, 90-92)

The alkylamino α -mangostin derivatives were synthesized by the reaction between each 3,6-O-dibromoalkyl α -mangostin derivative and *N,N*-diethylamine (in 1:2.5 mol eq.) in DMF at room temperature for about 24 h (Figure 14). Progress of the reaction was monitored by TLC using 30% acetone-hexane with a few drops of NH_4OH as eluting system. Purification of the dialkylamino products under silica gel column was unsuccessful. However, by using alumina (neutral) CC the pure products **89**, **36-39** and **90-92** were afforded in 50-95 % yields. The reaction conditions and % yields of the desired derivatives were summarized in Table 7. $^1\text{H-NMR}$ of the obtained 3,6-di-O-(*N,N*-diethylamino) alkyl derivatives, **89**, **36-39** and **90-92**, showed similar pattern. The $^1\text{H-NMR}$ data of **89** (Figure 21), taken as an example, was similar to that of the dibromoalkylated **79** except for the presence of four additional *N*-ethyl groups at δ_{H} 2.65 (q, 8H) and 1.07 (t, 12H). In addition, the two triplet signals of methylenes bromide shift from δ_{H} 3.74 and 3.68 in **79** to δ_{H} 2.95 in **89** confirm the structure.

Table 7 Reaction conditions and % yields for the synthesis of alkylamino α -mangostin derivatives

Compound	mmol	mmol of NHEt_2	Time (h.)	Product	n	% yield
79	0.01	0.03	24	89	2	75
80	0.03	0.07	30	36	3	50
81	0.04	0.10	24	37	4	52
82	0.03	0.07	20	38	5	84
83	0.03	0.07	21	39	6	57
84	0.06	0.16	26	90	7	50
85	0.07	0.19	27	91	8	95
86	0.05	0.14	20	92	9	75

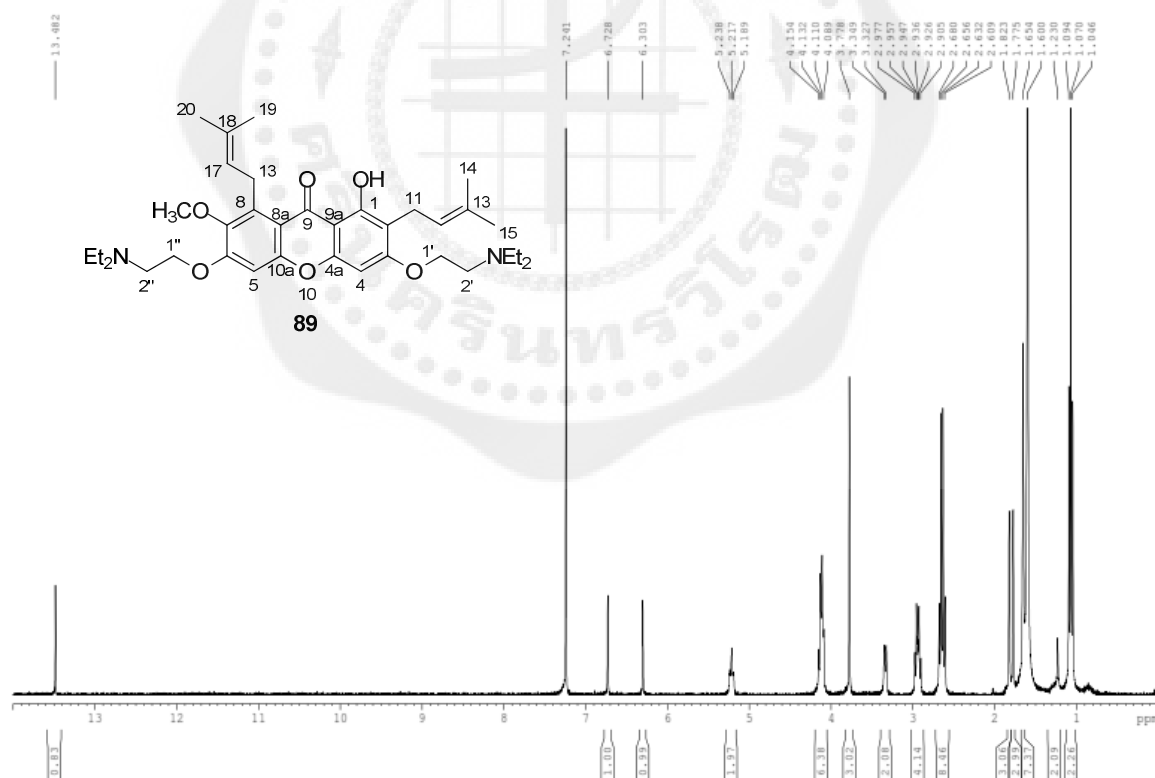


Figure 21 ^1H -NMR spectrum of 3,6-di-O-(2-(N,N-diethylamino) ethyl)- α -mangostin (**89**)

2.3 Synthesis of the mono-bromoalkylated and mono-alkylatedamino α -mangostin

By employing a similar reaction condition as for the dialkylated product preparation, the monoalkylated compound was yielded by reacting an equal mole ratio between α -mangostin, dibromobutane and K_2CO_3 in DMF at room temperature for only 2 h (Figure 13), in which the unreacted starting compound was left as observed in TLC reaction progress monitoring. The reaction mixture was then separated by column chromatography (silica gel) and followed by centrifugal thin-layer chromatography (silica gel) to yield two fractions of **A** and **B**. The major fraction **A** (23 %) was the mixture of the 6-O-bromobutyl (**87**) and the 3-O-bromobutyl (**88**) in a ratio of 11:1 by its ^1H -NMR data analysis (Figure 22A). The presence of only one set of a bromobutyl signal at δ_H 4.09 (H-1'), 3.50 (H-4') and 2.09 (H-2' and H-3'), in addition, with the NOESY correlations were observed between signals at δ_H 4.09 (H-1') and δ_H 6.70 (H-5) and δ_H 3.77 (7-OCH₃) confirmed that the major compound was 6-O-(4-bromobutyl) α -mangostin in fraction of **A**. The minor fraction **B** (2 %) was the mixture of **87** and **88** in a ratio of 1:14 by its ^1H -NMR data analysis (Figure 22B). In a comparison of their ^1H -NMR spectra (Figure 22), it was evident that the 1-OH of **87** (δ_H 13.81) was located at lower field than that of **88** (δ_H 13.41). In addition, the aromatic proton H-5 of **87** (δ_H 6.26) was upper field than that of **88** (δ_H 6.33).

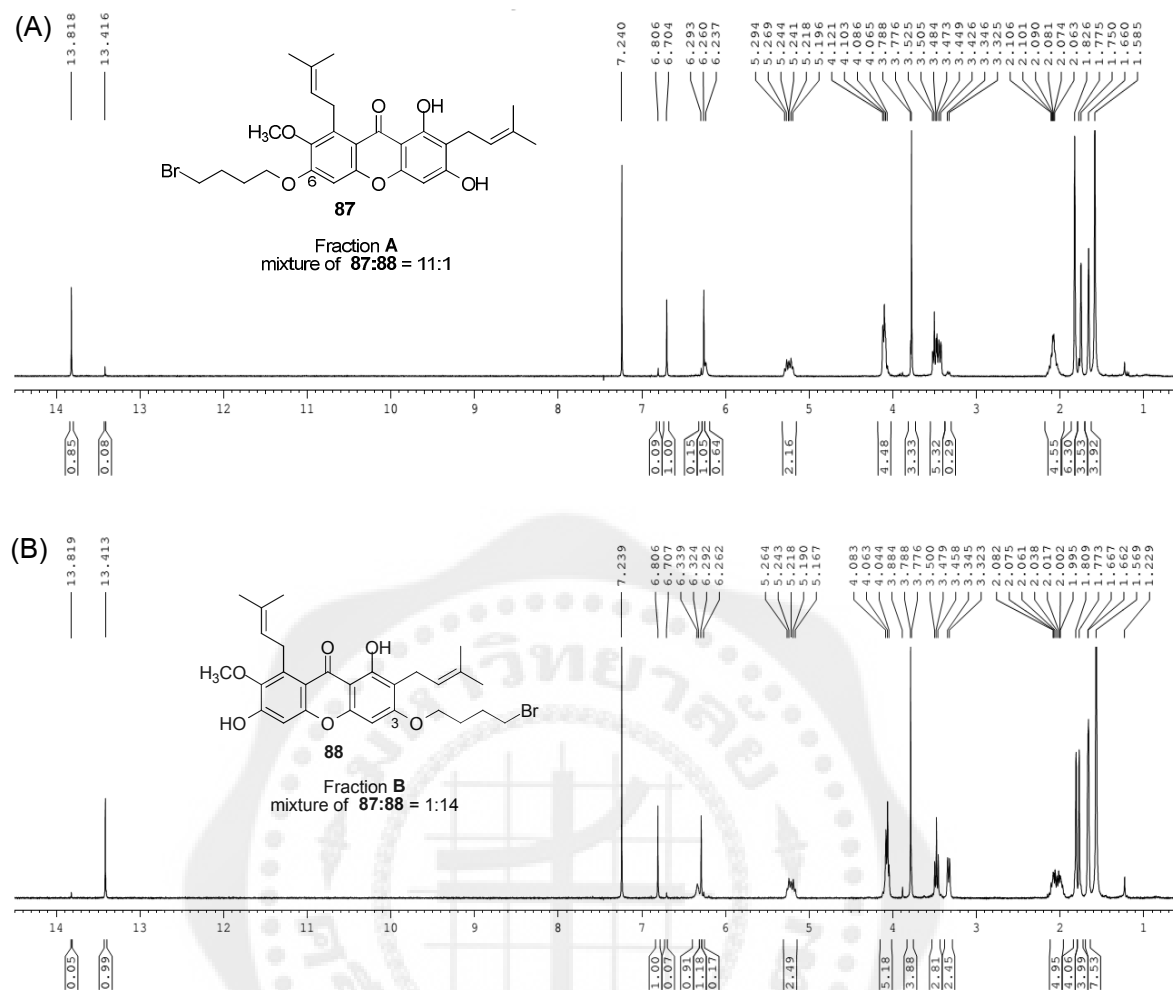


Figure 22 ¹H-NMR spectra of (A) the major fraction **A**, and (B) the minor fraction **B**

The major fraction **A** was further reacted with *N,N*-diethylamine (1: 1 eq.) by using the same method as described above (Figure 15) to give only the 6-*O*-(*N,N*-diethyl-*N*-buthylamino) α -mangostin (**93**) in 56 % yield after chromatographic separation. The ¹H-NMR data of **93** demonstrated two signals of *N*-ethyl groups at δ_H 2.65 (q, 8H) and 1.07 (t, 12H) confirmed the structures for 6-*O*-(*N,N*-diethyl-*N*-buthylamino) α -mangostin. The reaction condition and % yield of **93** were summarized in Table 8.

Table 8 Reaction conditions and % yields for the synthesis of monosubstituted of α -mangostin derivatives

Compound	mmol	Reagent	mmol	Time (h.)	Product	% yield
8	1.24	K ₂ CO ₃	1.24	2	87 + 88	10
		Br(CH ₂) ₄ Br	1.24			
87	0.03	NHEt ₂	0.03	24	93	56

2.4 Synthesis of tetrahydro α -mangostin derivatives (**103**)

In order to investigate the importance of double bond in the prenyl groups on α -mangostin analogue, therefore the tetrahydro α -mangostin (**31**) was synthesized in 98 % yield by catalytic hydrogenation using Parr Hydrogenator. The ¹H-NMR data of **31** was compatible with the reported values (Sudta; et al. 2013: 194-203). In a like manner as for α -mangostin analogue series, selected 3,6-di-O-(4-(*N,N*-diethylamino) butyl) tetrahydro α -mangostin (**103**) was prepared in 87 % yield from its corresponding tetrahydromangostin dibromide **102** (Figure 17). The reaction condition and % yields were summarized in Table 9. The ¹H-NMR data of **103** shows signals for tetrahydro α -mangostin and the *N*-ethyl groups at δ_H 2.53 (q, 8H) and 1.01 (t, 12H).

Table 9 Reaction conditions and % yields for the synthesis of tetrahydro α -mangostin derivatives

Compound	mmol	Reagent	mmol	Time (h.)	Product	% yield
8	1.23	H ₂	-	4	31	98
		Pd-C	cata.			
31	0.58	K ₂ CO ₃	1.46	22	102	30
		Br(CH ₂) ₄ Br	1.46			
102	0.08	NHEt ₂	0.20	24	103	87

2.5 Synthesis of α -mangostin ammonium iodide derivatives (**94-101**)

α -Mangostin ammonium iodide derivatives were prepared by reacting the alkylamino α -mangostin derivatives (**89**, **36-39** and **90-92**) with excess iodomethane in acetone at room temperature for 15-19 h (Figure 16). The products (**94-101**) were obtained in 51-99 % yields. The reaction condition and % yields of the ammonium iodides were summarized in Table 10. $^1\text{H-NMR}$ spectra of the α -mangostin ammonium iodide derivatives, **94-101**, were similar to those of the corresponding alkylamino α -mangostin compounds with additional of two singlets at δ_{H} 2.9-3.2 for the *N*-methyl ammonium groups. Moreover, the methylene protons connected to the ammonium groups were deshielded by its electron withdrawing inductive effect. $^1\text{H-NMR}$ spectrum of **94**, for example, was shown in Figure 23. ESIMS of **94** displays the base peak at m/z 319.4 $[\text{M}]^{2+}$ and m/z 765.2 $[\text{M}+\text{I}]^+$ which were compatible with molecular formula of $\text{C}_{38}\text{H}_{58}\text{N}_2\text{O}_6^{2+}$ and $\text{C}_{38}\text{H}_{58}\text{IN}_2\text{O}_6^+$, respectively. Thus these observed data confirmed the structures for **94** to be α -mangostin-3,6-di-*O*-(*N,N*-diethyl)-*N*-methylethylammonium iodide.

Table 10 Reaction conditions and % yields for the synthesis of α -mangostin diammonium iodide derivatives

Compound	mmol	Time (h.)	Product	n	% yield
89	0.005	15	94	2	98
36	0.04	16	95	3	99
37	0.07	18	96	4	51
38	0.02	15	97	5	94
39	0.01	16	98	6	78
90	0.02	18	99	7	88
91	0.01	19	100	8	99
92	0.01	18	101	9	96

3. Structure and activity relationship of oxygenated xanthenes against *Helicobacter pylori*

The isolated compounds from *G. fusca* were evaluated antibacterial activity against three stains of *H. pylori*, including metronidazole-resistant *H. pylori* ATCC 43504, *H. pylori* DMST 20165 and metronidazole-resistant clinical isolate *H. pylori* HP40. The results (Table 11) revealed that α -mangostin (**8**) (MIC 38.0 μ M) and β -mangostin (**9**) (MIC 36.6 μ M) exhibited similar effect against *H. pylori* ATCC 43504, though none of the compounds assayed was as effective as the control amoxicillin (MIC 5.3 μ M). The result of α -mangostin against the three strains *H. pylori* that was contrasted with the reported activity (MIC 3.80 μ M) (Hasegawa et al.1996). In the case of the *H. pylori* DMST 20165, cowaxanthone (**14**) (MIC 4.6 μ M) displayed the most activity and was about 2.5-fold more potent activity than metronidazole (MIC 11.1 μ M) but less activity than amoxicillin (MIC 0.6 μ M). Isojacareubin (**26**) demonstrated the most activity against *H. pylori* HP40 at MIC 23.9 μ M and was approximately two times more potent than that of amoxicillin (MIC 42.7 μ M), but much lower activity compared with that of clarithromycin (MIC 0.6 μ M).

Table 11 Antibacterial activity of the natural xanthones from *G. fusca* against three stains of *H. pylori*.

Compounds	MIC (μ M)		
	ATCC 43504	DMST 20165	HP 40
α -Mangostin (8)	38.0	19.0	76.1
β -Mangostin (9)	36.6	18.3	147.3
Cowanin (11)	261.2	16.3	130.6
Cowanol (12)	252.8	15.7	126.4
Cowaxanthone (14)	304.6	4.6	152.3
Fuscaxanthone A (17)	262.4	16.3	131.2
Fuscaxanthone G (23)	65.2	16.3	130.6
1,3,5,6-Tetrahydroxyxanthone (25)	>240.3	29.9	240.3
Isojacareubin (26)	47.8	23.9	23.9
Fuscaxanthone I (74)	61	15.2	122.0
Amoxicillin ^a	5.3	0.6	42.7
Metronidazole ^a	-	11.1	-
Clarithromycin ^a	41.8	-	0.6

^aPositive control.

4. Anti-*Helicobacter pylori* activity of the synthesized mangostin analogues and their structural and activity relationship study

Two series of nitrogen analogues of α -mangostin were synthesized and their antibacterial activity against *H. pylori* ATCC 43504 were evaluated (Table 12). The results showed that the alkylamino derivatives bearing 2-4 carbon linkers (**89**, **36**, **37**) were more potent activity than that of the parent compound (**8**) and the longer alkyl chains derivatives (**38**, **39**, **90-92**). Among them, the 3,6-di-O-(4-(*N,N*-diethylamino) butyl)- α -mangostin (**37**) was the most active compound (MIC 5.8 μ M), which was about the same potency as that of the positive control drug amoxicillin (MIC 5.3 μ M) and approximately 8-fold more active than that of clarithromycin (MIC 41.8 μ M). In addition, the disubstituted α -mangostin analogue **37** displayed about 5-fold more active than that of the 6-monosubstituted derivative **93** (MIC 29.0 μ M). The tetrahydro analogues in **31** (MIC 37.6 μ M) and **103** (MIC 5.8 μ M) showed no preference in activity over that of **8** (MIC 38.0 μ M) and **37** (MIC 5.8 μ M), respectively. The α -mangostin ammonium iodide series, **94-101**, exhibit highly increased anti-*H. pylori* activity with MIC ranges of 0.9-7.1 μ M. Compound with the highest anti-*H. pylori* potency (MIC 0.9 μ M) was approximately 5-fold and 40-fold more active than that of the positive control drugs, amoxicillin and claritromycin, respectively.

Table 12 MIC (μM) values of the synthetic xanthenes against *H. pylori* ATCC 43504.

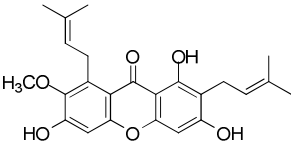
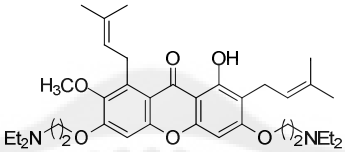
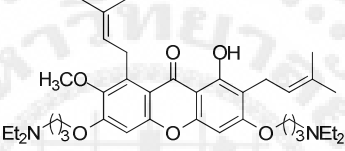
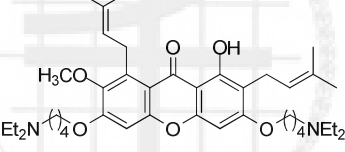
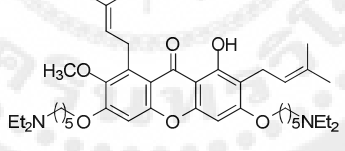
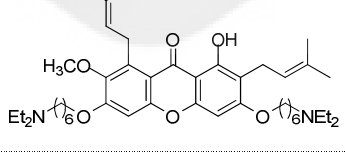
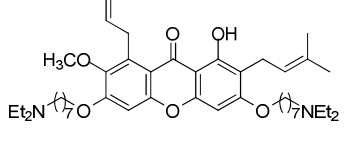
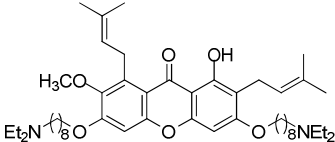
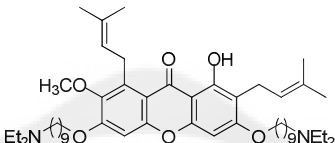
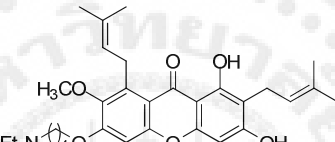
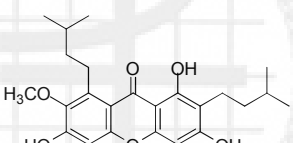
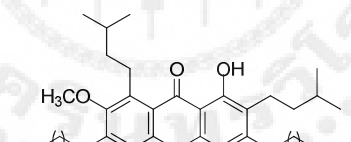
Compound	Structure	MIC (μM)
8		38.0
89		6.4
36		6.1
37		5.8
38		11.2
39		10.8
90		10.2

Table 12 (continued)

Compound	Structure	MIC (μ M)
91		40.2
92		38.8
93		29.0
31		37.6
103		5.8
-	Amoxicillin ^a	5.3
-	Clarithromycin ^a	41.8

^aPositive control.

CHAPTER 5

CONCLUSION

In order to search for the new drugs from the plant-based medicines for eradication of *H. pylori*, the ten natural oxygenated xanthenes from *G. fusca* were screened for the activity. The results showed that α -mangostin (**8**) and β -mangostin (**9**) exhibited moderate activity against *H. pylori* ATCC 43504 with MICs of 38.0 and 36.6 μ M, respectively. Cowaxanthone (**14**) was the most activity (MIC 4.6 μ M) against *H. pylori* DMST 20165 and was about 2.5-fold more potent activity than that of metronidazole. Among the tested compounds against *H. pylori* HP 40, isojacareubin (**26**) showed the highest activity (MIC 23.9 μ M) in the inhibition of bacterial growth. Nevertheless, the rest of compounds were less potency than that of amoxicillin for three strains of *H. pylori*.

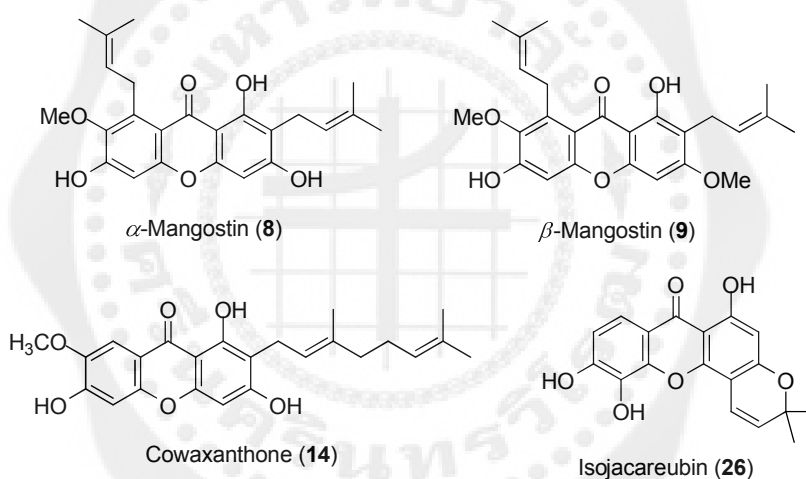
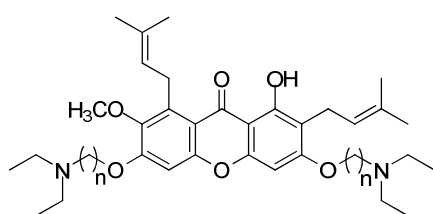


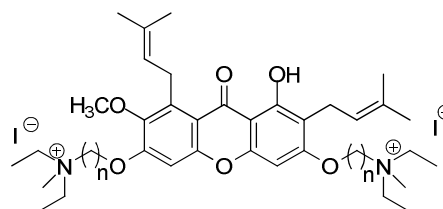
Figure 24 Active compounds from *G. fusca*

Due to the moderate activity against *H. pylori* of α -mangostin, the major metabolite which can be obtained in gram-scale from mangosteen pericarp. The aim of this study is therefore to structural modifications of α -mangostin to enhance its anti-*H. pylori* activity. α -Mangostin was derivatized at the free C3 and C6 hydroxy groups as the alkylamino derivatives with 2-9 carbons linkage and evaluation for their anti-*H. pylori* ATCC 43504 activity. We found that the disubstituted alkylamino group with 2-4 carbons linkage (**89**, **36**, and **37**) increased the activity with MIC ranges of 5.8-6.4 μ M. In addition, the disubstituted α -mangostin derivatives displayed more active than that of the 6-monosubstituted derivative. The isopentyl groups showed no preference in activity over that of the prenyl groups on the α -mangostin analogue. The α -mangostin ammonium iodide series, **94-101**, exhibit highly

increased anti-*H. pylori* activity with MIC ranges of 0.9-7.1 μM . Compound with the highest anti-*H. pylori* potency was 5-fold and 40-fold more active than that of the positive control drugs, amoxicillin and claritromycin, respectively.

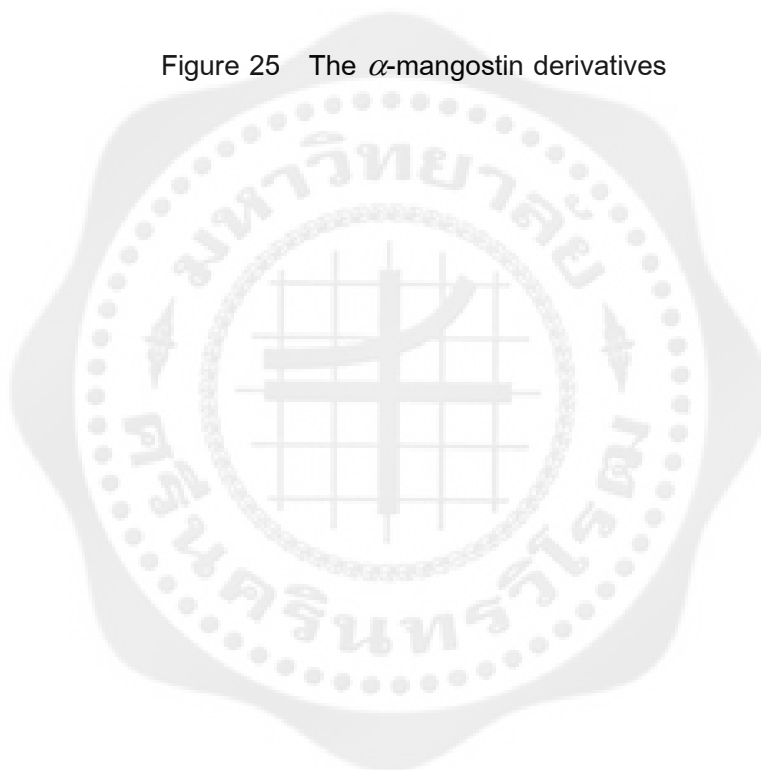


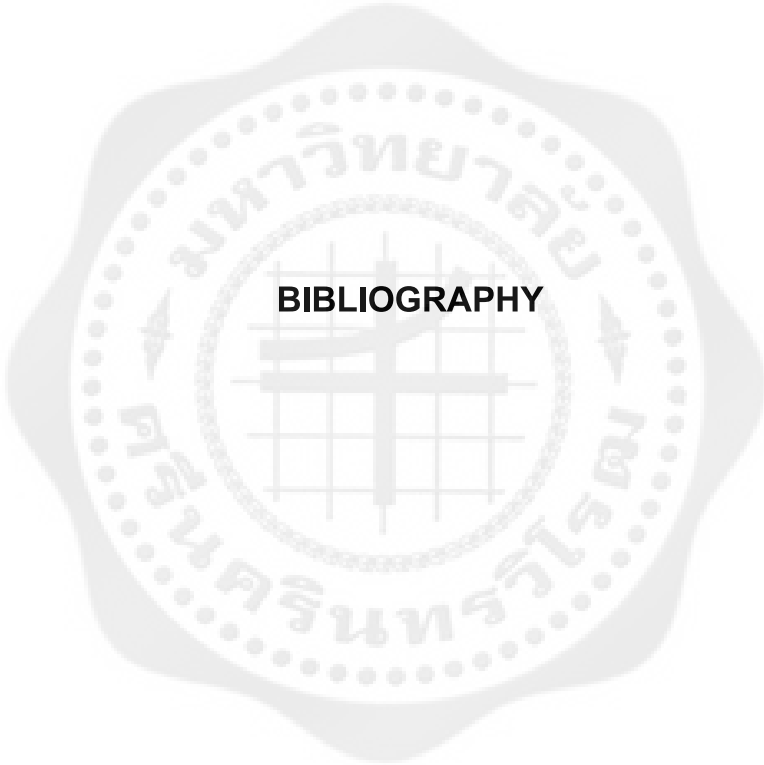
Alkylamino derivatives of α -mangostin



Ammonium iodide derivatives of α -mangostin

Figure 25 The α -mangostin derivatives





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BIBLIOGRAPHY

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LIST OF ABBREVIATIONS AND SYMBOLS

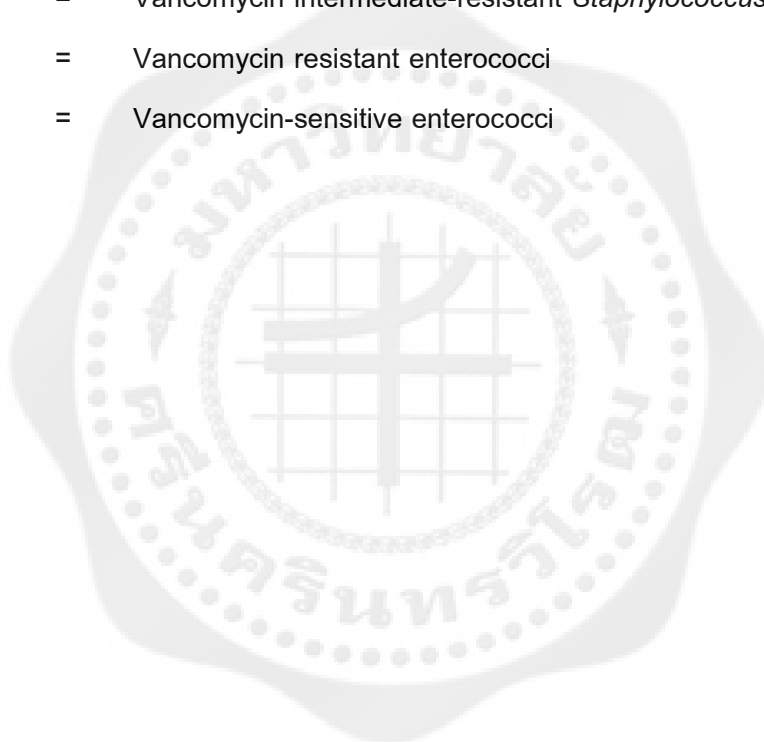
TPA	=	12-O-Tetradecanoylphorbol-13-acetate
α	=	Alpha
β	=	Beta
BHI	=	Brain Heart Infusion
CFU	=	Colony forming unit
CB	=	Columbia Blood agar Base
COSY	=	Correlation spectroscopy
COX-2	=	Cyclooxygenase-2
CagA	=	Cytotoxin associated protein
$^{\circ}\text{C}$	=	Degree Celsius
CDCl_3	=	Deuteriochloroform
CD_3OD	=	Deuteriomethanol
DMSO	=	dimethyl sulfoxide
DMSO-d_6	=	Dimethylsulfoxide- d_6
DEPT	=	Distortionless Enhancement by Polarization Transfer
d	=	Doublet (for NMR data)
dd	=	Doublet of doublets (for NMR data)
dt	=	Doublet of triplet (for NMR data)
EBV-EA	=	Epstein-Barr virus early antigen
EMP	=	Ethambutol
γ	=	Gamma
GSH	=	Glutathione
HC_{50}	=	Half maximal hemolysis concentration
IC_{50}	=	Half maximal inhibitory concentration
Hz	=	Hertz
HMBC	=	Heteronuclear multiple bond coherence

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

HMQC	=	Heteronuclear multiple quantum coherence
h	=	Hour
HIV	=	Human immunodeficiency virus
IR	=	Infrared
INH	=	Isoniazid
kg	=	Kilogram
MRSA	=	Methicillin resistant <i>Staphylococcus aureus</i>
μg	=	Microgram
μM	=	Micromolarity
mg	=	Milligram
mL	=	Milliliter
MIC	=	Minimum inhibitory concentration
mol	=	Mole
MDR	=	Multidrug-resistant
m	=	Multiplet (for NMR data)
nm	=	Nanometer
NSAIDs	=	Nonsteroidal antiinflammatory drugs
NMR	=	Nuclear magnetic resonance spectroscopy
NOESY	=	Nuclear Overhauser effect spectroscopy
PUD	=	Peptic ulcer disease
pmol	=	Picomole
PPI	=	Proton pump inhibitors
q	=	Quartet (for NMR data)
RMP	=	Rifampin
RT	=	Room temperature
s	=	Singlet (for NMR data)

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

SD	=	Standard deviation
SM	=	Streptomycin
TLC	=	Thin layer chromatography
t	=	Triplet (for NMR data)
UV	=	Ultraviolet
VacA	=	Vacuolating cytotoxin
VISA	=	Vancomycin intermediate-resistant <i>Staphylococcus aureus</i>
VRE	=	Vancomycin resistant enterococci
VSE	=	Vancomycin-sensitive enterococci



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1. Nontakham J, Charoenram N, **Upamai W**, Taweechotipatr M, Suksamrarn S. (2013). Anti-*Helicobacter pylori* xanthones of *Garcinia fusca*. *Arch. Pharm. Res.* doi: 10.1007/s12272-013-0266-4.
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1. **Upamai W**, Nontakham J, Jaratrungtawee A, Taweechotipatr M, Suksamrarn S. Anti-*Helicobacter pylori* activity of xanthones from *Garcinia fusca* and *Garcinia mangostana*. PERCH-CIC Congress VIII. Jomtien Palm Beach Hotel & Resort Pattaya, Chonburi, Thailand, May 5-8, 2013. Poster number S2-P45.
2. Pongpun N, Sinchai T, **Upamai W**, Wipasakornwarawut J, Worawit C, Decha S, Tipchakchaluk N, Wongskul T. Optimization of rapid extraction for determination of alprazolam, diazepam and lorazepam in urine sample by HPLC/UV. The 39th Congress on Science and Technology of Thailand. BITEC, Bangkok, Thailand, October 21-23, 2013. Poster number C1_C0103.

3. **Upamai W**, Nontakham J, Jaratrungtawee A, Taweechotipatr M, Suksamrarn S. Anti-*Helicobacter pylori* activity of xanthenes from *Garcinia fusca* and *Garcinia mangostana*. CRMA Exhibition 2013. Chulachomklao Royal Military Academy, Nakhonnayok, Thailand, November 20-21, 2013. page 91.

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