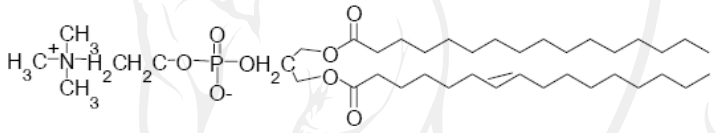
APPENDICES

APPENDIX A

Chemical and physical properties of substances used in this study

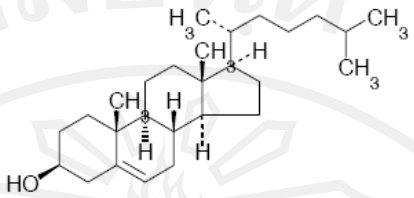
1. L-alpha dipalmitoyl phosphatidylcholine

Name	L- alpha dipalmitoyl phosphatidylcholine (DPPC)
Chemical name	1,2-palmitoyl-sn-glycero-3-phosphocholine
Synonyms	DPPC
Structure	
Empirical formula	$C_{24}H_{46}O_6$
Molecular weight	734.05
Description	white powder
Melting point	41 ^{o}C
Solubility	soluble in most organic solvents, insoluble but dispersible in water

2. Cholesterol

Name	Cholesterol
Chemical name	Cholest-5-en-3β-ol
Synonyms	Cholesterin

Structure



Empirical formula $C_{27}H_{46}O$

Molecular weight 386.67

Description White or faintly yellow, almost odorless, needles

Melting point 147 - 150 ^{o}C

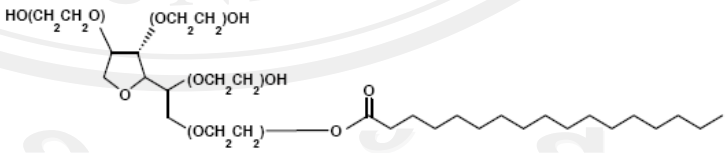
Solubility soluble in acetone, chloroform, ether, fixed oils, practically insoluble in water

3. Polyoxyethylene (4) sorbitan monostearate

Chemical name Polyoxyethylene (4) sorbitan monostearate

Synonyms Polysorbate 61, Tween61

Structure



Empirical formula $C_{32}H_{62}O_{10}$

Molecular weight 607

Description Tan solid

HLB 9.6

Solubility dispersible in water, soluble in ethanol

Hydroxyl value 170 – 200

4. Dimethyldioctadecylammonium bromide

Chemical name Dimethyldioctadecylammonium bromide

Synonyms DDAB, DODAB

Structure

Empirical formula $C_{38}H_{80}NBr$

Molecular weight 630.95

Description White powder

Solubility soluble in ethanol

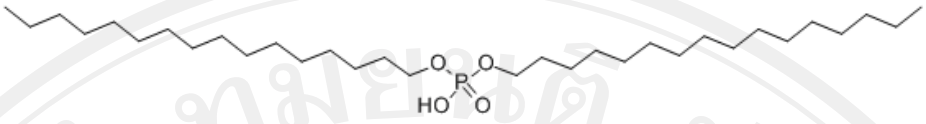
Melting point ~160°C

5. Dicetylphosphate

Chemical name Dicetylphosphate

Synonyms DP, dihexadecyl phosphate

Structure

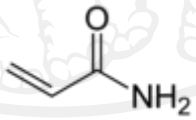


Empirical formula	$C_{32}H_{67}O_4P$
Molecular weight	546.85
Description	White powder
Solubility	Clear to slightly hazy colorless to faint yellow solution at 50 mg/ml in chloroform
Melting point	74-75°C

6. Acrylamide

Chemical name	prop-2-enamide
Synonyms	acrylic amide

Structure

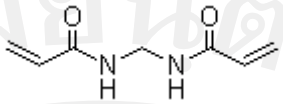


Empirical formula	C_3H_5NO
Molecular weight	71.08
Description	white odourless crystalline solid
Solubility	soluble in water, ethanol, ether, and chloroform
Melting point	84.5°C

7. N, N'-methylbisacrylamide

Chemical name	N, N'-Methylenebis (2-propenamide)
Synonyms	Bis-acrylamide, MBA, NAPP

Structure



Empirical formula $C_7H_{10}N_2O_2$

Molecular weight 154.17

Description white odourless powder

Solubility soluble in water and ethanol

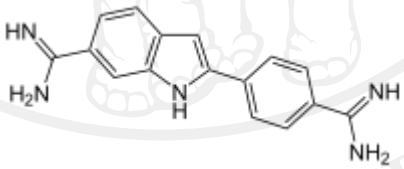
Melting point 185°C

8. 4', 6-diamidino-2-phenylindole (DAPI)

Chemical name 2-(4-amidinophenyl)-1H -indole-6-carboxamidine

Synonyms DAPI

Structure



Empirical formula $C_{16}H_{15}N_5$

Molecular weight 277.32

Description fluorescent stain, binds strongly to A-T rich regions in DNA, extensively use in fluorescence microscopy

Maximum absorption 358 nm

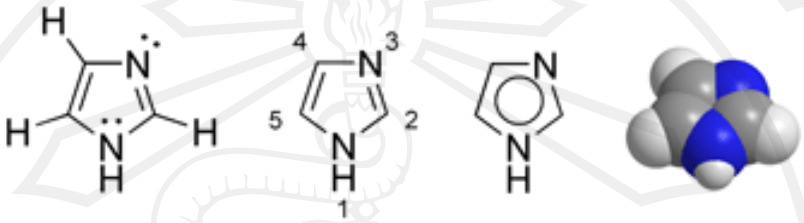
Maximum emission 461 nm

9. Imidazole

Chemical name 1H-Imidazole

Synonyms 1, 3-diazole, glyoxaline (archaic), 1, 3-diazacyclopenta-2,4-diene

Structure



Empirical formula $C_3H_4N_2$

Molecular weight 68.08

Description white to yellow crystals or powder, with an amine odour

Solubility soluble in water and other polar solvents

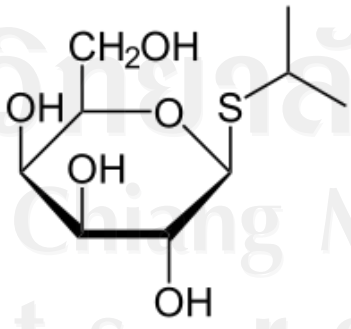
Melting point 89-91°C

10. Isopropyl-D-thiogalactopyranoside (IPTG)

Chemical name Isopropyl-β-D-1-thiogalactopyranoside

Synonyms IPTG

Structure

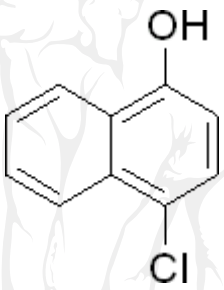


Empirical formula $C_9H_{18}O_5S$

Molecular weight	238.30
Description	white powder
Solubility	soluble in water
Melting point	105-115°C

11. 4-chloronaphthol

Chemical name	4-chloro-1-naphthol
Synonyms	4-CN; Chloronapthol; 4-chloro-1-Naphthalenol
Structure	



Empirical formula	$C_{10}H_7OCl$
Molecular weight	178.58
Description	off white to tan crystals
Solubility	soluble in methanol
Melting point	118-121°C

APPENDIX B

Plasmid map

Restriction sites of the GFP template plasmid (pWH105-gfpmut2) map

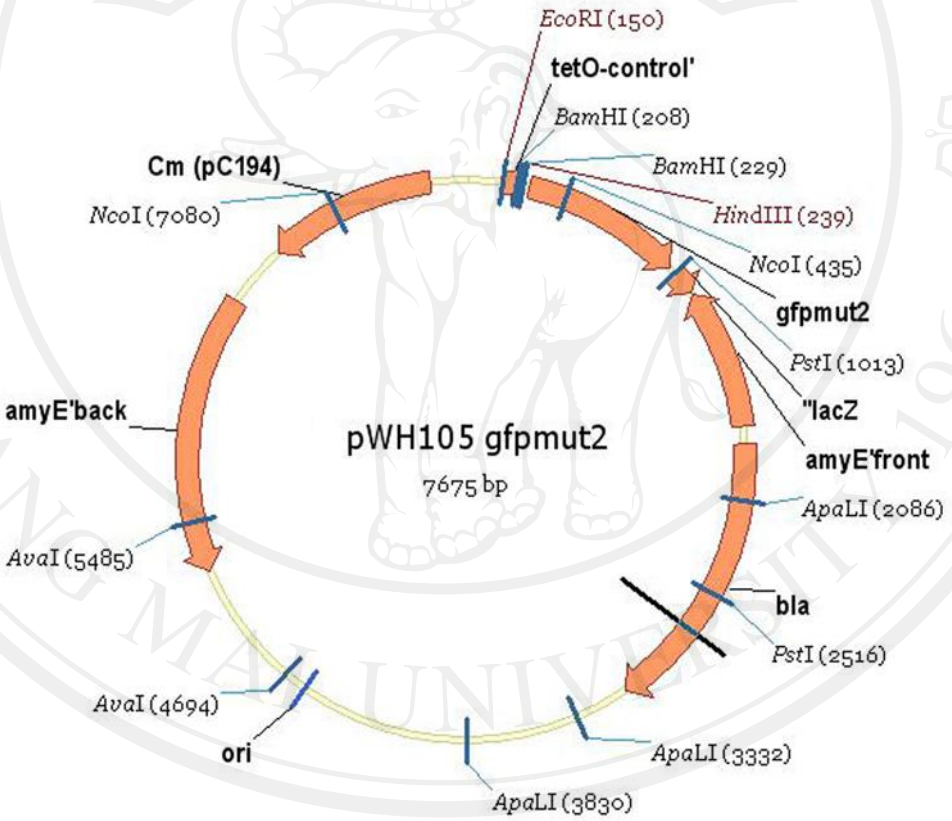


Figure B.1 Restriction sites of the pWH105-gfpmut2 map

Restriction sites of expression vector [pET28a(+)] map

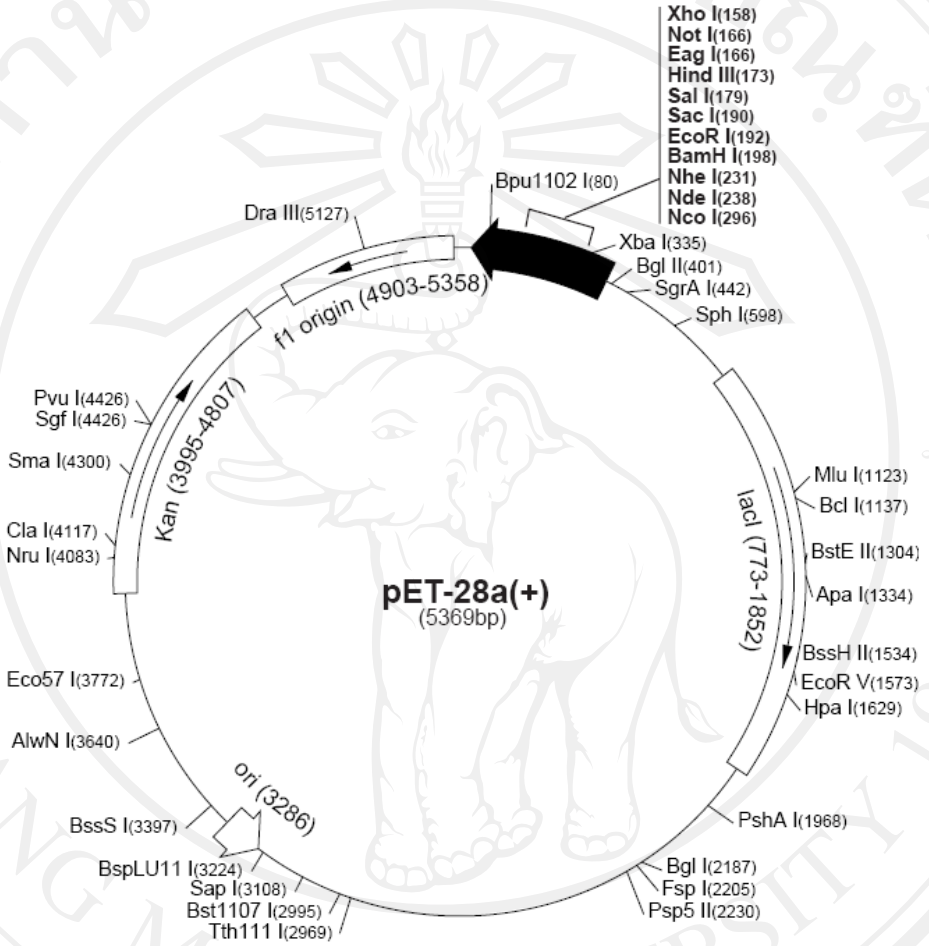


Figure B.2 Restriction sites of the pET28a(+) map under T7 promoter

APPENDIX C

Formulation of buffer and solution used in this study

1. Tris-Acetate-EDTA (TAE) buffer (50X)

Tris base	242 g
Acetic Acid	57.1 ml
0.5 M EDTA (shake vigorously before use)	100 ml

Add distilled H_2O to 1 Liter and adjust pH to 8.5 using KOH

2. Loading dye

0.5 M Tris-HCl, pH 6.8	1.2 ml
Glycerol	1 ml
Bromphenol blue	2.5 mg

Add distilled H_2O to 10 ml

3. Phosphate buffer saline, PBS (10X)

NaCl	8 g
KCl	0.2 g
$Na_2HPO_4 \cdot 2H_2O$	1.44 g
KH_2PO_4	0.24 g

Add distilled H_2O to 100 ml

4. Semi-dry transfer buffer

Tris base	1.51 g
Glycine	5.63 g
MeOH	50 ml

Add distilled H_2O to 500 ml and adjust pH to 8.3

5. TBS buffer

Tris-HCl	0.605 g
NaCl	4.385 g

Add distilled H_2O to 500 ml and adjust pH to 7.5

6. TBS-Tween buffer

Tris-HCl	1.21 g
NaCl	14.62 g
Tween 20	25 ml

Add distilled H_2O to 500 ml and adjust pH to 7.5

7. TBS-Tween/Triton buffer

Tris-HCl	1.21 g
NaCl	14.62 g
Tween 20	25 ml
Triton X-100	100 ml

Add distilled H_2O to 500 ml and adjust pH to 7.5

APPENDIX D

Amino acids sequence of GFPmut2

Table D Amino acid sequences of GFPmut2 (237 amino acids)

GFPmut2 amino acid sequence
MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGK LPVPWPTLVTTFAYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDG NYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADK QKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSK DPNEKRDHMVLLEFVTAAGITHGMDELYK

APPENDIX E

Calculation of nanovesicle compositions

The amount of each composition in nanovesicular formulations was calculated from the following equation:

Required amount (g) = molar ratio in the formulation × concentration of the nanovesicles (Molar) × required volume (L) × molecular weight

For example, to prepare 100 ml of neutral niosomes (20 mM) composed of Tween61: CHL at 1:1 molar ratio

The required amount of tween61 and CHL was as follows:

$$\text{Tween61 (g)} = \frac{1}{2} \times 20 \times 10^{-3} \times 100 \times 10^{-3} \times 1{,}311.7 = 1.3117 \text{ g}$$

$$\text{CHL (g)} = \frac{1}{2} \times 20 \times 10^{-3} \times 100 \times 10^{-3} \times 366.66 = 0.3667 \text{ g}$$

Table E Amounts of the composition in the prepared nanovesicles

Formulation	**Composition**	**Amount (g)***
Neutral niosomes	Tween61	0.2623
(Tween61:CHL = 1:1)	CHL	0.0733
Cationic niosomes	Tween61	0.2099
(Tween61:CHL:DDAB = 1:1:0.05)	CHL	0.0606
	DDAB	0.0587

Table E Amounts of the composition in the prepared nanovesicles (continued)

Formulation	Composition	Amount (g)
Anionic niosomes	Tween61	0.2099
(Tween61:CHL:DP = 1:1:0.05)	CHL	0.0606
	DP	0.0053
Neutral liposomes	DPPC	0.2055
(DPPC:CHL = 7:3)	CHL	0.0439
Cationic liposomes	DPPC	0.2055
(DPPC:CHL:DDAB = 7:2:1)	CHL	0.0309
	DDAB	0.0252
Anionic liposomes	DPPC	0.2055
(DPPC:CHL:DP = 7:2:1)	CHL	0.0309
	DP	0.0219

* The specified amount was for the preparation of 20 ml, 20 mM nanovesicles formulation

CURRICULUM VITAE

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Education

1995-2000 Secondary School Certificate
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2001-2005 Bachelor's degree in Pharmacy (B. Pharm, First Class Hons), Chiang Mai University, Thailand

2006-2011 A Ph.D candidate at Faculty of Pharmacy Chiang Mai University, Thailand under the RGJ-Ph.D Program of TRF

Scholarships and Awards

2004 Scholarship from Thailand Research Fund for the undergraduate student research project, "Development of cosmetic formulations containing extracts from *Centella asiatica* (Pennywort) and Allium cepa (onion) for scar treatment"

2005 Scholarship from Thailand Research Fund for the undergraduate student research project, "Development of nanoparticulate cosmetic formulations containing extracts from medicinal herbs for hair loss treatment and hair growth promotion"

2006 The Royal Golden Jubilee scholarship under Ph.D. Program, 2006-2011 (Thailand Research Fund)

Scientific Presentations

1. Aranya Manosroi, **Warangkana Lohcharoenkal**, Rolf G.Werner, Friedrich Götz and Jiradej Manosroi. The release profile of gallidermin from anionic liposomes evaluated by Franz diffusion cells, The RGJ Congress IX, Jomtien Palm Beach Resort Pattaya, Chonburi, Thailand, 4-6 April 2008 (Poster presentation)
2. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Friedrich Götz, Rolf G. Werner and Aranya Manosroi. Uptake efficiency enhancement of GFP into HT-29 cell line by HIV-Tat peptide, RGJ seminar series LXII, Faculty of Science, Chiang Mai University, 16 September 2009 (Oral presentation)
3. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Friedrich Götz, Rolf G. Werner and Aranya Manosroi. Uptake efficiency enhancement of GFP into HT-29 cell line by HIV-Tat peptide and an entrapment in nanovesicles, German-Thai Symposium on Nanoscience and Nanotechnology, Chiang Mai Orchid Hotel, Chiang Mai, Thailand, 21 September 2009 (Oral presentation)
4. Aranya Manosroi, **Warangkana Lohcharoenkal**, Rolf G.Werner, Friedrich Götz and Jiradej Manosroi. Potent cellular uptake enhancement of GFP-TAT fusion protein entrapped in elastic nanovesicles. RGJ-Ph.D. Congress XI, Jomtien Palm Beach Resort Pattaya, Chonburi, Thailand, 1-3 April 2010 (Oral presentation)
5. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Friedrich Götz, Rolf G. Werner and Aranya Manosroi. Polioviral receptor binding ligand: A novel and safe peptide drug carrier from polioviral capsid. RGJ-Ph.D. Congress XII,

Jomtien Palm Beach Resort Pattaya, Chonburi, Thailand, 1-3 April 2011 (Oral presentation)

6. **Warangkana Lohcharoenkal**, Aranya Manosroi, Friedrich Götz, Rolf G. Werner and Jiradej Manosroi. Potent enhancement of GFP uptake into HT-29 cells and rat skin permeation by co-incubation with Tat peptide. The 12th Barrier Function of Mammalian Skin 2011, Waterville Valley Resort, New Hampshire, USA, 7-12 August 2011 (Poster presentation)

Publications

1. Aranya Manosroi, Penpan Khanrin, Rolf G. Werner, Friedrich Götz, **Warangkana Lohcharoenkal**, Worapaka Manosroi and Jiradej Manosroi. Transdermal absorption enhancement through rat skin of Gallidermin loaded in niosomes. Int J Pharm. 2010; 392: 304-310.
2. Aranya Manosroi, **Warangkana Lohcharoenkal**, Friedrich Götz, Rolf G. Werner, Worapaka Manosroi and Jiradej Manosroi. Transdermal absorption enhancement of N-terminal Tat-GFP fusion protein (TG) loaded in novel low toxic elastic anionic niosomes. J Pharm Sci. 2011; 100 (4): 1525-1534.
3. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Friedrich Götz, Rolf G. Werner, Worapaka Manosroi and Aranya Manosroi. Cellular uptake enhancement of Tat-GFP fusion protein loaded in elastic niosomes. J Biomed Nanotechnol. 2011; 7: 366-376.
4. **Warangkana Lohcharoenkal**, Aranya Manosroi, Friedrich Götz, Rolf G. Werner and Jiradej Manosroi. Potent enhancement of GFP uptake into HT-29

cells and rat skin permeation by co-incubation with Tat peptide. J Pharm Sci. 2011; 100 (11): 4766-4773.

5. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Friedrich Götz, Rolf G. Werner, Worapaka Manosroi and Aranya Manosroi. Polioviral capsid protein: novel and safe carrier with oral peptide delivery potential. Drug Deliv. (Accepted 06/09/2011).
6. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Friedrich Götz, Rolf G. Werner, Worapaka Manosroi and Aranya Manosroi. Novel application of polioviral capsid: Development of a potent and prolonged oral calcitonin using polioviral receptor binding ligand and Tat peptide. Submitted.
7. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Friedrich Götz, Rolf G. Werner, Worapaka Manosroi and Aranya Manosroi. Enhancement of transdermal absorption and stability of salmon calcitonin by Tat peptide. Submitted.
8. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Parirat Khonsung, Worapaka Manosroi and Aranya Manosroi. Potent antihypertensive effects of Thai Lanna medicinal plant recipes selected from Manosroi II database. Submitted.
9. Jiradej Manosroi, **Warangkana Lohcharoenkal**, Parirat Khonsung, Worapaka Manosroi and Aranya Manosroi. Antihypertensive effect of Thai medicinal plant selected from Manosroi II database. Submitted.
10. Aranya Manosroi, **Warangkana Lohcharoenkal**, Bang-on Kietthanakorn, Worapaka Manosroi and Jiradej Manosroi. *In vitro* immunostimulating

activity of sap from fermented Thai rice on human and murine neutrophils. In preparation.