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APPENDICES

APPENDIX A

Chemical and physical properties of substances used in this study

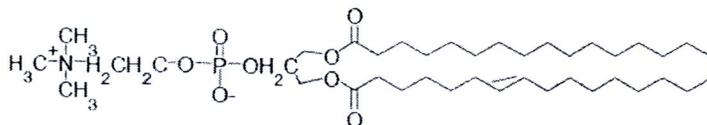
1. L-alpha dipalmitoyl phosphatidylcholine

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 $41^{\circ} C$

Solubility soluble in most organic solvents, insoluble but dispersible in water

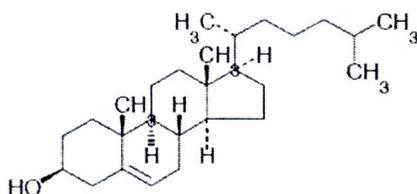
2. Cholesterol

Name Cholesterol

Chemical name Cholest-5-en-3 β -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 $147 - 150^{\circ}C$

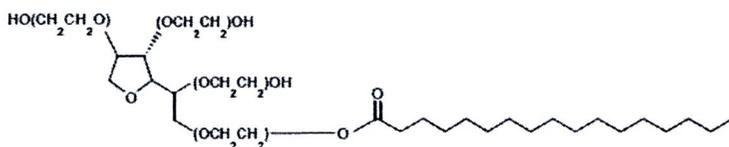
Solubility soluble in acetone, chloroform, ether, fixed oils, practically insoluble in water

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

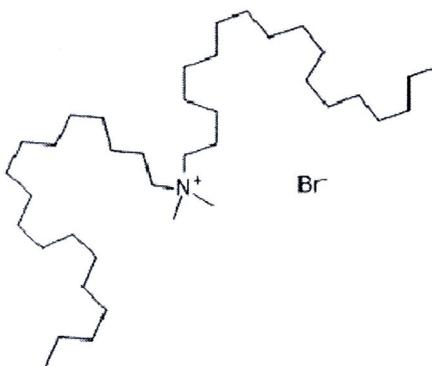
Solubility	dispersible in water, soluble in ethanol
Hydroxyl value	170 – 200

4. Dimethyldioctadecylammonium bromide

Chemical name Dimethyldioctadecylammonium bromide

Synonyms DDAB, DODAB

Structure



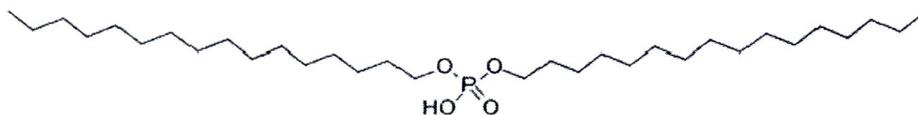
Empirical formula	$C_{38}H_{80}NBr$
Molecular weight	630.95
Description	White powder
Solubility	soluble in ethanol
Melting point	$\sim 160^{\circ}C$

5. Dicetylphosphate

Chemical name Dicetylphosphate

Synonyms DP, dihexadecyl phosphate

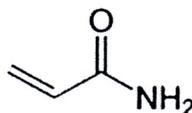
Structure



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	

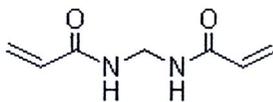


Empirical formula	C_3H_5NO
Molecular weight	71.08
Description	white odourless crystalline solid
Solubility	soluble in water, ethanol, ether, and chloroform
Melting point	84.5°C

7. N, N'-methylbisacrylamide

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

Structure

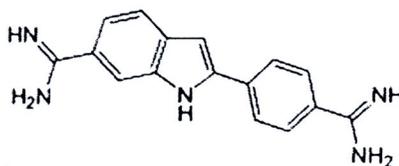


Empirical formula	$C_7H_{10}N_2O_2$
Molecular weight	154.17
Description	white odourless powder
Solubility	soluble in water and ethanol
Melting point	185°C



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

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

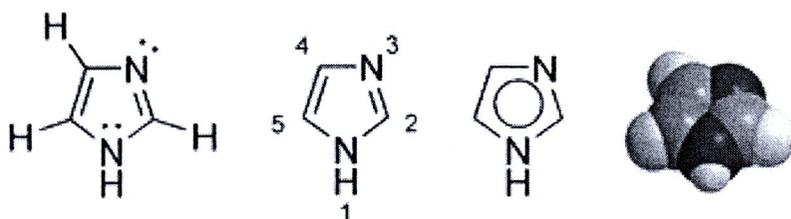


Empirical formula	$C_{16}H_{15}N_5$
Molecular weight	277.32
Description	fluorescent stain, binds strongly to A-T rich regions in DNA, extensively use in fluorescence microscopy
Maximum absorption	358 nm
Maximum emission	461 nm

9. Imidazole

Chemical name	1H-Imidazole
Synonyms	1, 3-diazole, glyoxaline (archaic), 1, 3-diazacyclopenta-2,4-diene

Structure

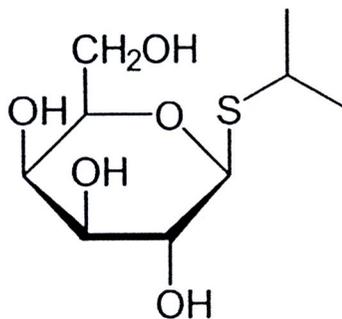


Empirical formula	$C_3H_4N_2$
Molecular weight	68.08
Description	white to yellow crystals or powder, with an amine odour
Solubility	soluble in water and other polar solvents
Melting point	89-91°C

10. Isopropyl-D-thiogalactopyranoside (IPTG)

Chemical name	Isopropyl- β -D-1-thiogalactopyranoside
Synonyms	IPTG

Structure



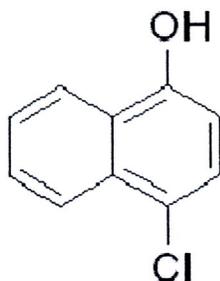
Empirical formula	$C_9H_{18}O_5S$
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Molecular weight	238.30
Description	white powder
Solubility	soluble in water
Melting point	105-115°C

11. 4-chloronaphthol

Chemical name	4-chloro-1-naphthol
Synonyms	4-CN; Chloronaphthol; 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

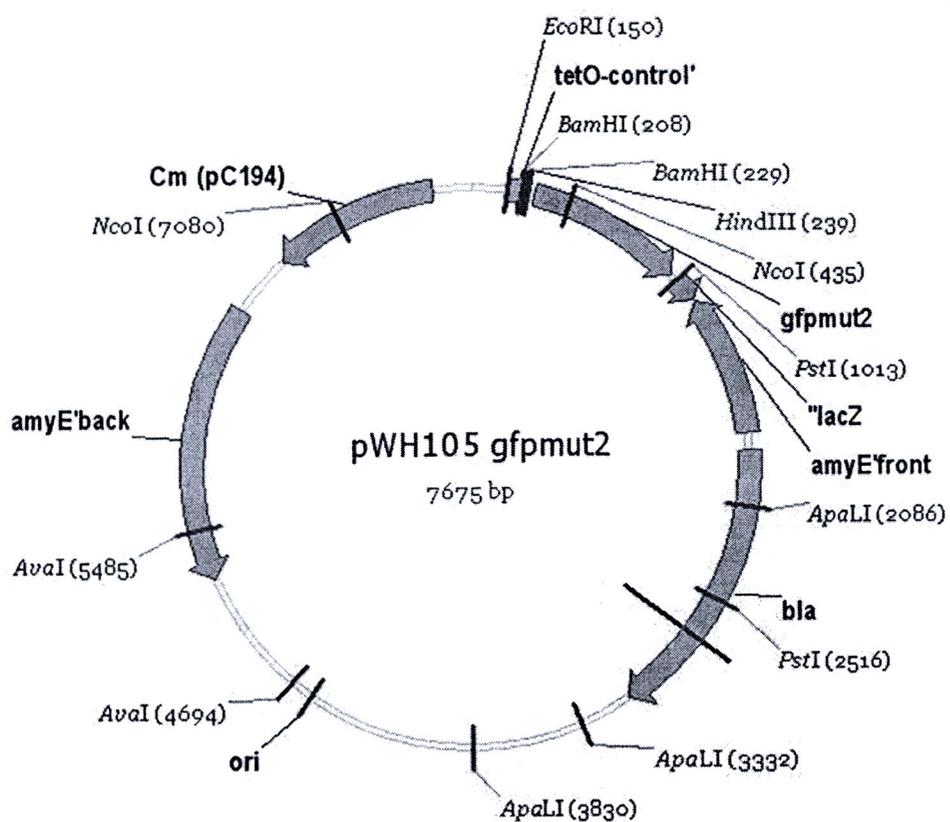


Figure B.1 Restriction sites of the pWH105-gfpmut2 map

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

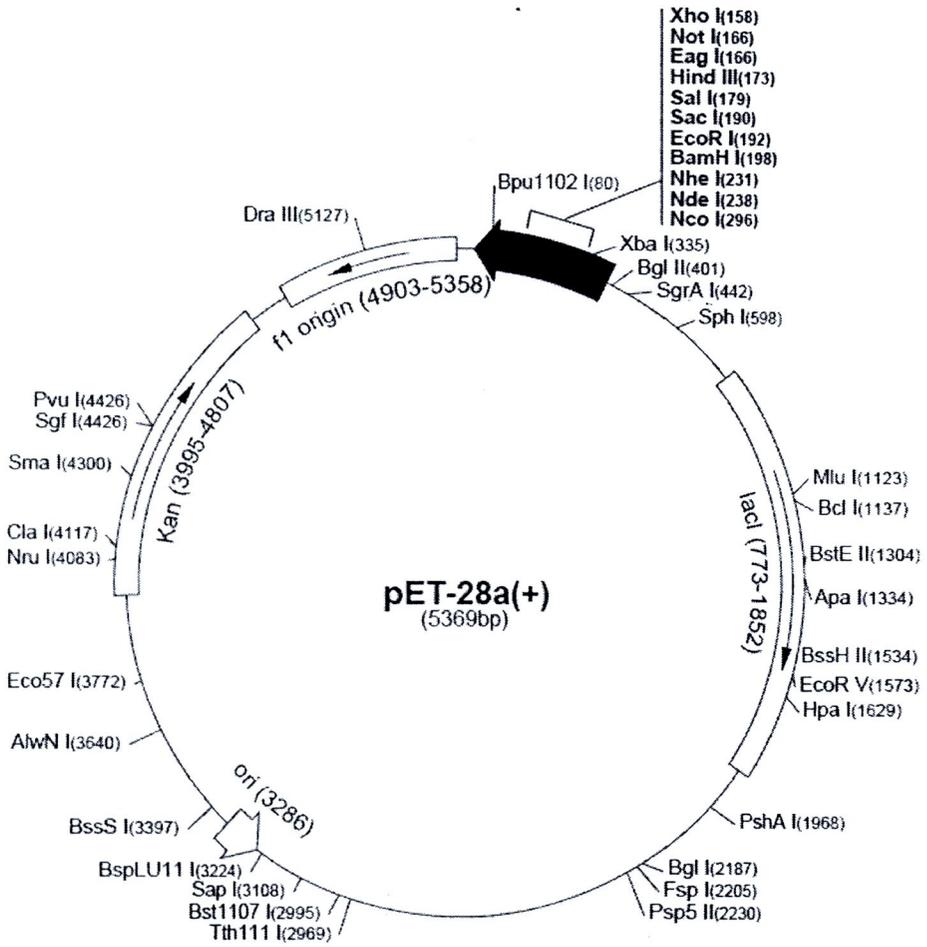


Figure B.2 Restriction sites of the pET28a(+) map under T7 promoter

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 ₂ O 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 ₂ O to 10 ml	

3. Phosphate buffer saline, PBS (10X)

NaCl	8 g
KCl	0.2 g
Na ₂ HPO ₄ ·2H ₂ O	1.44 g
KH ₂ PO ₄	0.24 g
Add distilled H ₂ O to 100 ml	

4. Semi-dry transfer buffer

Tris base	1.51 g
Glycine	5.63 g
MeOH	50 ml

Add distilled H₂O to 500 ml and adjust pH to 8.3

5. TBS buffer

Tris-HCl	0.605 g
NaCl	4.385 g

Add distilled H₂O 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 H₂O 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 H₂O 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
MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLLKFICTTGK LPVPWPTLVTTFAAYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDG NYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADK QKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSK DPNEKRDHMLLEFVTAAGITHGMDELYK



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 \times concentration of the nanovesicles (Molar) \times required volume (L) \times 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:CHL = 1:1)	Tween61	0.2623
	CHL	0.0733
Cationic niosomes (Tween61:CHL:DDAB = 1:1:0.05)	Tween61	0.2099
	CHL	0.0606
	DDAB	0.0587

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

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

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

CURRICULUM VITAE

Name	Ms. Warangkana Lohcharoenkal
Date of birth	8 th March 1984
Education	1995-2000 Secondary School Certificate Dara Academy, Chiang Mai, Thailand
	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 <i>Centella asiatica</i> (Pennywort) and <i>Allium cepa</i> (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.



