APPENDICES

APPENDIX A

List of chemicals and materials used in the study

Chemicals	Sources
2',7'-Dichlorohyddrofluorescin diacetate	Sigma-Aldrich, USA
Calcein-AM	Invitrogen,USA
Desferal (DFX)	GPO, Thailand
Desferrioxamine (DFO)	Switzerland Novartis
Desferrioxamine (DFP)	GPO, Thailand
D-glucose (Dextrose)	Amresco Inc., USA
Diethyl ether	LAB-SCAN, Australia
Dihydrogen phosphate potassium (KH_2PO_4)	Sigma-Aldrich, USA
Dimethyl sulfoxide (DMSO)	Fisher Scientific, UK
Disodium hydrogen phosphate (Na_2HPO_4)	Sigma-Aldrich, USA
D-sorbitol	Amresco Inc., USA
Ethanol (Absolute)	E. Merck, Germany
Gentamicin sulfate 40 mg/ml, 2 ml	Atlantic Laboratories, Thailand
Giemsa's azur eosin methylene blue solution	E. Merck, Germany
Glycerol	Univar, USA
HEPE	Sigma-Aldrich, USA
Hypoxanthine	Sigma-Aldrich, USA
Methanol	E. Merck, Germany
Potassium chloride (KCl)	Sigma-Aldrich, USA
Sodium bicarbonate (NaHCO3)	E. Merck, Germany
Sodium chloride (NaCl)	Sigma-Aldrich, USA
SYBR Green I	Invitrogen,USA
SYTO-61	Invitrogen,USA
RPMI 1640 medium	GIBCO, USA

APPENDIX B

List of instruments and equipments used in the study

Instruments and equipments	Sources
1.5 ml Microcentrifuge tube	Biologix
10 ml Glass Serological Pipette	Pyrex
15 ml Round-bottom centrifuge tube	Corning
5 ml Glass Serological Pipette	Pyrex
5 ml Round-bottom tube	BD (Becton, Dickinson and Company)
27 G × 1/2" needle	NIPRO
50 ml reservoir	SPL, Korea
50 ml round-bottom centrifuge tube	Corning
96 well-flat buttom	SPL, Korea
Autoclave	Tony autoclave SS-240
Automatic pipette	GILSON
Carbon dioxide incubator	Binder
Cell culture Petri dish (100×20 mm)	SPL
Cell culture Petri dish (60×15 mm)	SPL
Cellulose acetate membrane filter, pore size 0.2 μm	Sartorious AG, Germany
Cellulose acetate membrane filter, pore size 0.45 μm	Sartorious AG, Germany
Centrifuge, refrigerated	Andreas Hettich, UK
Cyogenic vial	Corning
Disposable syringe 1 ml	NIPRO
Electronic multichannel pipette	Eppendorf
Flow cytometer (FACSCanto II)	BD (Becton, Dickinson and Company)

Freezer -20 ˚C	Sharp
Hot air oven	Heraeus
Laminar Flow	Holten LaminAir
Liquid nitrogen tank	Taylor-Wharton
Lithium heparin tubes	BD (Becton, Dickinson and Company)
Microcetrifuge	Gyrozen
Micropipette Tip	Corning
Microscope slide	Sail Brand
Microscope	Olympus
Pasteur pipette	Pyrex
pH meter	Eutech Cybermetics
Pipette controller	Nichiryo
Plate shaker	Infinigen
Storage cane for cryotube vial	Nalge Nunc International
Water bath	Labline
Vortex mixture	Biosan

APPENDIX C

Preparation of reagents and buffers

1. Incomplete culture medium

RPMI 1640 powder with L-glutamine without $NHCO_3$	10.4 g
Hepes	5.94 g
Glucose	2 g
Gentamycin (80 mg/2 ml)	2 ml
Hypoxanthine	0.05 g

Dissolve in deionized water and adjust pH to 7.4. Filter the solution through 0.2 µm membrane filter. This solution mixture is stored at 4°C

2. Complete culture medium

Sterile 5% $NaHCO_3$	4.2 ml
Pooled human serum (inactived at 56°C for 30 min)	10 ml
Incomplete culture medium add up to	100 ml
Gentamycin (80 mg/2 ml)	2 ml
Hypoxanthine	0.05 g

This solution mixture is stored at 4°C

3. 5% $NaHCO_3$

$NaHCO_3$	5 g

Dissolve in deionized water (final volume 100 ml). Filter the solution through 0.2 µm membrane filter. This solution mixture is stored at 4°C

4. 5% Sorbitol

D-Sorbitol	5 g

Dissolve in deionized water (final volume 100 ml). Filter the solution through 0.2 µm membrane filter. This solution mixture is stored at 4°C

5. Thawing solution (3.5% NaCl)

NaCl	3.5 g

Dissolve in deionized water (final volume 100 ml). Filter the solution through 0.2 µm membrane filter. This solution mixture is stored at 4°C

6. Freezing solution for *P. falciprum*

Glycerol	28 ml
D-Sorbitol	3 ml
NaCl	0.65 g

Dissolve in deionized water (final volume 100 ml). Filter the solution through 0.2 µm membrane filter. This solution mixture is stored at 4°C

7. Freezing solution for *P. berghei*

Glycerol	30 ml

Dissolve in deionized water (final volume 100 ml). This solution mixture was stored at 4°C

8. Phosphate-buffered Saline (PBS) for Giemsa staining, 6.7 mM (pH 7.1)

Na_2HPO_4	0.41 g
$KH_2PO_4 \cdot H_2O$	0.65 g

Dissolve in deionized water (final volume 1 L)

9. Phosphate-buffered Saline (PBS) for cell culture, (pH 7.4)

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g

Dissolve in deionized water (final volume 1 L)

10. Calcein-AM solution

Calcein-AM stock solution (1 mM) is prepared by dissolving 1 mg calcein-AM (MW = 994.9) with DMSO (1 ml). Working Calcein-AM solution (50 µM) was freshly prepared by diluting the 1 mM calcein-AM solution with PBS.

11. DCFH-DA solution

DCFH-DA stock solution (1 mM) is prepared by dissolving 2.5 mg of DCFH-DA in methanol (5.0 ml) and store in the dark at -20°C.

APPENDIX D

Supplementary contents

Table S-1 Effect of DFO on growth of *P. falciparum*

Desferrioxamine (DFO)	
Concentration (µM)	**% Parasite growth (mean±SEM)**
0.00	100.0
3.99	97.2 ± 0.4
6.49	93.6 ± 3.0
10.56	83.5 ± 5.2
17.17	30.8 ± 6.9
27.91	7.3 ± 1.4
45.39	6.7 ± 1.5
73.80	6.1 ± 0.1
120.00	6.5 ± 0.5

Table S-2 Effect of DFP on growth of *P. falciparum*

Deferiprone (DFP)	
Concentration (µM)	**% Parasite growth (mean±SEM)**
0.00	100.0
24.09	98.3 ± 0.5
30.30	99.9 ± 1.4
38.11	93.4 ± 2.1
47.94	79.1 ± 1.8
60.30	50.9 ± 4.1
75.84	13.8 ± 0.3
95.40	9.9 ± 0.2
120.00	7.9 ± 0.9

Table S-3 Effect of CM1 on growth of *P. falciparum*

CM1	
Concentration (µM)	**% Parasite growth (mean±SEM)**
0.00	100.0
15.22	96.9 ± 0.8
19.15	96.8 ± 1.6
24.09	93.1 ± 2.4
30.30	79.3 ± 2.3
38.11	40.7 ± 4.3
47.94	12.3 ± 1.5
60.30	10.1 ± 1.4
75.84	10.0 ± 1.6

Table S-4 Effect of GTE on growth of *P. falciparum*

Green tea extract (GTE)	
Concentration (EGCG equivalent, µM)	**% Parasite growth (mean±SEM)**
0.00	100.0
3.99	96.0 ± 1.6
6.49	97.9 ± 0.7
10.56	96.6 ± 1.3
17.17	82.3 ± 2.2
27.91	18.8 ± 2.2
45.39	9.3 ± 0.7
73.80	7.4 ± 0.8
120.00	5.9 ± 1.1

Table S-5 Effect of DFX on growth of *P. falciparum*

Deferasirox (DFX)	
Concentration (µM)	**% Parasite growth (mean±SEM)**
0.00	100.0
12.51	97.4 ± 1.5
18.58	100.3 ± 0.6
27.61	96.6 ± 0.7
41.03	75.9 ± 0.9
60.96	10.4 ± 0.1
90.59	9.4 ± 0.1
134.60	8.6 ± 0.3
200.00	8.2 ± 0.5

Table S-6 Effect of PYR on growth of *P. falciparum*

Pyrimethamine (PYR)	
Concentration (nM)	**% Parasite growth (mean±SEM)**
0.00	100.0
4.98	98.0 ± 0.7
8.44	102.2 ± 1.2
14.30	102.9 ± 0.7
24.24	96.9 ± 2.1
41.08	44.2 ± 3.0
69.62	21.3 ± 1.4
118.00	10.5 ± 1.0
200.00	7.6 ± 0.6

Table S-7 Effect of DHA on growth of *P. falciparum*

Dihydroartemisinin (DHA)	
Concentration (nM)	**% Parasite growth (mean±SEM)**
0.00	100.00
0.30	97.79 ± 1.14
0.50	99.95 ± 1.09
0.82	94.56 ± 0.79
1.35	79.25 ± 2.04
2.23	44.80 ± 3.72
3.67	14.07 ± 1.34
6.06	9.76 ± 0.54
10.00	10.97 ± 0.53

Table S-8 Effect of PYR combined with CM1 on growth of *P. falciparum*

PYR + CM1		
Concentration		**% Parasite growth (mean±SEM)**
PYR (nM)	**CM1 (μM)**	
-	-	100.0
30	-	35.1 ± 3.3
-	30	34.6 ± 2.6
30	5	35.4 ± 4.3
30	10	38.9 ± 5.0
30	20	52.8 ± 7.3
30	35	44.0 ± 3.2
30	50	10.7 ± 1.6
30	100	10.7 ± 1.6
30	200	10.5 ± 1.3

Table S-9 Effect of PYR combined with GTE on growth of *P. falciparum*

PYR + GTE (EGCG equivalent)		
Concentration		**% Parasite growth (mean±SEM)**
PYR (nM)	**GTE (μM)**	
-	-	100.0
30	-	52.5 ± 2.9
-	20	64.0 ± 8.7
30	5	74.2 ± 2.5
30	10	82.2 ± 2.7
30	20	70.9 ± 5.0
30	35	13.5 ± 2.7
30	50	8.3 ± 0.5
30	100	7.6 ± 0.9
30	150	4.8 ± 0.9

Table S-10 Levels of LIP in *P. falciparum*-infected RBC treated with CM1 (0-200 µM)

CM1	
Concentration (µM)	**% Change of MFI (mean±SEM)**
0	100.0
25	2.9 ± 4.5
50	9.9 ± 3.2
100	19.6 ± 3.1
200	27.4 ± 4.5

Table S-11 Levels of LIP in *P. falciparum*-infected RBC treated with DFP (0-200 µM)

DFP	
Concentration (µM)	**% Change of MFI (mean±SEM)**
0	100.0
25	2.0 ± 3.0
50	1.9 ± 4.5
100	0.8 ± 4.0
200	9.6 ± 3.9

Table S-12 Levels of LIP in *P. falciparum*-infected RBC treated with GTE (0-200 µM)

GTE	
Concentration (EGCG equivalent, µM)	**% Change of MFI (mean±SEM)**
0	100.0
25	16.4 ± 1.9
50	21.4 ± 5.0
100	26.0 ± 7.0
200	26.6 ± 4.6

Table S-13 Levels of ROS in *P. falciparum*-infected RBC treated with CM1 (0-200 µM)

CM1	
Concentration (µM)	**% Change of MFI (mean±SEM)**
0	100.0
25	97.7 ± 3.4
50	101.4 ± 2.4
100	96.1 ± 7.6
200	98.7 ± 5.8

Table S-14 Levels of ROS in *P. falciparum*-infected RBC treated with DFP (0-200 µM)

DFP	
Concentration (µM)	**% Change of MFI (mean±SEM)**
0	100.0
25	103.1 ± 2.5
50	99.4 ± 3.7
100	95.7 ± 3.5
200	96.3 ± 3.3

Table S-15 Levels of ROS in *P. falciparum*-infected RBC treated with GTE (0-200 µM)

GTE	
Concentration (EGCG equivalent, µM)	**% Change of MFI (mean±SEM)**
0	100.0
25	68.8 ± 1.3
50	59.9 ± 3.8
100	56.5 ± 6.4
200	54.9 ± 3.4

MFI = Mean fluorescence intensity

Table S-16 Effect of PYR on *P. berghei* growth in infected mice

PYR	
Concentration (mg/kg)	**% Parasite growth (mean±SEM)**
0.0	95.7 ± 8.1
0.1	100.0 ± 8.2
0.5	72.4 ± 5.6
1.0	34.0 ± 9.0
5.0	11.7 ± 5.0

Table S-17 Effect of CM1 on *P. berghei* growth in infected mice

CM1	
Concentration (mg/kg)	**% Parasite growth (mean±SEM)**
0.0	100.0 ± 7.5
12.5	96.4 ± 1.4
25.0	93.7 ± 1.5
50.0	48.3 ± 0.1
100.0	26.7 ± 0.5

Table S-18 Effect of GTE on *P. berghei* growth in infected mice

GTE	
Concentration (EGCG equivalent, mg/kg)	**% Parasite growth (mean±SEM)**
0.0	100.0 ± 8.5
12.5	158.4 ± 13.2
25.0	134.3 ± 4.2
50.0	118.1 ± 2.2
100.0	114.3 ± 2.9

Table S-19 Effect of PYR combined with CM1 on *P. berghei* growth in infected mice

PYR + CM1		
Concentration (mg/kg)		**% Parasite growth (mean±SEM)**
PYR	**CM1**	
-	-	100.0 ± 17.5
0.6	-	23.4 ± 9.0
-	12.5	96.4 ± 1.4
0.6	12.5	20.2 ± 5.6
0.6	25.0	34.8 ± 1.9
0.6	50.0	12.0 ± 5.7
0.6	100.0	8.8 ± 3.1

Table S-20 Effect of PYR combined with GTE on *P. berghei* growth in infected mice

PYR + GTE (EGCG equivalent)		
Concentration (mg/kg)		**% Parasite growth (mean±SEM)**
PYR	**CM1**	
-	-	100.0 ± 17.5
0.6	-	23.4 ± 9.0
-	12.5	158.4 ± 13.2
0.6	12.5	15.1 ± 2.1
0.6	25.0	18.8 ± 1.5
0.6	50.0	19.4 ± 6.3
0.6	100.0	17.6 ± 2.8

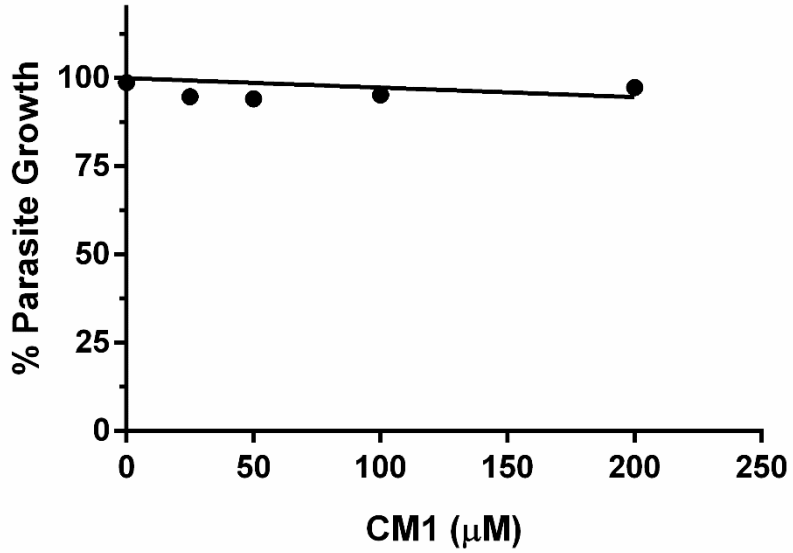

Figure S-1 The percentage of parasite growth after treatment with CM1 for 2 h, in experiment of measurement of LIP in *P. falciparum*-infected RBC. The percentage of parasite growth were calculated from parasitemia, determined using flow cytometry while LIP levels were being measured. Data was obtained from three independent experiments performed in triplicate and expressed as mean±SEM.

Table S-21 The percentage of parasite growth after treatment with CM1 for 2 h, in experiment of measurement of LIP in *P. falciparum*-infected RBC

CM1	
Concentration (μM)	**% Parasite growth (mean±SEM)**
0	98.8 ± 1.2
25	94.7 ± 0.2
50	94.1 ± 0.5
100	95.3 ± 0.6
200	97.3 ± 1.4

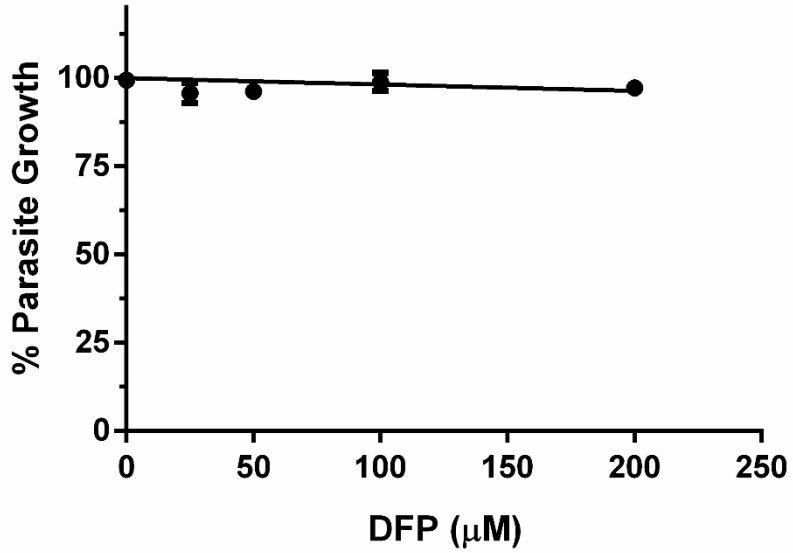

Figure S-2 The percentage of parasite growth after treatment with DFP for 2 h, in experiment of measurement of LIP in *P. falciparum*-infected RBC. The percentage of parasite growth were calculated from parasitemia, determined using flow cytometry while LIP levels were being measured. Data was obtained from three independent experiments performed in triplicate and expressed as mean±SEM.

Table S-22 The percentage of parasite growth after treatment with DFP for 2 h, in experiment of measurement of LIP in *P. falciparum*-infected RBC

DFP	
Concentration (μM)	**% Parasite growth (mean±SEM)**
0	99.4 ± 0.6
25	95.7 ± 2.9
50	96.3 ± 2.2
100	98.9 ± 2.6
200	97.2 ± 1.6

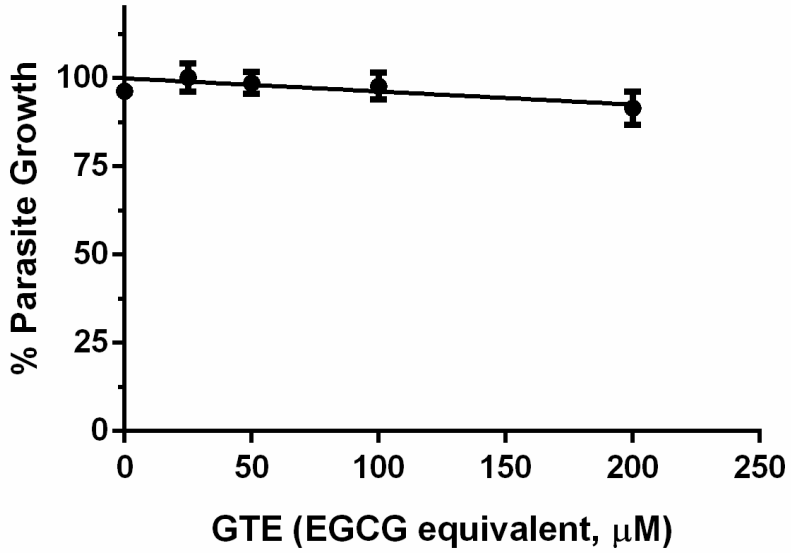

Figure S-3 The percentage of parasite growth after treatment with GTE for 2 h, in experiment of measurement of LIP in *P. falciparum*-infected RBC. The percentage of parasite growth were calculated from parasitemia, determined using flow cytometry while LIP levels were being measured. Data was obtained from three independent experiments performed in triplicate and expressed as mean±SEM.

Table S-23 The percentage of parasite growth after treatment with GTE for 2 h, in experiment of measurement of LIP in *P. falciparum*-infected RBC

GTE	
Concentration (EGCG equivalent, μM)	**% Parasite growth (mean±SEM)**
0	96.3 ± 1.9
25	100.2 ± 4.1
50	98.6 ± 3.1
100	97.8 ± 3.8
200	91.6 ± 4.7

CURRICULUM VITAE

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Poster presentation	Srichairatanakool S, **Thipubol S**, Tipsuwan W, Uthaipibull C. Inhibitory effect of novel iron chelator, 1-(*N*-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1) and green tea extract on growth of *Plasmodium falciparum*. *Malaria J* 2014, **13**(Suppl 1): P84	

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Experiences

Research Assistant of research grant through Assc. Prof. Dr. Somdet Srichairatanakool :

1) ELISA-based analysis of levels of antibody against *P. falciparum* and *P. vivax* in dry blood spot (DBS) of the population in Thailand endemic areas (2013)
2) Study of Nutritional value, biological and pharmacological activities of perilla (*Perilla frutescens* Linn. *Britt*) grain (2015)
3) ELISA-based analysis of serum human hepcidin (2015)
4) Assay of biochemical markers in the study animal and human serum samples (2015)