artemisinin-sensitive Cambodian clinical isolates and reference lines conferred varying degrees of in vitro resistance, suggesting additional contribution from the genetic background [50]. Importantly, Asian K13 mutations are generally not found in Africa where numerous additional but rare *Pfk13* alleles were identified [36, 57–59]. An exception is the SNP A578S, observed in many African countries [58–60] as well as Bangladesh [51] and Thailand [36], however this mutation is not associated with artemisinin resistance [36].

Apart from one study in a limited number of African children with severe malaria [61], none of the K13 mutations has been associated with clinical artemisinin resistance for the time being, despite evidence that introducing the C580Y mutation generates artemisinin resistance in vitro in the NF54 parasite strain considered to be of African origin [62]. Genetic determinants of artemisinin resistance have been reviewed recently by Fairhurst [63]. A search of mutations in the *k13 P. vivax* orthologue showed reduced polymorphism compared to *Pfk13* and the V552I mutation identified cannot be currently associated with any *P. vivax* drug resistance [64].

PfK13 activity

The exact function of PfK13 in *Plasmodium* is not known yet, but analogies are possible with the human Keap1 protein function, in particular, in the cell response to oxidative stress (Fig. 1). PfK13 and Keap1 share homologies in the C-terminal BTB/POZ and the 6-kelch propeller domains [33, 65]. The presence of a BTB/POZ domain suggests that PfK13 could dimerize like Keap1. Formation of a dimer has been experimentally confirmed in the recently solved crystal structure of the PfK13 BTB/POZ propeller domain [66]. Based on Keap1 function, a hypothetical model could be that in steady state conditions, the wild-type K13-propeller domain binds to uTF (a putative, unidentified transcription factor functionally equivalent to human Nrf2) allowing its ubiquitination and proteosomal degradation. This ubiquitination could be mediated by one of the *Plasmodium* ubiquitin ligases but this has not yet been investigated. Importantly, the putative uTF transcription factor remains to be identified in the *Plasmodium* genome [67]. Recently, *P. falciparum* phosphatidylinositol 3 kinase (PfPI3K) was shown to undergo a PfK13-dependent ubiquitinylation. It could be immuno-precipitated in a complex with PfK13 [68] and has been included as a complex with PfK13 illustrated in Fig. 1, but direct binding of PfPI3K to PfK13 remains to be demonstrated [68].

Biochemical consequences of a mutant K13 propeller

Based on Keap1 involvement in human lung cancer [69] and hypertension [70], it is possible to predict that K13-propeller mutations altering the propeller structure impair its biological function and interaction with partner proteins [33]. In particular, K13-propeller mutations would prevent fixation of uTF to the propeller domain, and as a consequence reduce the ubiquitinylation-dependent turnover, promoting translocation of uTF to the nucleus (Fig. 2).

In C580Y mutant parasites, PfPI3K was no longer ubiquitinylated, resulting in 1.5–twofold increased basal PI3P levels. This may reflect disrupted interactions between PfPI3K and PfK13 or disrupted interactions within the PfK13 associated protein complex (Fig. 2). Elevated basal phosphatidylinositol 3 phosphate (PI3P) levels appear central to artemisinin resistance, as K13 wild-type parasites became resistant upon elevation of PI3P levels induced by transgenic expression of human VPS34. Similarly transgenic elevation of PfAKT (also known as protein kinase B) in a wild-type parasite confers in vitro artemisinin resistance in connection with increased levels of PI3P by a feedback mechanism supposed [68]. As PfPI3K enzymatic activity is inhibited by artemisinins, the levels of PI3P likely drop rapidly upon exposure to artemisinins in sensitive parasites, whereas the PI3P level is already elevated in resistant parasites [68]. Additional downstream effectors likely come into play to orchestrate the quiescence response to artemisinin and withstand its toxicity.

PfK13 polymorphisms are also associated with modifications of endoplasmic reticulum homeostasis (Fig. 2). Indeed, transcriptome analysis of *P. falciparum* isolates revealed that artemisinin resistance is associated with increased expression of a network of molecular chaperones and major protein complexes belonging to the UPR pathway. In particular, immunoglobulin-binding protein (BiP) and cyclophilin B (CYPB), belonging to the *Plasmodium* reactive oxidative stress complex involved in protein folding and repair in the endoplasmic reticulum, are upregulated. Along the same line, the endoplasmic reticulum-resident calcium-binding protein (ERC) involved in endoplasmic reticulum Ca^{2+} homeostasis is also overexpressed [71, 72]. This upregulation of UPR in PfK13 mutants likely endows the parasite with increased ability to repair or degrade proteins damaged by alkylation and oxidation generated by artemisinin [73]. Based on homology with the human BiP-PERK-eIF2α pathway involved in the cell cycle arrest under stress conditions (Fig. 3) [74, 75], BiP bound to misfolded/altered proteins could dissociate from the PERK homologue, PfPK4, leading to its activation, and phosphorylation of eIF2α, which could trigger parasite cell cycle arrest via cyclin-dependent kinases, inhibition of protein synthesis, and the translocation of an unidentified transcription factor uTF into the nucleus. In mammalian cells, the PI3K/AKT

a The human Keap1 complex. The human Keap1 (Kelch-like ECH associated protein 1) is a repressor protein of Nrf2 (Nuclear factor erythroid 2-related factor 2) activity. The BTB/POZ domain (Broad-Complex, Tramtrack and Bric a Brac or Poxvirus or Zinc-finger domain) and the N-terminal portion of the IVR (Intervening Region) mediate Keap1 homodimerization allowing Cul3 (Cullin 3) binding with BTB domains, and Nrf2 binding, through its ETGE and DLG motifs, with kelch-propeller domains. In basal conditions the Keap1-Nrf2 complex leads to polyubiquitination (Ub) of Nrf2 mediated by the E2-ubiquitin ligase activity of Cul3 promoting Nrf2 degradation by the proteasome. Under stress conditions, modifications of cysteine residues of Keap1 induce a conformational change disrupting the interaction between the ETGE/DLG motifs and Kelch domains, and thus a release of Nrf2, which is no longer degraded and can translocate to the nucleus [96]. Inside the nucleus, Nrf2 controls, through the Antioxidant Response Elements (ARE), the expression of approximately 200 genes involved in the preservation of a healthy intracellular redox balance including the regulation of the expression of molecular chaperones as well as of proteasome subunits. The regulation of Nrf2 activity can also be mediated by the PI3K (phosphatidylinositol 3-kinase) pathway promoting Nrf2 translocation [97]. Thus the Keap1-Nrf2 complex is the major regulator of the cytoprotective response to any endogenous and exogenous stress caused by reactive oxygen species (ROS) and electrophiles. Some mutations of the Keap1-propeller cause a constitutive Nrf2 nuclear translocation increasing chemo-resistance and enhancing tumor cell growth [96]

b The *P. falciparum* K13 complex. A putative wild type PfK13 protein complex based on analogy with human Keap1 in steady state conditions. Known features are the capacity of PfK13 to form dimers, presence of PfPI3K in the PfK13 protein complex, and polyubiquitination of PfPI3K in steady state conditions [68]

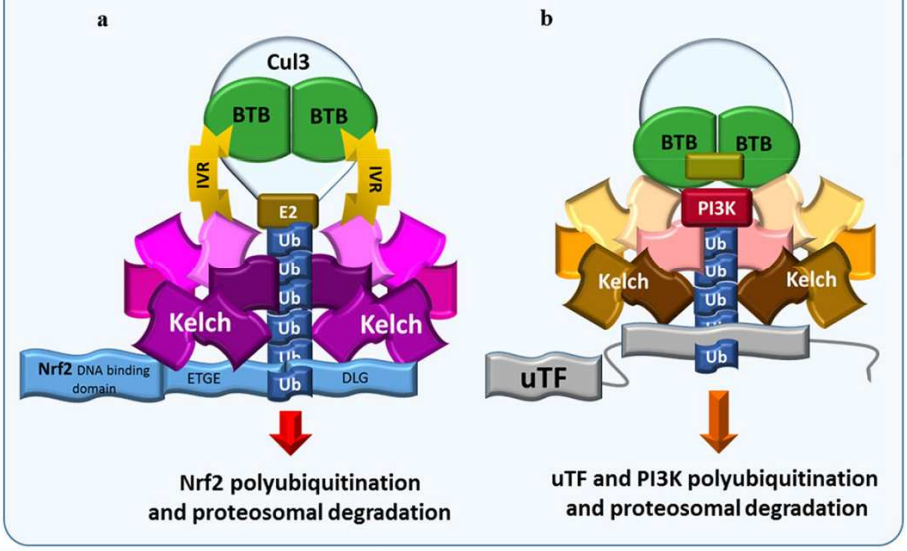

Fig. 1 The Keap1 complex in human cells and a hypothetical PfK13 complex

pathway participates in UPR regulation and the activated AKT protein (also known as protein kinase B) seems to be required for Protein kinase RNA-like endoplasmic reticulum kinase (PERK)-mediated eIF2α phosphorylation [76]. This is reminiscent of the *Plasmodium* cell cycle slow down and activation of eIF2α kinases (PfeIK1 and PfeIK2) induced by amino acid starvation and observed in sporozoite latency inside mosquito salivary glands [39, 77]. Translocation of uTF could, in addition, be favoured by its phosphorylation by PfPK4 and also, like in humans,

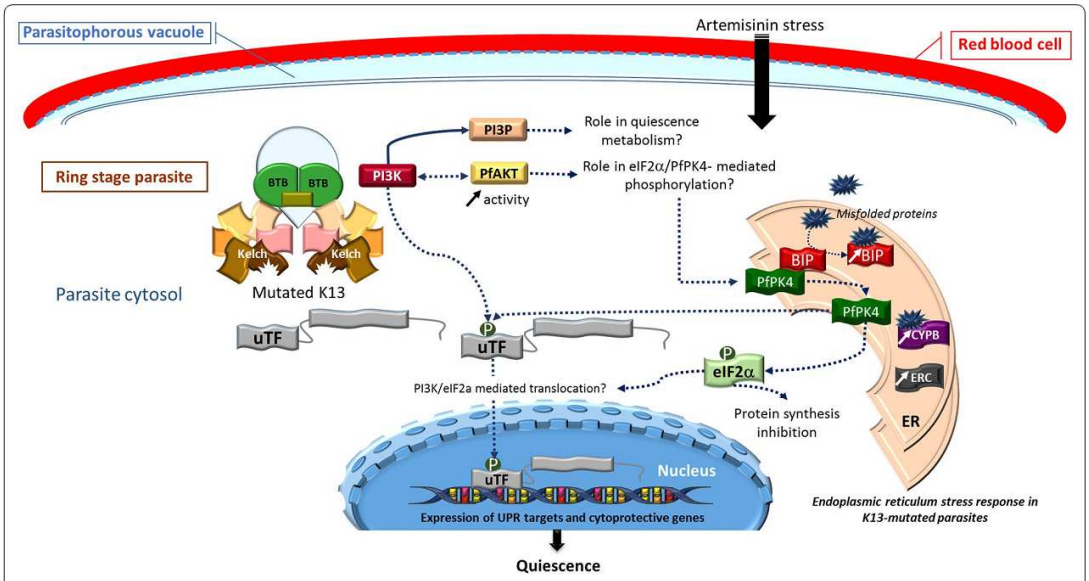

Fig. 2 Mechanism proposed for PfK13-mediated resistance to artemisinins in K13 mutated *Plasmodium falciparum* at young ring stage. K13-propeller mutation prevents the fixation of uTF and PI3K to the Kelch domain and their ubiquitination, leading to enhanced concentration of PI3P. The artemisinin-induced oxidative stress is responsible for the accumulation of misfolded proteins in the *ER* endoplasmic reticulum. Misfolded proteins bind to BiP, the complex BiP-PfPK4 is dissociated and PfPK4 phosphorylates uTF and eIF2α. eIF2α phosphorylation should allow the translocation of uTF in the nucleus to regulate UPR targets and cytoprotective gene expression and also inhibit protein synthesis [75]. *BiP* immunoglobulin-binding protein, *BTB* broad-complex, tramtrack and Bric a Brac, *CYPB* cyclophilin B, *eIF2a* eukaryotic translation initiation factor 2α, *ERC* endoplasmic reticulum resident calcium binding protein, *PfPK4 P. falciparum* protein kinase 4, *PI3K* phosphatidylinositol 3 kinase, *PI3P* phosphatidylinositol 3 phosphate, *uTF* unidentified transcription factor(s)

by PfPI3K, and could activate the expression of *Plasmodium* UPR and cytoprotective genes (Fig. 2).

This model does not exclude the existence of more than one uTF. Moreover, the PfPK4/PfPI3K downstream regulation cascades remain to be elucidated and experimentally demonstrated (Fig. 2). The involvement of the PfAKT pathway in artemisinin resistance, like PI3P involvement, still remains to be detailed. Moreover, the exact kinetics of events once the parasites are exposed to artemisinin oxidative and alkylating damage needs to be clarified, in particular as PI3K is inhibited by artemisinins [68] and should no longer contribute to refuelling PI3P levels.

Artemisinin resistance possibly includes a combination of the increased degradation of misfolded proteins/repair of damaged proteins especially those involved in the cell cycle progression and the 'PfPK4/PfPI3K' mediated response to the artemisinins' oxidative stress. This cellular response of K13 mutant parasites to artemisinin stress could lead to a sub-set of ring stage parasites to enter into a quiescence state (Fig. 2). Why only a sub-set and a variable fraction of ring stages is involved needs to be understood. It may reflect a finely tuned balance between different cellular effectors that is unequally distributed between individual infected cells.

Artemisinin-induced quiescence and *Plasmodium* metabolism

Dihydroartemisinin (DHA)-induced quiescence in non-mutated PfK13 parasites is associated with suspended RNA, DNA and protein synthesis [49]. Folate metabolism, isoprenoid metabolism, lactate dehydrogenase activity and glycolysis, the main metabolic pathway producing adenosine triphosphate (ATP) and phosphoenolpyruvate (PEP) in *Plasmodium*, are severely down-regulated [49]. But, even though the metabolism of quiescent parasites is largely down-phased, some pathways remain active and require another source of ATP and PEP, depending on pyruvate production by the apicoplast and on mitochondrial activity (Fig. 4) [49, 78]. In DHA-induced quiescent rings, among the genes that remain actively transcribed, are genes coding for enzymes of the pyruvate metabolism (pyruvate kinase 2, pyruvate dehydrogenase E1 beta subunit), the fatty acid metabolism by FASII -fatty acid synthesis type II- pathway (biotin acetyl-CoA carboxylase, enoyl-acyl carrier

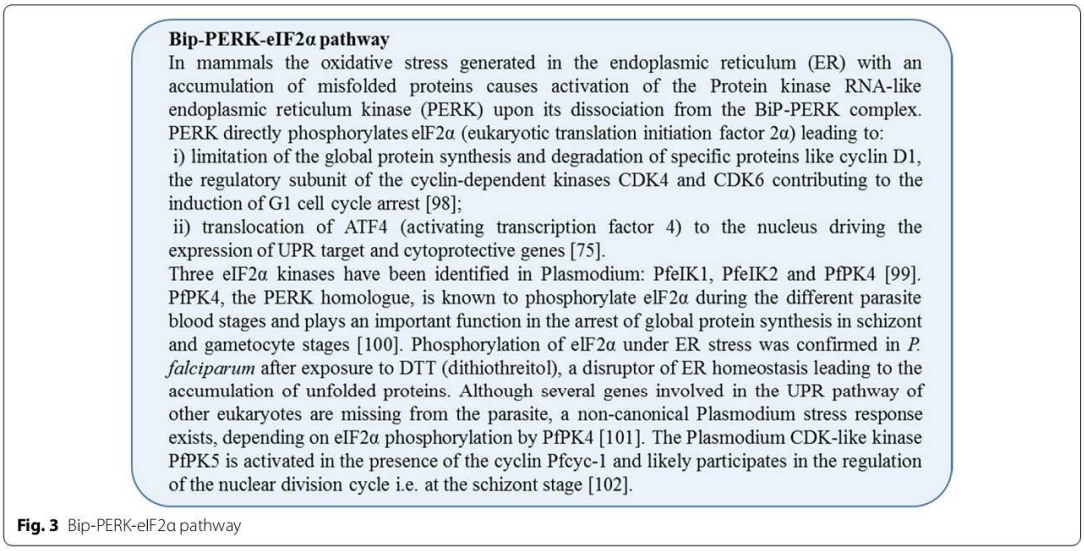

Fig. 3 Bip-PERK-eIF2α pathway

reductase/FabI) and for the lipoic acid metabolism (lipoic acid synthase) [49]. The mitochondrial tricarboxylic acid cycle is down-regulated but remains active and genes encoding the proteins of the electron transport chain also remain transcribed at a normal level (cytochrome c subunit II, NADH-ubiquinone oxidoreductase II, flavoprotein sub-unit of succinate dehydrogenase and ubiquinol cytochrome c reductase iron sulfur sub-unit) [49]. This was confirmed by rhodamine staining of parasite after DHA treatment: only rhodamine-positive parasites resumed growth [42]. In stress conditions, depletion of host-derived fatty acids induces an upregulation of the *Plasmodium* FASII pathway [79]. PI3P is present in the apicoplast membrane and can play a role in exchanges of protein and likely lipids [80–82] with the adjacent mitochondrion. The elevated production of PI3P in resistant parasites [68] could play a role in the maintenance of this minimal energetic metabolism based on mitochondrial and apicoplast activity and also found in quiescent resistant parasites [49]. Indeed these parasites cannot survive and resume growth in the presence of atovaquone, an inhibitor of the mitochondrial activity, and the use of haloxyfob, an acetyl-CoA carboxylase inhibitor, as well as triclosan, an inhibitor of FabI delays the recovery of DHA-induced quiescent parasites [35, 42, 49, 83, 84], demonstrating that this minimum active metabolism during the induced quiescence state by artemisinins is essential for the survival and the recovery of the parasites from dormancy.

Thus, the quiescent state induced at the young ring stage by artemisinin in sensitive and resistant parasites likely seems to involve the same cellular mechanisms, but the PfK13 mutation shifts the intra-population distribution so as to allow more parasites to become quiescent in the case of resistant parasites, and to quickly resume growth after the drug removal in vitro or drug elimination in patients.

Conclusions

It is increasingly clear that the *P. falciparum* resistance to artemisinin and its derivatives is not due to efflux modulation or target modifications as described for other anti-malarials, but is based on increased capacity of PfK13-mutant parasites to manage oxidative damage thanks to greater UPR mobilization. The over-expression of UPR target genes should impact three key points: (an) unidentified transcription factor(s) (uTF) regulating transcription of UPR/oxidative response genes, the PI3 K/PI3P/AKT pathway activity and the PfPK4/eIF2α cascade. This would allow parasite entrance into a quiescence state with minimal energy metabolism provided by the apicoplast and the mitochondrion, maintained by alternative tricarboxylic acid cycle and FASII metabolism, until drug removal/excretion when parasites can resume growth. Recently, the artemisinin-resistant F32-ART line, selected by long-term drug pressure with solely artemisinin, was shown to display an extended age range of stages surviving artemisinin treatment extending to older ring stages (13–16 h) and even trophozoite stages. These parasites were also able to survive lethal doses of diverse classes of anti-malarial drugs, including molecules used as partners in currently recommended ACT, in the

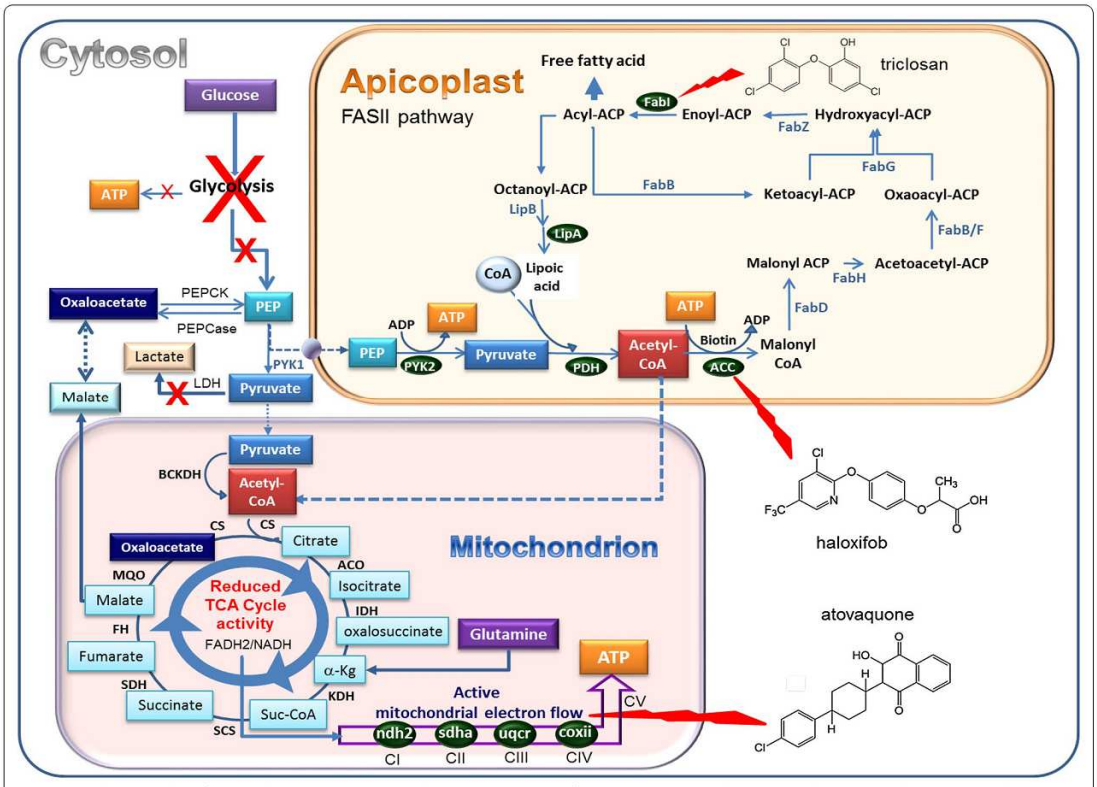

Fig. 4 Synthetic model of *Plasmodium* quiescence metabolism. Quiescent *P. falciparum* parasites demonstrated an arrested glycolysis pathway leading to suspended production of ATP and phosphoenolpyruvate. Basal metabolism is maintained in quiescent parasites due to FASII metabolism in the apicoplast coupled with ATP production in the mitochondrion. Haloxifob, triclosan and atovaquone can disrupt these biochemical pathways [49, 95]. *Green* protein/enzyme with maintained expression in quiescent rings [49]. *ACC* acetyl-CoA carboxylase, *ACO* aconitase, *BCKDH* branched-chain keto acid dehydratase, *coxii* cytochrome c sub-unit II, *CS* citrate synthase, *FabI* enoyl-ACP reductase, *FabZ* β-hydroxyacyl-ACP dehydratase, *FabG* β-ketoacyl-ACP reductase, *FabB/F* β-ketoacyl-ACP synthase I/II, *FabH* β-ketoacyl-ACP synthase III, *FabD* malonyl-CoA:ACP transacylase, *FASII* fatty acid synthesis type II, *FH* fumarate hydratase, *IDH* isocitrate dehydrogenase, *KDH* α-ketoglutarate dehydrogenase, *LDH* lactate dehydrogenase, *LipA* lipoic acid synthase, *LipB* octanoyl-ACP protein transferase, *MQO* malate quinone oxidoreductase, *ndh2* NADH-ubiquinone oxidoreductase II, *PEP* phosphoenolpyruvate, *PEPCase* PEP carboxylase, *PEPCK* PEP carboxykinase, *PDH* pyruvate dehydrogenase, *PYK* pyruvate kinase, *SCS* succinyl-CoA synthase, *SDH* succinate dehydrogenase, *sdha* flavoprotein subunit of succinate dehydrogenase, *TCA* Tri carboxylic acid, *uqcr* iron sulfur sub-unit of ubiquinol Cytochrome c reductase [49, 83, 95]

absence of the 'classical' mutations of the target genes for these drugs. Thus, long-term in vitro artemisinin exposure selects a novel multidrug tolerant phenotype, which could represent a major threat to anti-malarial drug policy in the field [35]. This threat is exacerbated by the fact that artemisinin-resistant parasites seem able to infect and be transmitted by a large panel of *Anopheles* species, including the major African species *Anopheles gambiae* [85]. This is yet a another reason to urgently clarify the cellular network of ART resistance in order to identify new therapeutic modalities (to protect the molecules currently used), and/or novel drug development, in order to avoid entrance into a quiescent state, target quiescent parasites and promote restart of the cell cycle, rescuing drug susceptibility of parasites.

Additional file

Additional file 1. ACT recommended by WHO and the newly approved combination DHA-piperaquine.

Authors' contributions

JMA and FBV designed the review; all authors contributed to acquisition, analysis and interpretation of data. LP, APR, OMP, JMA, and FBV have been

involved in drafting and revising the manuscript critically. All authors read and approved the final manuscript.

Authors' information
OM-P and FB-V are co-inventors on the pending patents #US61/904651 and US62/062439, filed by Institut Pasteur. These patents cover the use of K13 mutations as a molecular marker of *P. falciparum* ART resistance.

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Competing interests
The authors declare that they have no competing interests.

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References

1. Nobel price. http://www.nobelprize.org/nobel_prizes/medicine/laureates/2015/. 2015.
2. WHO. Status report on artemisinin and ACT resistance. Geneva: World Health Organization; 2015.
3. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2014;371:411–23.
4. Duru V, Khim N, Leang R, Kim S, Domergue A, Kloeung N, et al. *Plasmodium falciparum* dihydroartemisinin-piperaquine failures in Cambodia are associated with mutant K13 parasites presenting high survival rates in novel piperaquine in vitro assays: retrospective and prospective investigations. BMC Med. 2015;13:305.
5. Lubell Y, Dondorp A, Guerin PJ, Drake T, Meek S, Ashley E, et al. Artemisinin resistance–modelling the potential human and economic costs. Malar J. 2014;13:452.
6. Fitch CD. Ferriprotoporphyrin IX, phospholipids, and the antimalarial actions of quinoline drugs. Life Sci. 2004;74:1957–72.
7. Robert A, Benoit-Vical F, Claparols C, Meunier B. The antimalarial drug artemisinin alkylates heme in infected mice. Proc Natl Acad Sci USA. 2005;102:13676–80.
8. Kessl JJ, Meshnick SR, Trumpower BL. Modeling the molecular basis of atovaquone resistance in parasites and pathogenic fungi. Trends Parasitol. 2007;23:494–501.
9. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. Pharmacol Rev. 2005;57:117–45.
10. Bruce-Chwatt L. Chemotherapy of malaria. Geneva: World Health Organization; 1986. p. 245.
11. Maude RJ, Nguon C, Dondorp AM, White LJ, White NJ. The diminishing returns of atovaquone-proguanil for elimination of *Plasmodium falciparum* malaria: modelling mass drug administration and treatment. Malar J. 2014;13:380.
12. Durand R, Prendki V, Cailhol J, Hubert V, Ralaimazava P, Massias L, et al. *Plasmodium falciparum* malaria and atovaquone-proguanil treatment failure. Emerg Infect Dis. 2008;14:320–2.
13. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med. 2008;359:2619–20.
14. Noedl H, Socheat D, Satimai W. Artemisinin-resistant malaria in Asia. N Engl J Med. 2009;361:540–1.
15. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2009;361:455–67.
16. Phyo AP, Nkhoma S, Stepniewska K, Ashley EA, Nair S, McGready R, et al. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. Lancet. 2012;379:1960–6.
17. WHO. Guidelines for the treatment of malaria. Geneva: World Health Organization; 2010.
18. WHO. World malaria report 2014. Geneva: World Health Organization; 2014.
19. Na-Bangchang K, Muhamad P, Ruaengweerayut R, Chaijaroenkul W, Karbwang J. Identification of resistance of *Plasmodium falciparum* to artesunate-mefloquine combination in an area along the Thai-Myanmar border: integration of clinico-parasitological response, systemic drug exposure, and in vitro parasite sensitivity. Malar J. 2013;12:263.
20. Saunders DL, Vanachayangkul P, Lon C. Dihydroartemisinin-piperaquine failure in Cambodia. N Engl J Med. 2014;371:484–5.
21. Leang R, Taylor WR, Bouth DM, Song L, Tarning J, Char MC, et al. Evidence of *Plasmodium falciparum* malaria multidrug resistance to artemisinin and piperaquine in Western Cambodia: dihydroartemisinin Piperaquine open-label multicenter clinical assessment. Antimicrob Agents Chemother. 2015;59:4719–26.
22. Spring MD, Lin JT, Manning JE, Vanachayangkul P, Somethy S, Bun R, et al. Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: an observational cohort study. Lancet Infect Dis. 2015;15:683–91.
23. WWARN. Clinical determinants of early parasitological response to ACTs in African patients with uncomplicated falciparum malaria: a literature review and meta-analysis of individual patient data. BMC Med 2015;13:212.
24. Packard RM. The origins of antimalarial-drug resistance. N Engl J Med. 2014;371:397–9.
25. Smith Gueye C, Newby G, Hwang J, Phillips AA, Whittaker M, MacArthur JR, et al. The challenge of artemisinin resistance can only be met by eliminating *Plasmodium falciparum* malaria across the Greater Mekong subregion. Malar J. 2014;13:286.
26. Samarasekera U. Countries race to contain resistance to key antimalarial. Lancet. 2009;374:277–80.
27. Brown TS, Jacob CG, Silva JC, Takala-Harrison S, Djimde A, Dondorp AM, et al. *Plasmodium falciparum* field isolates from areas of repeated emergence of drug resistant malaria show no evidence of hypermutator phenotype. Infect Genet Evol. 2015;30:318–22.
28. Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, et al. Genetic architecture of artemisinin-resistant *Plasmodium falciparum*. Nat Genet. 2015;47:226–34.
29. Khim N, Bouchier C, Ekala MT, Incardona S, Lim P, Legrand E, et al. Countrywide survey shows very high prevalence of *Plasmodium falciparum* multilocus resistance genotypes in Cambodia. Antimicrob Agents Chemother. 2005;49:3147–52.
30. Anderson TJ, Nair S, Nkhoma S, Williams JT, Imwong M, Yi P, et al. High heritability of malaria parasite clearance rate indicates a genetic basis for artemisinin resistance in western Cambodia. J Infect Dis. 2010;201:1326–30.
31. Witkowski B, Lelievre J, Barragan MJ, Laurent V, Su XZ, Berry A, et al. Increased tolerance to artemisinin in *Plasmodium falciparum* is mediated by a quiescence mechanism. Antimicrob Agents Chemother. 2010;54:1872–7.
32. Imwong M, Dondorp AM, Nosten F, Yi P, Mungthin M, Hanchana S, et al. Exploring the contribution of candidate genes to Artemisinin resistance in *Plasmodium falciparum*. Antimicrob Agents Chemother. 2010;54:2886–92.
33. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014;505:50–5.
34. Looareesuwan S, Wilairatana P, Viravan C, Vanijanonta S, Pitisuttithum P, Kyle DE. Open randomized trial of oral artemether alone and a sequential combination with mefloquine for acute uncomplicated falciparum malaria. Am J Trop Med Hyg. 1997;56:613–7.
35. Ménard S, Ben Haddou T, Ramadani AP, Ariey F, Iriart X, Beghain J, et al. Induction of multidrug tolerance in *Plasmodium falciparum* by extended artemisinin pressure. Emerg Infect Dis. 2015;21:1733–41.

36. Ménard D, Khim N, Beghain J, Adegnika A, Alam M, Amodu O et al. A worldwide map of *Plasmodium falciparum* artemisinin resistance. N Engl J Med. 2016. (in press).
37. Hoshen MB, Na-Bangchang K, Stein WD, Ginsburg H. Mathematical modelling of the chemotherapy of *Plasmodium falciparum* malaria with artesunate: postulation of 'dormancy', a partial cytostatic effect of the drug, and its implication for treatment regimens. Parasitology. 2000;121:237–46.
38. Daignan-Fornier B, Sagot I. Proliferation/quiescence: the controversial "aller-retour". Cell Div. 2011;6:10.
39. Babbitt SE, Altenhofen L, Cobbold SA, Istvan ES, Fennell C, Doerig C, et al. *Plasmodium falciparum* responds to amino acid starvation by entering into a hibernatory state. Proc Natl Acad Sci USA. 2012;109:E3278–87.
40. Dembele L, Franetich JF, Lorthiois A, Gego A, Zeeman AM, Kocken CH, et al. Persistence and activation of malaria hypnozoites in long-term primary hepatocyte cultures. Nat Med. 2014;20:307–12.
41. Witkowski B, Khim N, Chim P, Kim S, Ke S, Kloeung N, et al. Reduced artemisinin susceptibility of *Plasmodium falciparum* ring stages in western Cambodia. Antimicrob Agents Chemother. 2013;57:914–23.
42. Peatey CL, Chavchich M, Chen N, Gresty KJ, Gray KA, Gatton ML, et al. Mitochondrial membrane potential in a small subset of Artemisinin-induced dormant *Plasmodium falciparum* parasites *in vitro*. J Infect Dis. 2015;212:426–34.
43. Klonis N, Crespo-Ortiz MP, Bottova I, Abu-Bakar N, Kenny S, Rosenthal PJ, et al. Artemisinin activity against *Plasmodium falciparum* requires hemoglobin uptake and digestion. Proc Natl Acad Sci USA. 2011;108:11405–10.
44. Dogovski C, Xie SC, Burgio G, Bridgford J, Mok S, McCaw JM, et al. Targeting the cell stress response of *Plasmodium falciparum* to overcome artemisinin resistance. PLoS Biol. 2015;13:e1002132.
45. Wang J, Zhang CJ, Chia WN, Loh CC, Li Z, Lee YM, et al. Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*. Nat Commun. 2015;6:10111.
46. Hott A, Casandra D, Sparks KN, Morton LC, Castanares GG, Rutter A, et al. Artemisinin-resistant *Plasmodium falciparum* parasites exhibit altered patterns of development in infected erythrocytes. Antimicrob Agents Chemother. 2015;59:3156–67.
47. Saralamba S, Pan-Ngum W, Maude RJ, Lee SJ, Tarning J, Lindegardh N, et al. Intrahost modeling of artemisinin resistance in *Plasmodium falciparum*. Proc Natl Acad Sci USA. 2011;108:397–402.
48. Teuscher F, Gatton ML, Chen N, Peters J, Kyle DE, Cheng Q. Artemisinin-induced dormancy in *Plasmodium falciparum*: duration, recovery rates, and implications in treatment failure. J Infect Dis. 2010;202:1362–8.
49. Chen N, LaCrue AN, Teuscher F, Waters NC, Gatton ML, Kyle DE, et al. Fatty acid synthesis and pyruvate metabolism pathways remain active in dihydroartemisinin-induced dormant ring stages of *Plasmodium falciparum*. Antimicrob Agents Chemother. 2014;58:4773–81.
50. Straimer J, Gnadig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, et al. Drug resistance. K13-propeller mutations confer artemisinin resistance in *Plasmodium falciparum* clinical isolates. Science. 2015;347:428–31.
51. Mohon AN, Alam MS, Bayih AG, Folefoc A, Shahinas D, Haque R, et al. Mutations in *Plasmodium falciparum* K13 propeller gene from Bangladesh (2009-2013). Malar J. 2014;13:431.
52. Huang F, Takala-Harrison S, Jacob CG, Liu H, Sun X, Yang H, et al. A single mutation in K13 predominates in Southern China and is associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment. J Infect Dis. 2015;212:1629–35.
53. Takala-Harrison S, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, et al. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. J Infect Dis. 2015;211:670–9.
54. Nyunt MH, Hlaing T, Oo HW, Tin-Oo LL, Phway HP, Wang B, et al. Molecular assessment of artemisinin resistance markers, polymorphisms in the k13 propeller, and a multidrug-resistance gene in the eastern and western border areas of Myanmar. Clin Infect Dis. 2015;60:1208–15.
55. Amaratunga C, Witkowski B, Dek D, Try V, Khim N, Miotto O, et al. *Plasmodium falciparum* founder populations in western Cambodia have reduced artemisinin sensitivity in vitro. Antimicrob Agents Chemother. 2014;58:4935–7.
56. Amaratunga C, Witkowski B, Khim N, Menard D, Fairhurst RM. Artemisinin resistance in *Plasmodium falciparum*. Lancet Infect Dis. 2014;14:449–50.
57. Sibley CH. Understanding artemisinin resistance. Science. 2015;347:373–4.
58. Kamau E, Campino S, Amenga-Etego L, Drury E, Ishengoma D, Johnson K, et al. K13-propeller polymorphisms in *Plasmodium falciparum* parasites from sub-Saharan Africa. J Infect Dis. 2015;211:1352–5.
59. Taylor SM, Parobek CM, DeConti DK, Kayentao K, Coulibaly SO, Greenwood BM, et al. Absence of putative artemisinin resistance mutations among *Plasmodium falciparum* in sub-Saharan Africa: a molecular epidemiologic study. J Infect Dis. 2014;211:680–8.
60. Maiga-Ascofare O, May J. Is the A578S Single-Nucleotide Polymorphism in K13-propeller a marker of emerging resistance to artemisinin among *Plasmodium falciparum* in Africa? J Infect Dis. 2016;213:165–6.
61. Hawkes M, Conroy AL, Opoka RO, Namasopo S, Zhong K, Liles WC, et al. Slow clearance of *Plasmodium falciparum* in severe pediatric Malaria, Uganda, 2011–2013. Emerg Infect Dis. 2015;21:1237–9.
62. Ghorbal M, Gorman M, Macpherson CR, Martins RM, Scherf A, Lopez-Rubio JJ. Genome editing in the human malaria parasite *Plasmodium falciparum* using the CRISPR-Cas9 system. Nat Biotechnol. 2014;32:819–21.
63. Fairhurst RM. Understanding artemisinin-resistant malaria: what a difference a year makes. Curr Opin Infect Dis. 2015;28:417–25.
64. Popovici J, Kao S, Eal L, Bin S, Kim S, Menard D. Reduced polymorphism in the Kelch propeller domain in *Plasmodium vivax* isolates from Cambodia. Antimicrob Agents Chemother. 2015;59:730–3.
65. Adams J, Kelso R, Cooley L. The kelch repeat superfamily of proteins: propellers of cell function. Trends Cell Biol. 2000;10:17–24.
66. Protein Data Bank. Crystal structure analysis of Kelch protein from *Plasmodium falciparum*. http://www.rcsb.org/pdb/explore/explore.do?structureId=4yy8. 2015.
67. Winzeler EA, Manary MJ. Drug resistance genomics of the antimalarial drug artemisinin. Genome Biol. 2014;15:544.
68. Mbengue A, Bhattacharjee S, Pandharkar T, Liu H, Estiu G, Stahelin RV, et al. A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria. Nature. 2015;520:683–7.
69. Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, et al. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Mol Cell. 2006;21:689–700.
70. Boyden LM, Choi M, Choate KA, Nelson-Williams CJ, Farhi A, Toka HR, et al. Mutations in kelch-like 3 and cullin 3 cause hypertension and electrolyte abnormalities. Nature. 2012;482:98–102.
71. Morita M, Sanai H, Hiramoto A, Sato A, Hiraoka O, Sakura T, et al. *Plasmodium falciparum* endoplasmic reticulum-resident calcium binding protein is a possible target of synthetic antimalarial endoperoxides, N-89 and N-251. J Proteome Res. 2012;11:5704–11.
72. Mok S, Ashley EA, Ferreira PE, Zhu L, Lin Z, Yeo T, et al. Population transcriptomics of human malaria parasites reveals the mechanism of artemisinin resistance. Science. 2015;347:431–5.
73. Liu Y, Lok CN, Ko BC, Shum TY, Wong MK, Che CM. Subcellular localization of a fluorescent artemisinin derivative to endoplasmic reticulum. Org Lett. 2010;12:1420–3.
74. Gosline SJ, Nascimento M, McCall LI, Zilberstein D, Thomas DY, Matlashewski G, et al. Intracellular eukaryotic parasites have a distinct unfolded protein response. PLoS One. 2011;6:e19118.
75. Kansanen E, Jyrkkänen H-K, Levonen A-L. Activation of stress signaling pathways by electrophilic oxidized and nitrated lipids. Free Radical Bio Med. 2012;52:973–82.
76. Blaustein M, Perez-Munizaga D, Sanchez MA, Urrutia C, Grande A, Risso G, et al. Modulation of the Akt pathway reveals a novel link with PERK/eIF2alpha, which is relevant during hypoxia. PLoS One. 2013;8:e69668.
77. Zhang M, Fennell C, Ranford-Cartwright L, Sakthivel R, Gueirard P, Meister S, et al. The *Plasmodium* eukaryotic initiation factor-2alpha kinase IK2 controls the latency of sporozoites in the mosquito salivary glands. J Exp Med. 2010;207:1465–74.
78. Salcedo-Sora JE, Caamano-Gutierrez E, Ward SA, Biagini GA. The proliferating cell hypothesis: a metabolic framework for *Plasmodium* growth and development. Trends Parasitol. 2014;30:170–5.
79. Botte CY, Yamaryo-Botte Y, Rupasinghe TW, Mullin KA, MacRae JI, Spurck TP, et al. Atypical lipid composition in the purified relict

plastid (apicoplast) of malaria parasites. Proc Natl Acad Sci USA. 2013;110:7506–11.
80. Bhattacharjee S, Stahelin RV, Speicher KD, Speicher DW, Haldar K. Endoplasmic reticulum PI(3)P lipid binding targets malaria proteins to the host cell. Cell. 2012;148:201–12.
81. Tawk L, Chicanne G, Dubremetz JF, Richard V, Payrastre B, Vial HJ, et al. Phosphatidylinositol 3-phosphate, an essential lipid in *Plasmodium*, localizes to the food vacuole membrane and the apicoplast. Eukaryot Cell. 2010;9:1519–30.
82. Kobayashi T, Sato S, Takamiya S, Komaki-Yasuda K, Yano K, Hirata A, et al. Mitochondria and apicoplast of *Plasmodium falciparum*: behaviour on subcellular fractionation and the implication. Mitochondrion. 2007;7:125–32.
83. Shears MJ, Botte CY, McFadden GI. Fatty acid metabolism in the *Plasmodium* apicoplast: drugs, doubts and knockouts. Mol Biochem Parasitol. 2015;199:34–50.
84. Ralph SA, van Dooren GG, Waller RF, Crawford MJ, Fraunholz MJ, Foth BJ, et al. Tropical infectious diseases: metabolic maps and functions of the *Plasmodium falciparum* apicoplast. Nat Rev Microbiol. 2004;2:203–16.
85. St Laurent B, Miller B, Burton TA, Amaratunga C, Men S, Sovannaroth S, et al. Artemisinin-resistant *Plasmodium falciparum* clinical isolates can infect diverse mosquito vectors of Southeast Asia and Africa. Nat Commun. 2015;6:8614.
86. Wongsrichanalai C, Pickard AL, Wernsdorfer WH, Meshnick SR. Epidemiology of drug-resistant malaria. Lancet Infect Dis. 2002;2:209–18.
87. Witkowski B, Berry A, Benoit-Vical F. Resistance to antimalarial compounds: methods and applications. Drug Resist Updat. 2009;12:42–50.
88. Sanchez CP, Dave A, Stein WD, Lanzer M. Transporters as mediators of drug resistance in *Plasmodium falciparum*. Int J Parasitol. 2010;40:1109–18.
89. Koenderink JB, Kavishe RA, Rijpma SR, Russel FG. The ABCs of multidrug resistance in malaria. Trends Parasitol. 2010;26:440–6.
90. Ibraheem ZO, Abd Majid R, Noor SM, Sedik HM, Basir R. Role of different Pfcrt and Pfmdr-1 mutations in conferring resistance to antimalaria drugs in *Plasmodium falciparum*. Malar Res Treat. 2014;2014:950424.
91. Summers RL, Nash MN, Martin RE. Know your enemy: understanding the role of PfCRT in drug resistance could lead to new antimalarial tactics. Cell Mol Life Sci. 2012;69:1967–95.
92. Setthaudom C, Tan-ariya P, Sitthichot N, Khositnithikul R, Suwanditakul N, Leelayoova S, et al. Role of *Plasmodium falciparum* chloroquine resistance transporter and multidrug resistance 1 genes on in vitro chloroquine resistance in isolates of Plasmodium falciparum from Thailand. Am J Trop Med Hyg. 2011;85:606–11.
93. Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, et al. Mefloquine resistance in *Plasmodium falciparum* and increased pfmdr1 gene copy number. Lancet. 2004;364:438–47.
94. Ubben D, Poll EM. MMV in partnership: the Eurartesim(R) experience. Malar J. 2013;12:211.
95. Ke H, Lewis IA, Morrisey JM, McLean KJ, Ganesan SM, Painter HJ, et al. Genetic investigation of tricarboxylic acid metabolism during the *Plasmodium falciparum* life cycle. Cell Rep. 2015;11:164–74.
96. Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL. The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer. Redox Biol. 2013;1:45–9.
97. Nakaso K, Yano H, Fukuhara Y, Takeshima T, Wada-Isoe K, Nakashima K. PI3K is a key molecule in the Nrf2-mediated regulation of antioxidative proteins by hemin in human neuroblastoma cells. FEBS Lett. 2003;546:181–4.
98. Raven JF, Koromilas AE. PERK and PKR: old kinases learn new tricks. Cell Cycle. 2008;7:1146–50.
99. Fennell C, Babbitt S, Russo I, Wilkes J, Ranford-Cartwright L, Goldberg DE, et al. PfeIK1, a eukaryotic initiation factor 2alpha kinase of the human malaria parasite *Plasmodium falciparum*, regulates stress-response to amino-acid starvation. Malar J. 2009;8:99.
100. Zhang M, Mishra S, Sakthivel R, Rojas M, Ranjan R, Sullivan WJ Jr, et al. PK4, a eukaryotic initiation factor 2alpha(eIF2alpha) kinase, is essential for the development of the erythrocytic cycle of *Plasmodium*. Proc Natl Acad Sci USA. 2012;109:3956–61.
101. Chaubey S, Grover M, Tatu U. Endoplasmic reticulum stress triggers gametocytogenesis in the malaria parasite. J Biol Chem. 2014;289:16662–74.
102. Doerig C, Endicott J, Chakrabarti D. Cyclin-dependent kinase homologues of *Plasmodium falciparum*. Int J Parasitol. 2002;32:1575–85.

Annex 2. Plant Determination Certificate: *T. diversifolia*

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DEPARTMENT OF PHARMACY
UNIVERSITAS ISLAM INDONESIA
Jl. Kaliurang km 14,5 Ngemplak Sleman, Yogyakarta
Phone: (0274)895920 ext 4033, 4034

CERTIFICATE

Number: 007-02/L-PB/UII/2013

Customer name : Arba Pramundita Ramadani

Address : Dept of Pharmacology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Phone : +62-81804209884

Date of determination : August 1st 2013

The undersigned explained that the plant determination was performed using aerial part of *kembang bulan* in the Laboratory of Pharmaceutical Biology.

The result was :

Thithonia diversifolia

I certify that this letter is a true and accurate record of the certificate of plant determination.

Yogyakarta, August 7th 2013
Head of Pharmaceutical Biology Laboratory

LABORATORIUM BIOLOGI FARMASI

Hady Anshory T, M.Sc.,Apt

Annex 3. Plant Determination Certificate: *C. barbata*

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DEPARTMENT OF PHARMACY
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CERTIFICATE

Number: 008-03/L.S-PB/UII/2013

Customer name : Arba Pramundita Ramadani

Address : Dept of Pharmacology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Phone : +62-81804209884

Date of determination : August 1st 2013

The undersigned explained that the plant determination was performed using aerial part of *Cincau* in the Laboratory of Pharmaceutical Biology.

The result was :

Cyclea barbata

I certify that this letter is a true and accurate record of the certificate of plant determination.

Yogyakarta, August 7th 2013
Head of Pharmaceutical Biology Laboratory

UNIVERSITAS ISLAM INDONESIA
LABORATORIUM BIOLOGI FARMASI
FAKULTAS KEDOKTERAN

Hady Anshory T, M.Sc.,Apt

Annex 4. Plant Determination Certificate: *T. crispa*

LABORATORY OF PHARMACEUTICAL BIOLOGY
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CERTIFICATE

Number: 009-03/S-PB/UII/2013

Customer name : Arba Pramundita Ramadani

Address : Dept of Pharmacology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Phone : +62-81804209884

Date of determination : August 3rd 2013

The undersigned explained that the plant determination was performed using aerial part of *Brotowali* in the Laboratory of Pharmaceutical Biology.

The result was :

***Tinospora crispa* (L.) Miers**

I certify that this letter is a true and accurate record of the certificate of plant determination.

Yogyakarta, August 7th 2013
Head of Pharmaceutical Biology Laboratory

LABORATORIUM BIOLOGI FARMASI

Hady Anshory T, M.Sc.,Apt