The potential role of CI-679 against artemisinin-resistant *Plasmodium falciparum*

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Research

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Abstract

Background

The emergence of artemisinin-resistant *Plasmodium falciparum (P. falciparum)* in the southeast of Asia hampers the control of malaria. It is urgent to develop novel antimalarial drug with mechanism different from that of artemisinin.

Methods

The efficacy of Nitroquine (CI-679), which is thought to antifolate, against blood stage parasites in the *Plasmodium yoelii (P. yoelii)* -infected BALB/C mice model was firstly identified. Then, *in vitro* assay was performed to investigate its killing effect on 3D7 *P. falciparum* strain. Finally, the potential effect of CI-679 was also observed on artemisinin-resistant *P. falciparum*, which is constructed by introducing the C580Y mutation in K13 of the 3D7 using the CRISPR-cas9 technology.

Results

CI-679 could significantly suppress the growth of rodent malaria parasite *P. yoelii* BY265 at a dose-dependent manner, and even potently kill the parasites in mice when administrated at the peak of parasitemia. Furthermore, CI-679 could also efficiently inhibit the growth of *P. falciparum in vitro*, with more sensitivity against late phase of parasite. Strikingly, CI-679 can even kill the artemisinin-resistant *P. falciparum*, which is constructed by the mutation of C580Y at K13 gene, as sensitive as the 3D7 parent parasites.

Conclusion

CI-679 could be explored as an alternative antimalaria drug against artemisinin-resistant *P. falciparum*, as well as a potential partner of artemisinin-based combination therapies against malaria parasite in the future.

Background

Malaria is the most important parasitic disease of human beings. It is transmitted in 87 endemic countries, affected about 3 billion people and caused 229 million cases and 409 thousand deaths in 2019, the majority being children under 5 years old living in Sub-Saharan Africa [1]. This situation is expected to worsen as the ongoing SARS-COV-2 pandemic affects malaria treatment and prevention measures [2]. Five species of the genus *Plasmodium* cause all malarial infections in human beings. *P. vivax* is the most widespread species, whereas *P. falciparum* is responsible for the most deaths [1]. Pregnant women and nonimmune naïve hosts are particularly susceptible to severe malaria, which is

associated with complications such as severe anemia and/or sequestration of parasitized red blood cells (RBCs) into key organs, including brain [3]. The deployment of first-line artemisinin-based combination therapies (ACTs) has made a substantial contribution to promoting control and elimination strategies [4]. In the absence of an effective licensed malaria vaccine, the strategies are critically reliant on the continued clinical efficacy of ACTs [3].

However, the initial observation of artemisinin clinical failure in Southeast Asia along the Thai-Myanmar and Thai-Cambodia borders was reported in 2008 [5, 6]. Great efforts have been made to reveal the artemisinin-resistant mechanism of *P. falciparum*, and kelch 13 (*Pf*k13) was identified as the artemisinin resistance (AR) molecular marker [7]. *Pf*k13 resides on chromosome 13 of the *P. falciparum* genome, mapping to the β-propeller domain of the encoded kelch-like protein, *PF3D7_1343700* [7]. The propeller domain of the kelch superfamily encodes proteins harbor multiple protein-protein interaction sites and mediates diverse cellular functions, including ubiquitin-regulated protein degradation and oxidative stress responses [8] and the *Pf*k13 is associated with slow parasite clearance rates [9–11]. Over 20 mutations in *Pf*k13 have been identified after treatment with artemisinin derivatives across 15 locations in Southeast Asia [12], among which, at least 11 kelch13 mutations has been validated as molecular markers for artemisinin resistance according to WHO criteria [13, 14] and the most prevalent *Pf*k13 mutation is C580Y [7, 15–17]. The emergency and spread of resistance to artemisinin has posed a major hindrance to the strategies towards malaria control and elimination [17–20] and potentially derailing current control efforts. Thus, there is an urgent need for new antimalarial drugs with different mechanism, especially those that act against currently artemisinin resistant strains.

In the early 1960s, a collaborative program of synthesis of folic acid analogs in which the pteridine ring was replaced by a quinazoline moiety was launched by the Departments of Chemistry of Parke Davis and Company, Ann Arbor, Michigan and Hounslow, Middlesex, England [21]. There interests soon focused on 2,4-diamino-6-substituted derivatives for their promising properties to inhibit the activities of dihydrofolate reductases and thymidylate synthetases of mammal and bacteria [22, 23]. Representative 2,4-diamino-6-substituted quinazolines were further determined for antiparasitic activities and PAM-1392 [2,4-diamino-6-(3,4-dichlorobenzylamino)-quinazoline] [22] and CI-679 (2,4-diamino-6-[(3,4-dichlorobenzyl)-nitrosoamino]-quinazoline) [24] attracted special attention. CI-679 was approximately 40 times as effective as PAM-1392 in suppressing infections with the parent sensitive strain of *Plasmodium berghei* and approximately 10 times as effective as PAM-1392 in suppressing and curing infections with blood schizonts of *Plasmodium cynomolgi* [25, 26]. Then, CI-679, along with artemisinin of the same time, was selected as an important part of Project 523, a national project set up by the Chinese government in 1967 [27, 28]. However, the research and development of CI-679 were retarded by the success in artemisinin. As the developing and spread resistance to artemisinin, and the mechanism of killing parasite by CI-679 is divergent from that of artemisinin, there has been a resurgence of interest in CI-679.

Here, we describe the development of CI-679 for activity against *P. yoelii* in murine model and artemisinin-sensitive *P. falciparum* 3D7 and artemisinin-resistant *P. falciparum* C580Y *in vitro*.

Methods

Study design

The program objectives were to characterize the efficacy of CI-679. Efficacy was evaluated against the *P. yoelii* in murine model *in vivo* and artemisinin-sensitive *P. falciparum* 3D7 and artemisinin-resistant *P. falciparum* C580Y *in vitro*. Studies *in vivo* were designed to evaluate efficacy and how fast the compound kills parasites. Studies *in vitro* were designed to evaluate efficacy across the full range of blood stage of *P. falciparum* 3D7 and against *P. falciparum* C580Y.

Parasite and mice

BALB/C female mice at 6 weeks of age, were purchased from Beijing Huafukang Bioscience Company Limited. *P. yoelii* strain BY-265 is maintained in our laboratories. It has been used for several decades to test susceptibility to a wide range of antimalarial drugs. For infection, BALB/C female mice were injected with 1×10^6 rodent malaria parasite *P. yoelii* strain BY-265 via intravenous injection (i.v.) [29]. Animal experiments were approved by the institutional animal care and use committees for each of the experimental sites. All studies were conducted according to the appropriate legislation and institutional policies on the care and use of animals.

Parasite culture and the establishment of P. falciparum C580Y

3D7 *P. falciparum* were cultured as previously described with 5% O_2 and 5% CO_2 at 37 ℃ [30]. Parasites were synchronized with 5% sorbitol solution for ring stage or purified on a 40/70% percoll discontinuous gradient for schizont stage.

The resistant strain *P. falciparum* C580Y was constructed through mutation of C580Y in K13 of 3D7 using Crispr-Cas9 technology [31]. The K13_C580Y mutation (with shield mutations) and the Sir2B shield mutations were introduced into the Cas9i system by plasmid *pL6cs-K13-Sir2B*. For transfection, fresh human type O erythrocytes were electroporated in cytomix (120 mM KCl, 10 mM KH_2PO_4, 25 mM HEPES, 2 mM EGTA, 0.15 mM $CaCl_2$, 5 mM $MgCl_2$, pH 7.6) with 100 μg of each plasmid under standard electroporation parameters [32] and enriched late schizonts were mixed with the electroporated erythrocytes immediately. Positive selection drugs were applied 72 h post-transfection, and media and drugs were refreshed every day for the first 7 days. The final concentrations of drugs were 2.5 μg/ml for BSD (Sigma), 2.5 nM for WR99210 (Sigma), and 1.5 μM for DSM1 [33], respectively. The desired transgenic 3D7 strain *K13::Sir2B-mut*^{Cas9i} was obtained in about 20 days. The correct introduction of K13_C580Y mutation and shield mutations was identified as reported [31].

In vivo efficacy of CI-679 in the P. yoelii-infected BALB/C mice

BALB/C mices were infected with *P. yoelii* strain BY-265 as described above. To observe the dose-dependent efficacy of CI-679, the mice were treated with or without 2.5, 5.0 and 10.0 mg/kg/day of CI-679 (dissolve in DMSO for storage and dissolve in PBS when used) via intragastric injection (i.g.) respectively for 3-day-treatment. To investigate the treatment effect of CI-679, the mice were treated with 2.5 mg/kg/day of CI-679 via intragastric injection on 5-6 days post infection (dpi) and 3-5 dpi for its role on peak and rising stage of parasitemia, respectively, for 3-day-treatment.

The determination of parasitemia and mice survival rate

Blood smears were made daily from the tail venous blood of mice and stained by Giemsa. Parasitemia was counted from at least 5,000 RBCs by microscopy. Blood smears and mortality data were taken on 1 dpi and thereafter till clear of parasitemia of blank control, at which time all treated mice surviving that were blood film negative were considered cured.

In vitro blood-stage parasites inhibition assay

A total of 0-3 h ring-stage survival assay ($RSA^{0-3\,h}$) assay was performed to evaluate the activity of CI-679 against the *P. falciparum* sensitive 3D7 and artemisinin resistance C580Y as previously described [34]. In brief, parasites were synchronized several times to acquire the accurate 0-3 h post-invasion rings. The $RSA^{0-3\,h}$ was done immediately with 0-3 h post-invasion rings, the trophozoite-stage survival assay ($TSA^{24-27\,h}$) with 24-27 h post-invasion rings, and the schizont-stage survival assay ($SSA^{36-39\,h}$) with 36-39 h post-invasion trophozoites. In each assay, the accurately synchronized parasites were exposed to 0/640 ng/ml discontinuous gradient (0, 0.01, 0.04, 0.16, 0.64, 2.5, 10, 40, 160, 640 ng/ml) CI-679 or 0.1% DMSO (the solvent) for 6 h, washed with incomplete medium to remove the drug, and cultured for another 66 h, 42 h and 30 h, respectively, after drug removal. Blood films were made to assess the survival rates of the strains microscopically. Each assay was repeated for 3 times independently, with each group setting three technical repetitions. Each technical repetition was counted for 40,000 erythrocytes.

For EC50 determinations, data were fitted to the log(inhibitor) versus response – variable slope (four-parameter) model in GraphPad Prism 9.0.

Statistical analysis

Graphical representation and statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Inc.). Unless otherwise stated, the results are shown as means ± s.e.m. from three independent experiments in duplicate. The two-way ANOVA was used to compare parasitemia between CI-679 treatments and the control group mice. The two-tailed Student's *t* - test was used to test the differences of proliferation *in vitro*. The log-rank (Matel–Cox) test was used to compare the survival distributions of two groups. For parasite EC50 determination, data were fitted to the specified model as described in Materials and Methods using nonlinear least squares analysis. A *P* value threshold of 5% was used to indicate statistical significance. Biological replicates (n) indicated in figure legends refers to the number of mice or number of independent cultures. Sample sizes were chosen on the basis of historical data, no statistical methods were used to predetermine sample size.

Results

CI-679 suppresses the growth of P. yoelii in a dose-dependent manner

Firstly, we had an insight into the efficacy of CI-679 against rodent malaria. We undertook a detailed analysis of the intragastric dose-dependent profile in the *P. yoelii* BALB/C mouse 3-day dosing model. Mice in all groups were inoculated intravenously with 1×10^6 parasitized red blood cells (pRBCs). Parasitized mice in experimental groups treated with CI-679 by intragastric injection (i.g.) at 2.5, 5.0, 10.0 mg per kg of body weight 2 hours after inoculation for 3 consecutive days thereafter. The control group was given equal volume of PBS. All mice survived the challenge were able to control parasitemia without the need for antimalarial drug treatment (Fig. 1a). Parasitemia was evaluated from 1 day post infection (dpi) until the control mice were cleared of parasitemia. We found that as low as a dose of 2.5 mg per kg was fully protective (Fig. 1a) and all the mice were survived (Fig. 1b) in experimental groups.

CI-679 efficiently inhibit the growth of P. yoelii even administrated at the peak of parasitemia

The previous experiment suggested parasites infected mice treated with CI-679 by intragastric injection at 2.5 mg per kg of body weight for 3 consecutive days could be cured and the first parasitemia peak in control group was reached between 5 to 7 dpi. We choose this window to investigate the role of CI-679 even administrated at the peak of parasitemia. We found that all mice treated with CI-679 were clear of parasitemia soon after administration (7 dpi) (Fig. 2a) and all survived till the mice in control were free from parasitemia (Fig. 2b). We further investigated the role of CI-679 as the parasitemia rising up (3-5 dpi). As expected, all mice treated with CI-679 were clear of parasitemia after administration (6 dpi) (Fig. 2c) and survived (Fig. 2d).

CI-679 efficiently inhibit the growth of sensitive P. falciparum strain in vitro

To further identify the efficacy of CI-679 against different blood stages of *P. falciparum*, we evaluated the activity of CI-679 against the ring, trophozoite and schizont stages using highly synchronized *P. falciparum* parasites drug-sensitive strain 3D7. Parasites were synchronized to ring, trophozoite and schizont stage in a classical manner [35]. Parasites were exposed to CI-679 at 0/640 ng/ml discontinuous gradient for a 6-h treatment at ring (0-6 hours post invasion, hpi), trophozoite (24-30 hpi) and schizont (36-42 hpi) stages (Fig. 3a). Further measurements of parasitemia at 72 hpi indicated that the treatment at the trophozoite and schizont stages were more active to parasite killing than that at ring stage (Fig. 3b). This suggested that the mechanism of CI-679 may be associated with the development of trophozoites and schizonts in red blood cells.

CI-679 efficiently kills artemisinin-resistant P. falciparum strain

Having demonstrated the excellent inhibitory activities of CI-679 on *P. falciparum* drug sensitive 3D7, we wanted to determine the effect of CI-679 on artemisinin-resistant parasites. As the mutation of C580Y in K13 gene confer the resistance of *P. falciparum* to artemisinin, and C580Y mutation has been confirmed

to be correlated to artemisinin resistance characterized by high $RSA^{0-3\,h}$ survival rates[7], we introduced the K13_C580Y mutation into the Cas9i system by plasmid *pL6cs-K13-Sir2B* (Fig. 4a). The level of artemisinin resistance was assessed as previously reported [31]. Both *P. falciparum* 3D7 and C580Y parasites were exposed to CI-679 at 0/640 ng/ml discontinuous gradient. Using the standard 72 h drug assay, CI-679 exhibited similar activities against both *P. falciparum* 3D7 and C580Y parasites (Fig. 4b). Taken together, our results suggested CI-679 had a potent inhibitory activity against artemisinin-resistant malaria parasites.

Discussion

The *P. falciparum* parasites have independently developed partial resistance to artemisinin, the core compound of the best available antimalarial drugs, in several foci in the Greater Mekong subregion (GMS), Africa, Oceania and Latin America [1, 36, 37]. As a consequence, the increasing prevalence of artemisinin-resistance and the consequent emergence of resistance to the partner drugs in ACTs may well reverse the substantial recent gains in malaria control [15]. New antimalarial drugs are urgently needed.

Recent data indicate that the average cost and time for a new drug from idea to market are about 3 billion dollars and 14 years, respectively [38, 39]. Although the malaria drug development pipeline is in a healthier state for many years at present, there is only one 8-aminoquinoline, tafenoquine, newly approved by FDA against liver stages of malaria [40, 41]. An alternative strategy is to evaluate the antimalarial activity of molecules that are already licensed for new medical indications, an approach known as drug repurposing that has been successfully applied in oncology [42]. Since the drugs are already in routine clinical use for other purposes early phase clinical trials can be bypassed, saving time and money. Thus, this strategy offers a promising alternative to traditional drug development pipelines, and can help fast and less expensive solutions to realize malaria elimination and eradication.

Patients with *P. falciparum* hyperparasitemia are at increased risk for treatment failure, severe malaria and death [43]. Parasitemia level can serve as a predictor for severe malaria [44]. Herein, we show that CI-679 could suppress the growth of rodent malaria parasite *P. yoelii* BY265 at a dose-dependent manner, and even rapidly kill the parasite in mice when administrated at the peak of parasitemia. Furthermore, CI-679 has a wide therapeutic window, both effective at the rising and peak stage of parasitemia, makes it can be widely available as a partner drug in a fixed-combination. The property could make severe malaria and death much less likely.

Continued research and development to make new drugs available to replace current drugs as resistance emerges is one of the several high priority research areas identified to be addressed urgently regardless of whether the world mobilizes for a renewed effort to eliminate malaria [45]. As the increasing threat of the emergency and spread of resistance to artemisinin, finding the optimal combination of interventions to maximize impact and mitigate the risk of resistance is a critical challenge in malaria elimination [46]. For eradication to be effective, the concept of a drug combination Single Encounter Radical Cure and Prophylaxis (SERCaP) was developed [45], signify clinical candidates will necessarily require combination

with mechanistically distinct drug partner(s) to alleviate the potential for drug resistance. CI-679 can kill the artemisinin-resistant *P. falciparum*, which is constructed by the mutation of C580Y at K13 gene. Therefore, CI-679 could be explored as an alternative antimalarial agent against artemisinin-resistant *P. falciparum* and a potential combination partner of ACTs against malaria parasite.

The ring stages can develop into schizonts through the trophozoites and the schizonts burst and release daughter merozoites by the end of blood stage, each of which can invade blood cells and repeat the cycle [3]. In the blood stage, *Plasmodium* parasites replicate rapidly and may replicate exponentially to > 10^{12} parasites per patient. This rapid growth requires sustained pools of nucleotides for the synthesis of DNA and RNA [47]. Folate is essential for the survival and growth of the malaria parasite and DNA synthesis [48] and the folate metabolic pathway constitutes an antimalarial target. CI-679 could efficiently inhibit the growth of *P. falciparum in vitro*, with more sensitivity against late phase of blood stage. This result was as expected, for CI-679 was a product of the program of the synthesis of folic acid analogs. The development of parasites in late phase of blood stage might be impeded by the insufficient DNA synthesis ascribed to the administration of CI-679.

Although CI-679 is potent against *P. yoelii* and *P. falciparum*, even artemisinin-resistant C580Y, further pharmacokinetic properties, tolerability and safety need to be characterized to support its preclinical development and to enable the compound to advance to human clinical trials. In addition, as the rising concern of resistance to antifolates, possible drug resistance mechanisms based on *in vitro* selection should also be included.

Conclusions

In summary, CI-679 could be explored as an alternative antimalarial drug against artemisinin-resistant *P. falciparum*, as well as a potential combination partner of ACTs against *Plasmodium* parasite. As the money- and time-consuming progress in the antimalarial drugs research and development, repurposing the agents already known might be an efficient strategy to combat the *P. falciparum* drug resistance issues.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

WYX, QFZ, JZ and NT conceived and designed the experiments. NT, YMZ, YD, YF and HXL performed the experiments. NT and YMZ analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. WHO. World malaria report. 2020. Geneva: World Health Organization. 2020.
2. Sherrard-Smith E, Hogan AB, Hamlet A, Watson OJ, Whittaker C, Winskill P, et al. The potential public health consequences of COVID-19 on malaria in Africa. Nat Med. 2020;26(9):1411–6.
3. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria The Lancet. 2014;383(9918):723–35.
4. WHO. Global technical strategy for malaria 2016-2030. Geneva: World Health Organization; 2015.
5. 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(24):2619–20.
6. 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.
7. 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(7481):50–5.

8. Adams J, Kelso R, Cooley L. The kelch repeat superfamily of proteins: propellers of cell function. Trends Cell Biol. 2000;10:17–24.
9. Cheeseman IH, Miller BA, Nair S, Nkhoma S, Tan A, Tan JC, et al. A major genome region underlying artemisinin resistance in malaria. Science. 2012;336(6077):79–82.
10. Takala-Harrison S, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, et al. Genetic loci associated with delayed clearance of Plasmodium falciparum following artemisinin treatment in Southeast Asia. PNAS. 2013;110(1):240–5.
11. Miotto O, Almagro-Garcia J, Manske M, Macinnis B, Campino S, Rockett KA, et al. Multiple populations of artemisinin-resistant Plasmodium falciparum in Cambodia. Nat Genet. 2013;45(6):648–55.
12. 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(3):226–34.
13. WHO. Report on antimalarial drug efficacy, resistance and response 10 years of surveillance (2010–2019). Geneva: World Health Organization; 2020.
14. Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana S-I, Yamauchi M, et al. Evidence of artemisinin-resistant malaria in Africa. N Engl J Med. 2021;385(13):1163–71.
15. 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(5):411–23.
16. Menard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, et al. A worldwide map of Plasmodium falciparum K13-propeller polymorphisms. N Engl J Med. 2016;374(25):2453–64.
17. Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, Rekol H, et al. The spread of artemisinin-resistant Plasmodium falciparum in the Greater Mekong subregion: a molecular epidemiology observational study. Lancet Infect Dis. 2017;17(5):491–7.
18. Schmedes SE, Patel D, Dhal S, Kelley J, Svigel SS, Dimbu PR, et al. Plasmodium falciparum kelch 13 Mutations, 9 Countries in Africa, 2014-2018. Emerg Infect Dis. 2021;27(7):1902–8.
19. 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. The Lancet. 2012;379(9830):1960–6.
20. Haldar K, Bhattacharjee S, Safeukui I. Drug resistance in Plasmodium. Nat Rev Microbiol. 2018;16(3):156–70.
21. Davoll J, Johnson AM. Quinazoline analogues of folic acid. J Chem Sco. 1970;C:997–1002.
22. Davoll J, Johnson AM, Davies HJ, Bird D, Clarke J, Elslager EF. Folate antagonists. 2. 2,4-diamino-6-([aralkyl and (heterocyclic)methyl] amino)quinazolines, a novel class of antimetabolites of interest in drug-resistant malaria and Chagas' disease. J Med Chem. 1972;15(8):812–26.
23. Bird OD, Vaitkus JW, Clarke J. 2-amino-4-hydroxyquinazolines as inhibitors of thymidylate synthetase. Mol Pharmocol. 1970;6:573–5.

24. Elslager EF, Clarke J, Werbel LM, Worth DF, Davoll J. Folate antagonists. 3. 2,4-diamino-6-(heterocyclic)quinazolines, a novel class of antimetabolites with potent antimalarial and antibacterial activity. J Med Chem. 1972;15(8):827–36.
25. Thompson PE, Bayles A, Olszewski B. PAM 1392 [2,4-diamino-6-(3,4-dichlorobenzylamino) quinazoline] as a chemotherapeutic agent: Plasmodium berghei, P. cynomolgi, P. knowlesi, and Trypanosoma cruzi. Exp Parasitol. 1969;25:32–49.
26. Thompson PE, Bayles A, Olszewski B. Antimalarial activity of 2,4-diamino-6-[(3,4-dichlorobenzyl) nitros-amino] quinazoline (CI-679 base) and CI-679 acetate. Am J Trop Med Hyg. 1970;19(1):12–26.
27. Wang J, Xu C, Liao FL, Jiang T, Krishna S, Tu Y. A temporizing solution to “artemisinin resistance”. N Engl J Med. 2019;380(22).
28. Group 523 in the Military Medical University. The introduction of new antimalaria drug nitroquine. Acta Acadiemiae Midicinae Militaris Tertiae. 1979;1(4):1–7.
29. Hearn J, Rayment N, Landon DN, Katz DR, Souza, JBd. Immunopathology of cerebral malaria: morphological evidence of parasite sequestration in murine brain microvasculature. Infect Immun. 2000;68(9):5364–76.
30. Ghorbal M, Gorman M, Macpherson CR, Martins RM, Scherf A, Lopez-Rubio J-J. Genome editing in the human malaria parasite Plasmodium falciparum using the crisPr-cas9 system. Nat Biotechnol. 2014;32:819–21.
31. Zhao Y, Wang F, Wang C, Zhang X, Jiang C, Ding F, et al. Optimization of CRISPR/Cas system for improving genome editing efficiency in Plasmodium falciparum. Front Microbiol. 2021;11:625862.
32. Fidock DA, Wellems TE. Transformation with human dihydrofolate reductase renders malaria parasites insensitive to WR99210 but does not affect the intrinsic activity of proguanil. PNAS. 1997(94):10931–6.
33. Phillips MA, Gujjar R, Malmquist NA, White J, Mazouni FE, Baldwin J, et al. Triazolopyrimidine-based dihydroorotate dehydrogenase inhibitors with potent and selective activity against the malaria parasite Plasmodium falciparum. J Med Chem. 2008;51(12):3649–53.
34. Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, et al. Novel phenotypic assays for the detection of artemisininresistant Plasmodium falciparum malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect Dis. 2013;13:1043–9.
35. Lambros C, Vanderberg JP. Synchronization of Plasmodium falciparum erythrocytic stages in cultures. J Parasitol. 1979;65(3):418–20.
36. Mathieu LC, Cox H, Early AM, Mok S, Lazrek Y, Paquet J-C, et al. Local emergence in Amazonia of Plasmodium falciparum k13 C580Y mutants associated with in vitro artemisinin resistance. eLife. 2020;9.
37. Miotto O, Sekihara M, Tachibana SI, Yamauchi M, Pearson RD, Amato R, et al. Emergence of artemisinin-resistant Plasmodium falciparum with kelch13 C580Y mutations on the island of New Guinea. PLoS Pathog. 2020;16(12):e1009133.

38. Schuhmacher A, Gassmann O, Hinder M. Changing R&D models in research-based pharmaceutical companies. J Transl Med. 2016;14(1):105.
39. Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov. 2010;9(3):203–14.
40. Ashley EA, Phyo AP. Drugs in development for malaria. Drugs. 2018;78(9):861–79.
41. Llanos-Cuentas A, Lacerda MVG, Hien TT, Velez ID, Namaik-Larp C, Chu CS, et al. Tafenoquine versus primaquine to prevent relapse of Plasmodium vivax malaria. N Engl J Med. 2019;380(3):229–41.
42. Bertolini F, Sukhatme VP, Bouche G. Drug repurposing in oncology - patient and health systems opportunities. Nat Rev Clin Oncol. 2015;12:732–42.
43. WHO. Guidelines for the Treatment of Malaria. 3rd edn. 2015. Geneva: World Health Organization. 2015.
44. Tangpukdee N, Krudsood S, Kano S, Wilairatana P. Falciparum malaria parasitemia index for predicting severe malaria. Int J Lab Hematol. 2012;34:320–7.
45. The malERA Consultative Group on Drugs. A research agenda for malaria eradication: drugs. PLoS Med. 2011;8(1):e1000402.
46. Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J, et al. malERA: An updated research agenda for malaria elimination and eradication. PLoS Med. 2017;14(11):e1002456.
47. Phillips MA, Burrows JN, Huijsduijnen RHv, Manyando C, Voorhis WCV, Wells TNC. Malaria. Nat Rev Dis Primers. 2017;3:17050.
48. Metz J. Folic acid metabolism and malaria. Food Nutr Bull. 2007;28(4 Suppl):540-9.

Figures

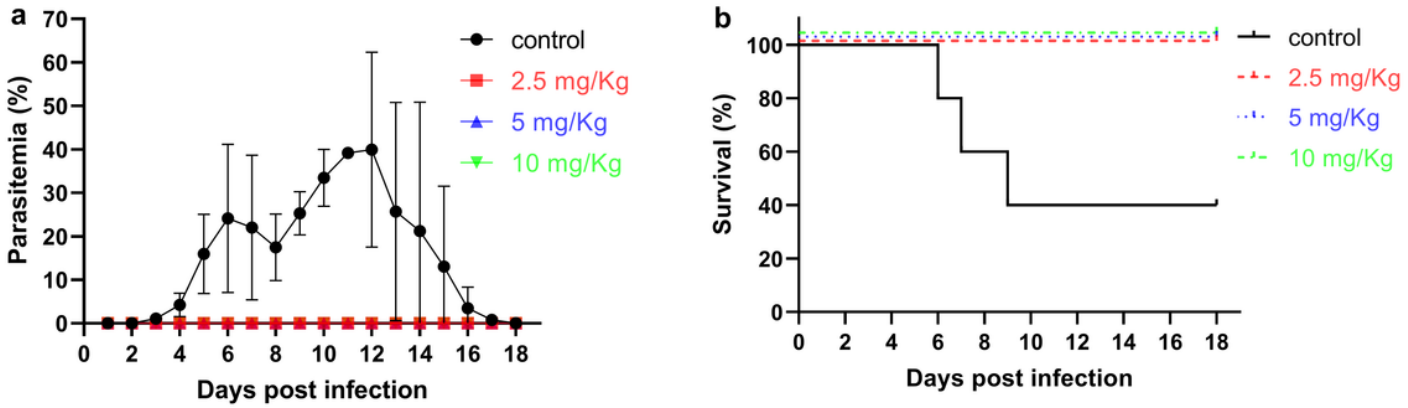

Figure 1

In vivo dose-dependent efficacy of CI-679 in the P. yoelii-infected BALB/C mice. CI-679 was dosed as the acetate salt once daily for 3 consecutive days from the day of infection. (a) Blood parasitemia versus days after infection for administered doses (mg/kg per day). The results are shown as means ± s.e.m.; n=5 in 3 independent experiments. (b) Survival of parasites infected BALB/c mice treated with different doses of CI-679 and the control group mice.

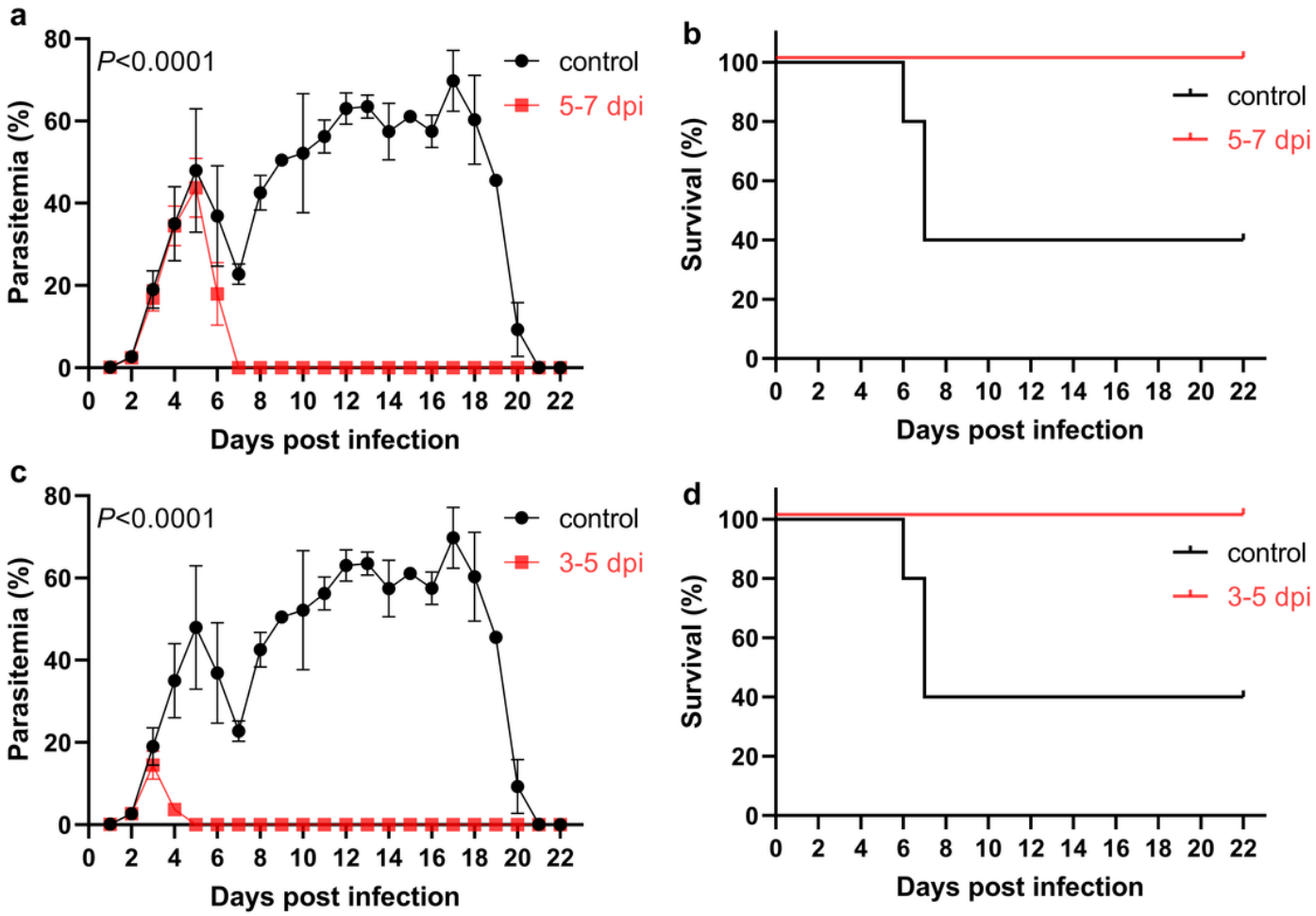

Figure 2

Efficacy of CI-679 on peak and rising stage of parasitemia in the P. yoelii-infected BALB/C mice. (a) Blood parasitemia versus days after infection for administered doses at the peak of parasitemia (5-7 dpi). The results are shown as means ±s.e.m.; n=5 in 3 independent experiments; P values were determined by two-way ANOVA. (b) Survival of parasites infected BALB/c mice treated with CI-679 (5-7 dpi) and the control group mice. (c) Blood parasitemia versus days after infection for administered doses at the rising stage of parasitemia (3-5 dpi). The results are shown as means ± s.e.m.; n=5 in 3 independent experiments; P values were determined by two-way ANOVA. (d) Survival of parasites infected BALB/c mice treated with CI-679 (3-5 dpi) and the control group mice.

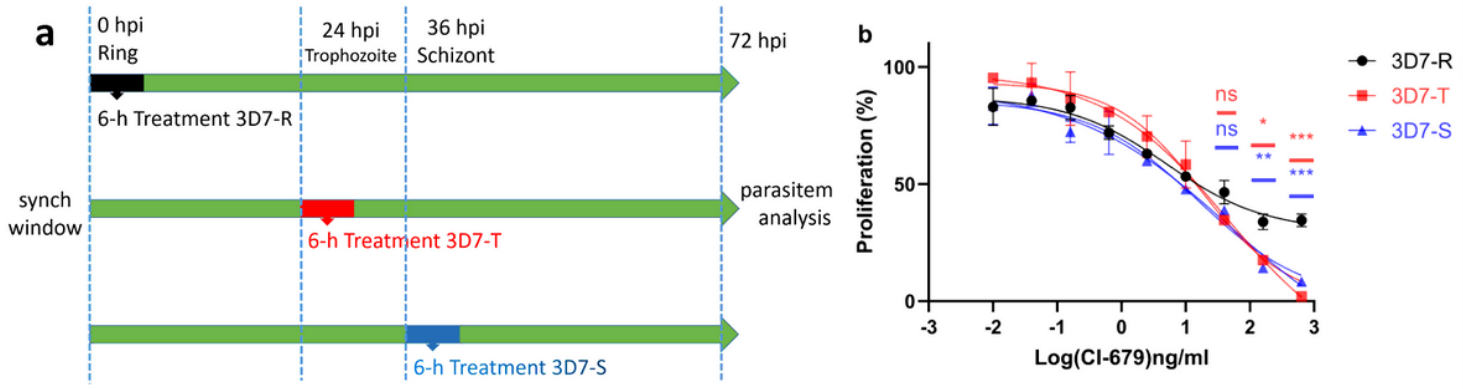

Figure 3

In vitro P. falciparum 3D7 growth inhibition. (a) Schematic of the experiment design. Highly synchronized P. falciparum 3D7 were treatment for 6h with CI-679 at 0/640 ng/ml discontinuous gradient (0, 0.01, 0.04, 0.16, 0.64, 2.5, 10, 40, 160, 640 ng/ml) for three consecutive periods of the blood stage (the ring, trophozoite and schizont stage). Parasitemia was analyzed at 72 hpi. (b) Fitted EC50s were 5.04 ng/ml, 17.98 ng/ml, and 15.74 ng/ml for 3D7-R, 3D7-T, and 3D7-S, respectively. Three technical replicates per concentration and 27-30 data points per fit; the plot shows mean ± s.e.m.; P values were determined by two-tailed Student's t-test. *P < 0.05, **P < 0.01, ***P < 0.001.

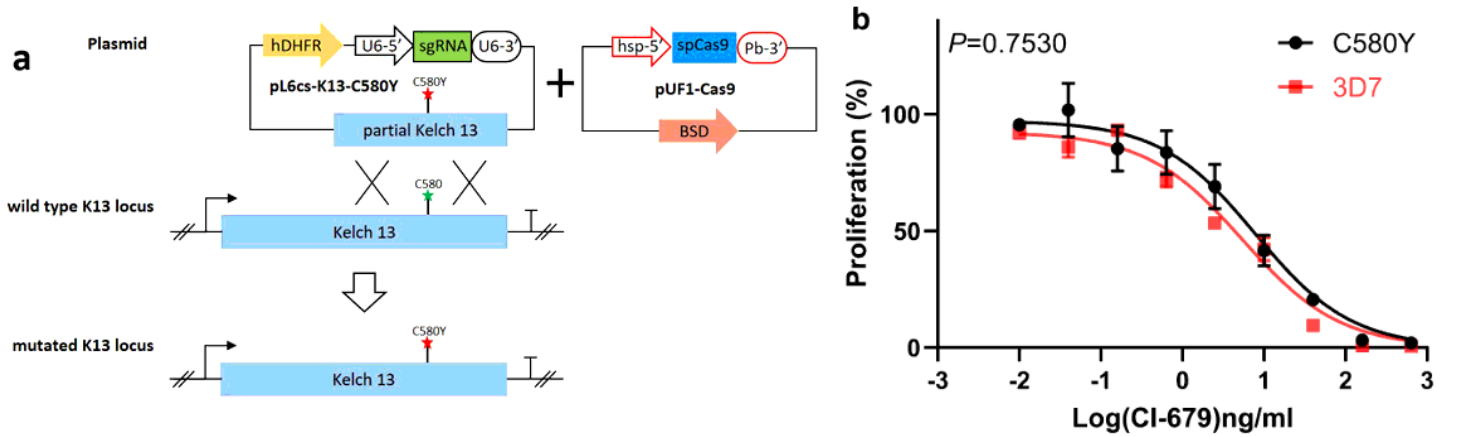

Figure 4

in vitro P. falciparum C580Y growth inhibition. (a) Sketch of pL6cs-K13-C580Y and pUF1-Cas9. pUF1-Cas9 expresses the Cas9 protein with the BSD drug-selectable marker. pL6cs-K13-C580Y carries the K13-C580Y cassette, the donor DNA including the hDHFR marker. (b) Fitted EC50s were 5,471 ng/ml, and 7.886 ng/ml for 3D7 and C580Y, respectively. Three technical replicates per concentration and 18-20 data points per fit; the plot shows mean ± s.e.m.; P values were determined by two-tailed Student's t-test.

Supplementary Files

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