Structural basis for the inhibition of *Plasmodium falciparum* hexose transporter

Malaria remains one of most devastating disease in tropic and subtropic regions around the world, leading approximately 409,000 mortalities during 2019. Five parasites of *Plasmodium spp.* cause human malaria, of which *Plasmodium falciparum* is the deadliest form and is responsible for more than half of total malaria cases. Administration of effective antimalarials, like chloroquine and artemisinin-based combination therapies (ACTs), has markedly decreased malaria-related toll in the past decades. However, the commence and rapid spread of drug-resistance parasites raise increasing concerns to malarial eradication, which results in urgent needs for novel antimalarial chemotherapies [1].

The blood-stage malaria parasites primarily uptake glucose from host erythrocyte as energy source. In particular, *P. falciparum* levers the hexose transporter PfHT1, a single-copy gene without close paralogues, to achieve this goal, which makes it possible to cut off energy supply of asexual stage parasite by inhibiting the transport activity of PfHT1 (Fig. 1(A)). Previous investigation on substrate specificity of PfHT1 yielded a glucose analog, Compound 3361 (C3361), that can moderately and selectively inhibit PfHT1 rather than its human homologue hGLUT1 [2]. However, efforts for chemotherapeutic development based on C3361 has long been hampered due to lack of structural information of PfHT1. Here, we elucidated the molecular model of PfHT1 by solving a 2.6 Å resolution crystal structure of PfHT1 bound with D-glucose. We also successfully revealed a novel ligand-binding-induced allosteric pocket through a 3.7 Å crystal structure of PfHT1-C3361 complex, followed by rational design on the basis of C3361. We succeeded in obtaining a high potent and selective PfHT1 inhibitor, **HTI-1**, which simultaneously targets the orthosteric and allosteric sites of PfHT1 [3] (Fig. 1(B)).

To elucidate the structure of PfHT1 in complex with D-glucose, codon-optimized cDNA of PfHT1 was expressed in Sf9 insect cell. Recombinant PfHT1 was purified through combined biochemical approaches, followed by crystallization using hanging-drop vapor diffusion method. Crystals of PfHT1-glucose complex were screened at SSRF BL18U and diffraction data was collected at SPring-8 **BL32XU**. The structure of PfHT1-glucose complex was solved by molecular replacement, using modified initial model of hGLUT3, at a final resolution 2.6 Å. Similar to other sugar porter (SP) family members, PfHT1 exhibits a canonical major facilitator superfamily (MFS) fold with 12 transmembrane segments (TMs) form two six-helical bundles. The intervening sequence between N and C domains, together with C-terminal segment, constitutes an intracellular helical (ICH) domain (Fig. 2(A)).

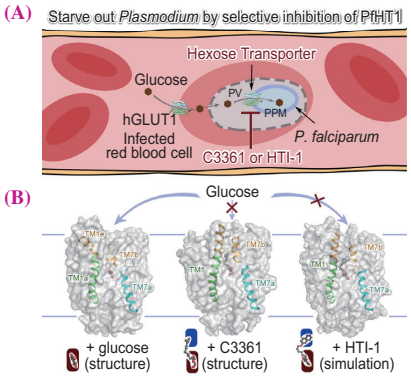

Fig. 1. Schematic diagram of inhibition of PfHT1. **(A)** Cutting off the glucose uptake of *P. falciparum* by selectively inhibiting PfHT1. **(B)** Structural models for C3361 and HTI-1 inhibition.

Analysis of central pocket revealed a highly conserved glucose binding pattern; the sequence and acting mode of glucose binding residues are almost identical to hGLUT1 and hGLUT3 (Fig. 2(B)).

On the other hand, PfHT1 possesses unique features in several aspects. The second half of TM7, designated TM7b, bends to a larger degree toward substrate binding site, which seals the entrance tunnel from extracellular side. Together with the closed intracellular tunnel, PfHT1-glucose complex presents a novel occluded state (Fig. 2(C)). Despite the extracellular half of TM1 also forms a membrane-parallel helix, the TM1e of PfHT1 is much shorter than it in hGLUTs. Two long extracellular loops, L1-2 between TM1 and TM2 and L5-6 between TM5 and TM6, coordinate with each other through H-bonds between Arg67 of L1-2 and Glu196 and Lys191 of L5-6. In addition, TM1 and TM2 is linked by a disulfide bound between Cys61 and Cys70, which is important for the transport activity of PfHT1 (Fig. 2(D)).

The structure determination of PfHT1 in complex with C3361 was unexpectedly challenging. Using the aforementioned vapor diffusion method, we could only obtain tiny crystals without an acceptable diffraction. We turned to Lipid cubic phase (LCP) method and screened thousands of micro-crystals manually or with the help of ZOO system at BL32XU using micro-focus beam. Finally, a 3.7 Å PfHT1-C3361 complex structure was resolved by merging 172 data sets with wedges of 10 degree for each LCP crystals. Structure of PfHT1 bound to C3361 retained occluded state; However, an unprecedented conformational shift was induced by C3361 binding (Fig. 2(E)). Compared to D-glucose bound PfHT1, TM1e and TM7b straighten up upon C3361 association. TM2 and TM4 slightly swap away from center pocket (Fig. 2(F)). Consequently, the

aliphatic tail of C3361 accommodated to a hydrophobic tunnel and a novel allosteric pocket formed at the end of the tail (Fig. 2(G)). Nevertheless, the coordination between PfHT1 and glucose moiety of C3361 is largely unchanged comparing to PfHT1-glucose complex.

Targeting the C3361-induced allosteric pocket, we designed dozens of compounds with modifications on sugar moiety, aliphatic linker, and functional group at the end of its tail. Three compounds, designated **1a**, **1b**, and **1c** (also named as **HTI-1** for its highest potency), stood out for their high efficiency to inhibit the transport activity of PfHT1 (Fig. 3(A)). Among these compounds, an aromatic group (**1a**, phenyl; **1b**, 2-naphthyl) or a heteroaromatic group (**1c**, 6-quinolyl) is attached to the C8 alkyl chain through an ether linker to substitute the vinyl group in C3361. The IC50 decreases in order of C3361, **1a**, **1b**, and **1c**, referring the substitution gradually fits into allosteric site. In addition, all three compounds retain selectivity of PfHT1 over hGLUT1 (Fig. 3(B)), which makes it possible to apply these compounds to selectively kill parasites with minimal effect on human cells. As expected, all rational-designed compounds demonstrate high potency to multi-drug-resistant strain (Dd2) of *P. falciparum* while moderate cytotoxicity is observed on HEK293T/17 cell line (Fig. 3(C)). Besides, an excellent correlation between IC50 and EC50 values of rational-designed C3361 derivatives indicates the suppression of parasites growth is caused by inhibition of PfHT1 activity (Fig. 3(D)).

Taken together, the emergence of multi-drug-resistant malaria necessitates development of next-generation antimalarials that act with alternative molecular target. PfHT1, the unique hexose transporter mediates glucose uptake of *P. falciparum*, was characterized as potential drug target to “starve out the malaria parasites” through cutting off their energy supply. Previous lead optimization was hindered by enigmatic structure of PfHT1. Our insights into PfHT1 in complex with D-glucose or C3361 illuminate the mechanism of C3361 inhibition and reveal a novel ligand-induced allosteric pocket, which establishes a foundation for structural-guided drug discovery. Given the molecular model of central cavity and allosteric pocket, we rational-designed and obtained a high potency and low cytotoxicity lead, **HTI-1**, which effectively suppressed the growth of parasites by blocking their glucose uptake. Our results serve as proof of principle for an orthosteric-allosteric dual inhibition of PfHT1 and pave the way to next-generation antimalarials targeting PfHT1.

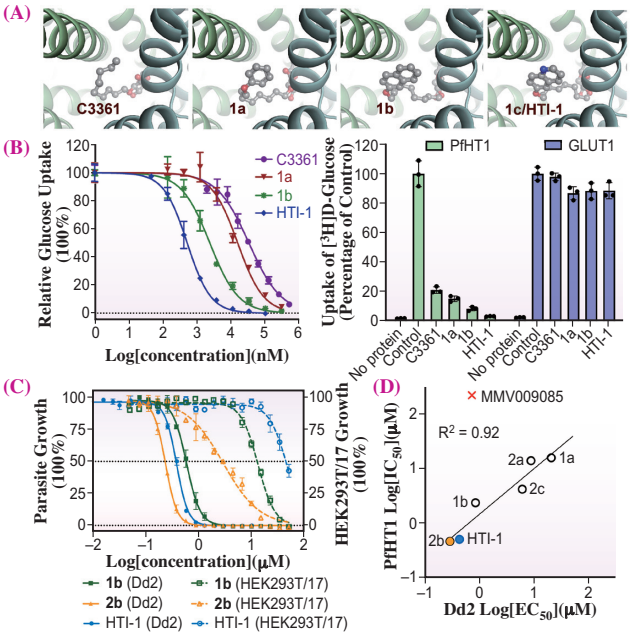

Fig. 3. Rational design and characterization of PfHT1 selective inhibitors. **(A)** Representative of docking results of C3361 and designed inhibitors. **(B)** Proteoliposome based inhibition assay to measure the potency of PfHT1 inhibitors. **(C)** Antiparasitic effect and cytotoxicity of represent PfHT1 inhibitors. **(D)** Correlation between PfHT1 inhibition and parasites growth suppression reveal an on-target effect.

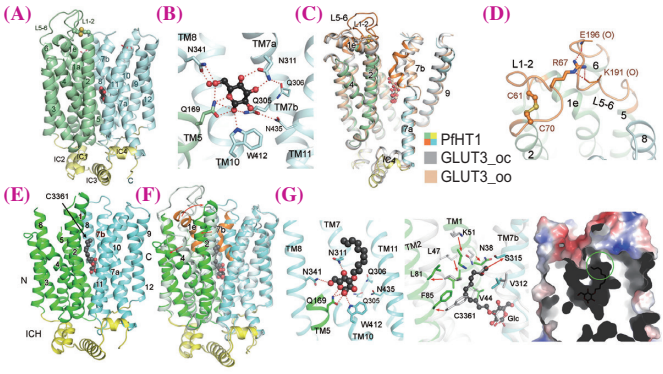

Fig. 2. Crystal structures of PfHT1 bound with D-glucose or C3361. **(A)** Overall structure of PfHT1-glucose complex. **(B)** Coordination of D-glucose in PfHT1. **(C)** Structural comparison between PfHT1 and hGLUT3. **(D)** Unique extracellular loops and disulfide bound of PfHT1. **(E)** Occluded state of PfHT1 in complex with C3361. **(F)** Conformational shift of PfHT1 upon C3361 binding. **(G)** Coordination of C3361 and ligand-induced-allosteric pocket.

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References

[1] K. Haldar *et al.*: Nat. Rev. Microbiol. **16** (2018) 156.
[2] T. Joet *et al.*: Proc. Natl. Acad. Sci. USA **100** (2003) 7476.
[3] *X. Jiang, Y. Yuan, J. Huang, S. Zhang, S. Luo, N. Wang, D. Pu, N. Zhao, Q. Tang, K. Hirata, X. Yang, Y Jiao, T. Sakata-Kato, J.-W. Wu, C. Yan, N. Kato, H. Yin, N. Yan: Cell **183** (2020) 258. e12.*