Crystal Structure of *Plasmodium falciparum* cGMP-Dependent Protein Kinase (*Pf*PKG): Insights into Selective Kinase Inhibition for Antimalarial Drug Discovery

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Malaria is an infectious disease caused by protozoa of the genus *Plasmodium* and transmitted by *Anopheles* spp. mosquitoes. Despite global control efforts, the WHO reported more than 282 million cases and nearly 610,000 deaths in 2024. The most severe and lethal cases are primarily attributed to *P. falciparum*, especially among children under five in sub-Saharan Africa. Although artemisinin-based combination therapies (ACTs) remain the frontline treatment, the emergence and spread of resistance from Southeast Asia to Africa threaten current control strategies. These challenges reinforce the need to identify parasite-specific molecular vulnerabilities that can support the development of new antimalarial agents with innovative mechanisms of action. One central regulator of asexual blood-stage signaling is the cGMP-dependent protein kinase PfPKG, a serine/threonine kinase essential for calcium-dependent events that drive microneme secretion, parasite egress, and invasion. Here, we combined structural biology, biophysical/biochemical characterization, and inhibitor screening to validate PfPKG and enable structure-based drug discovery. Recombinant PfPKG was successfully expressed in insect cells and purified, exhibiting catalytic activity and robust stability in kinetic and thermal-shift assays. Using Differential Scanning Fluorimetry (DSF), we identified stabilizing buffer conditions and confirmed ligand engagement, observing marked thermal stabilization in the presence of ibrutinib (ΔT_m = 6.2 °C) and IBT6A (ΔT_m = 7.9 °C). Steady-state kinetics yielded K_M values of 130 ± 30 μM for ATP and 110 ± 20 μM for the peptide substrate, consistent with reported parameters. Crystallization of apo *Pf*PKG enabled X-ray diffraction data collection at the Brazilian Synchrotron Light Laboratory (LNLS) and structure determination at 2.31 Å resolution, with an RMSD of 0.32 Å relative to the reference *Pf*PKG model (PDB 5DYK), revealing the canonical bilobal kinase architecture, with the N-lobe and C-lobe connected by the hinge region. Comparative structural inspection highlighted features within and around the catalytic site that differ from human kinases, supporting opportunities for selective inhibitor design. In parallel, inhibition screening identified sapanisertib (IC_{50} = 914 nM), ibrutinib (IC_{50} = 676 ± 30 nM), and IBT6A (IC_{50} = 139 ± 30 nM) as *Pf*PKG inhibitors, and ibrutinib and IBT6A showed *in vitro* antiplasmodial activity against the chloroquine-sensitive 3D7 strain (IC_{50}^{3D7} = 3.1 ± 0.8 μM and 1.9 ± 0.3 μM, respectively). Altogether, these results establish an integrated functional and structural framework for *Pf*PKG and provide a strong foundation for structure-guided discovery and optimization of selective *Pf*PKG inhibitors as next-generation antimalarial hits.