Targeted inhibition of the *Plasmodium falciparum* Vitamin B6 producing enzyme Pdx1 and the biochemical and functional consequences thereof

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Summary

Malaria is caused by the parasite *Plasmodium falciparum* and still plagues many parts of the world. To date, efforts to control the spread of the parasites have been largely ineffective. Due to development of resistance by the parasites to current therapeutics there is an urgent need for new classes of therapeutics. The vitamin B_6 biosynthetic pathway consists of a PLP synthase which produces pyridoxal 5'-phosphate (PLP) within the parasite. The absence of this pathway in humans makes it attractive for selective targeting using small chemical molecules. The PLP synthase condenses D-ribose 5-phosphate (R5P) and DL-glyceraldehyde 3-phosphate (G3P) with ammonia to form PLP. Two proteins make up this PLP synthase – *Pf*Pdx1 and *Pf*Pdx2. Computational modelling of *Pf*Pdx1, and mapping of the R5P-binding site pharmacophore facilitated the identification of several ligands with predicted favourable binding interactions. Confirmatory testing of these on the purified *Pf*Pdx1 *in vitro* revealed D-erythrose 4-phosphate (E4P) and an analogue 4-phospho-D-erythronhydrazide (4PEHz) were capable of dose-dependently inhibiting the enzyme. The acyclic tetrose scaffold of E4P, with both aldehyde and phosphate group moieties, was thought to affect R5P imine bond formation in *Pf*Pdx1, possibly allowing the molecule to enter the R5P-binding site of *Pf*Pdx1. This hypothesis was supported by molecular docking simulations, and suggested that 4PEHz could similarly enter the R5P-binding site. 4PEHz was detrimental to the proliferation of cultured *P. falciparum* intraerythrocytic parasites and had an inhibitory concentration (IC_{50}) of 10 µM. The selectivity of 4PEHz in targeting *Pf*Pdx1 was investigated using transgenic cell lines over-expressing *Pf*Pdx1 and *Pf*Pdx2, revealing that complementation of PLP biosynthesis rescued the parasites from the detrimental effects of 4PEHz. Functional transcriptomic and proteomic characterisation of 4PEHz-treated parasites revealed that the expression of *Pf*Pdx2 increased during 4PEHz treatment, moreover showed that other PLP-related processes were affected. These results supported that *Pf*Pdx1 is targeted by 4PEHz, and affected PLP biosynthesis *de novo*. Results from this study allude to alternative regulation of *de novo* PLP biosynthesis within the parasites by E4P. Moreover, contributions from this work showed that the *de novo* vitamin B_6 pathway of *P. falciparum* is chemically targetable, and a potential strategy for the development of newer antimalarials.

Abbreviations

4PEHz	4-phospho-D-erythronhydrazide
ACT	artemisinin combinational therapeutics
ALAS	delta-aminolevulinic acid synthetase (ALAS)
ANCOVA	analysis of covariance
AspAT	aspartate aminotransferase or aspartate transaminase
ATP	adenosine triphosphate
BNI	Bernhard-Nocht Institute for Tropical Medicine
*Bs*Pdx1	*Bacillus subtilis* Pdx1
CDC	Centers for Disease Control
CPS	carbamoyl phosphate synthetases
CQ	chloroquine
CS	circumsporozoite
Cyc	cyclophillin
DDT	dichlorodiphenyltrichloroethane
DHA	dihydroartemisinin
DHAP	dihydroxyacetone phosphate
DHFR-TS	dihydrofolate reductase-thymidylate synthase
DHPS	dihydropteroate synthase
DOPE	discrete optimized protein energy
DS	Discovery Studio
DXP	deoxy-D-xylulose 5-phosphate
E4P	D-erythrose 4-phosphate
FC	fold change
F6P	D-fructose 6-phosphate
G3P	DL-glyceraldehyde 3-phosphate
GMAP	Global Malaria Action Plan
GO	Gene ontology
GSEA	Gene set enrichment analysis
Hb	haemoglobin
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
HDAC	histone deactelyase
HPI	hours post invasion

HTS	high-throughput screening
IDC	intra-erythrocytic development cycle
IRS	indoor residual spraying
ITN	insecticide treated bed nets
KEGG	Kyoto encyclopaedia of genes and genomes
LDH	Lactate dehydrogenase
LLIN	long lasting impregnated nets
LPM	ligand pharmacophore mapping
MMV	The Medicines for Malaria Venture
MSA	multiple protein sequence alignment
MPD	(4*S*)-2-methyl-2,4-pentanediol
OAT	L-ornithine aminotransferase
ODC	L-ornithine decarboxylase
PABA	*p*-aminobenzoic acid
PAGE	polyacrylamide gel electrophoresis
*Pb*Pdx1	*Plasmodium berghei* Pdx1
*Pf*Pdx1	*Plasmodium falciparum* Pdx1
PfPdx2	*Plasmodium falciparum* Pdx2
PCR	polymerase chain reactions
PDF	probability density function
Pdx1	SNO glutamine amidotransferase family protein
Pdx2	SNO glutamine amidotransferase family protein
PdxK	pyridoxine kinases
PdxP	pyridoxal phosphatases
PfCRT	chloroquine resistance transporter
*Pf*MDR	multidrug resistance transporter 1
PL	pyridoxal
PLP	pyridoxal 5'-phosphate
PM	pyridoxamine
PN	pyridoxine
PNPase	4-nitrophenyl phosphatase
PMSF	phenylmethylsulphonyl fluoride
PPP	pentose phosphate pathway
PQ	primaquine

PS	phosphatidylserine
PSDC	phosphatidylserine decarboxylase
RBM	Roll Back Malaria Partnership
RMSD	root mean square deviation
RQI	RNA quality indicator
rRNA	ribosomal RNAs
R5P	D-ribose 5-phosphate
Ru5P	D-ribulose-5-phosphate
SAR	structure activity relationship
SCD	sickle cell disease
*Sc*Pdx1	*Sacchromyces cerevisiae* Pdx1
SDS	Sodium dodecyl sulphate
SHMT	serine hydroxymethyltransferase
SP	sulpha doxine-pyrimethamin
SPT	serine C-palmitoyltransferase
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
StSyn	seryl-tRNA synthetase
T	Treated
THF	tetrahydrofolate
TIM	triosephosphate isomerases
*Tm*Pdx2	*Thermotoga maritima* Pdx1
TPP	thiamine pyrophosphate
*Tt*Pdx1	*Thermus thermophilus* Pdx1
UNICEF	United Nations Children's Fund
UT	Untreated
WHO	World Health Organization
WT	wildtype

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