A ‘multi-omics’ analysis of the novel aminobenzimidazoles reveals a depletion in haemoglobin derived peptides

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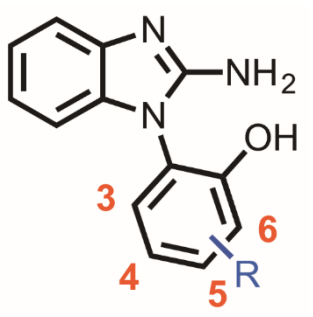
Introduction

- Current anti-malarial treatments are failing due to the emergence of resistance to frontline treatments.
- Identification of new anti-malarial compounds with novel mechanisms of action (MOA) is urgently needed (1).
- Aminobenzimidazoles (ABIs) are a novel class of anti-malarial, possessing excellent potency against blood stage *P. falciparum* malaria (Fig 1) and activity against multiple resistant parasite strains.

a



b

Compound #	R=	IC_{50} (nM)
MIPS-2244	H	77 ± 4
MIPS-2808	5-Me	42 ± 4
MIPS-2862	5-OMe	42 ± 3
MIPS-2869	5-F	188 ± 23

Fig 1: a) Scaffold for ABI compounds **b)** IC_{50}s of selected ABI analogues

Metabolomics

- Untargeted metabolomics was performed on MIPS-2862 and a panel of current anti-malarials with known and unknown MOAs.
- Parasites were incubated with 1 µM of the test compounds for 1 h.
- Samples were analysed via high-res LCMS, data analysis was performed using IDEOM (2) and multivariate analysis was performed using Metaboanalyst (3) (Fig 2).

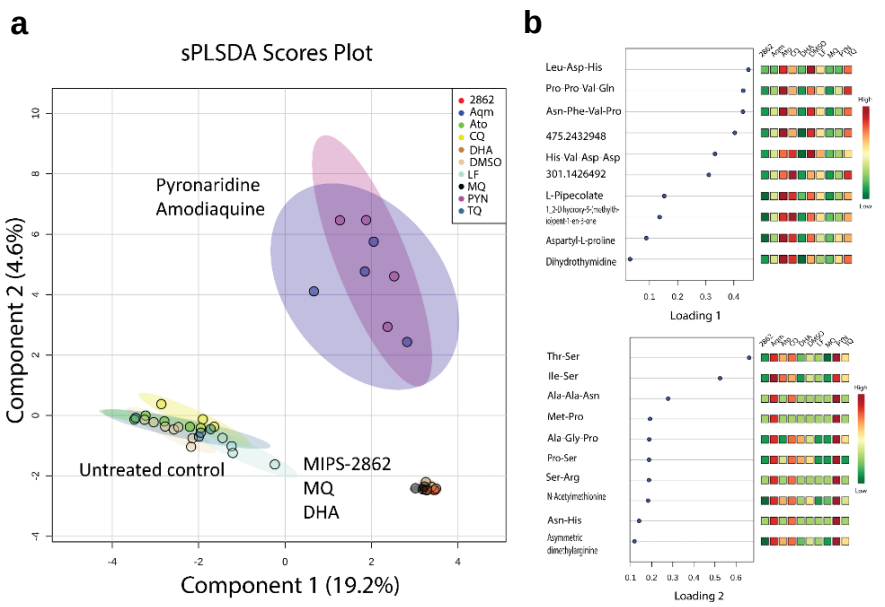


Fig 2: a) sPLSDA analysis of top 10 changed metabolites **b)** Loadings plot of metabolites conferring component 1 separation (x-axis) and component 2 separation (y-axis)

- MIPS-2862, MQ and DHA cluster tightly and are separated from DMSO largely by a depletion in short chain peptides.
- These depleted peptides can be mapped to haemoglobin (Hb), suggesting that ABIs may disrupt Hb digestion.
- The 4-aminoquinolones, Pyronaridine and Amodiaquine were separated from MIPS-2862 and DMSO by an increase in a subset of short chain peptides, suggesting a different MOA.

Peptidomics

- Untargeted peptidomics was performed on parasites incubated with 1 µM of MIPS-2862 or chloroquine (CQ) or 10 nM DHA for 5 hrs to analyse longer chain Hb-derived peptides not detected using the metabolomics workflow.
- A complete depletion of longer chain Hb-derived peptides was observed for MIPS-2862 and DHA. In contrast, CQ treatment resulted in a slight accumulation of Hb peptides.

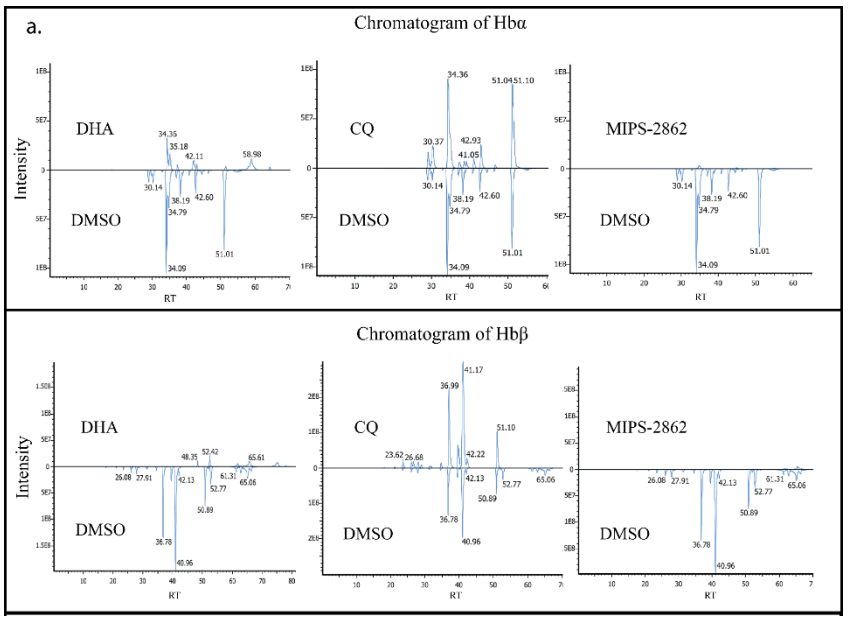


Fig 3: a) Mirrored chromatograms of peptides originating from either Hb alpha chain (Hbα) or Hb beta chain (Hbβ)

Proteomics

- Untargeted proteomics was performed on parasites incubated with 1 µM of MIPS-2862 or a DMSO control for 5 h.
- Translation initiation, the ubiquitin-proteasome system, DNA repair and regulation and surface related proteins were among the largest groups. affected (Fig 4).

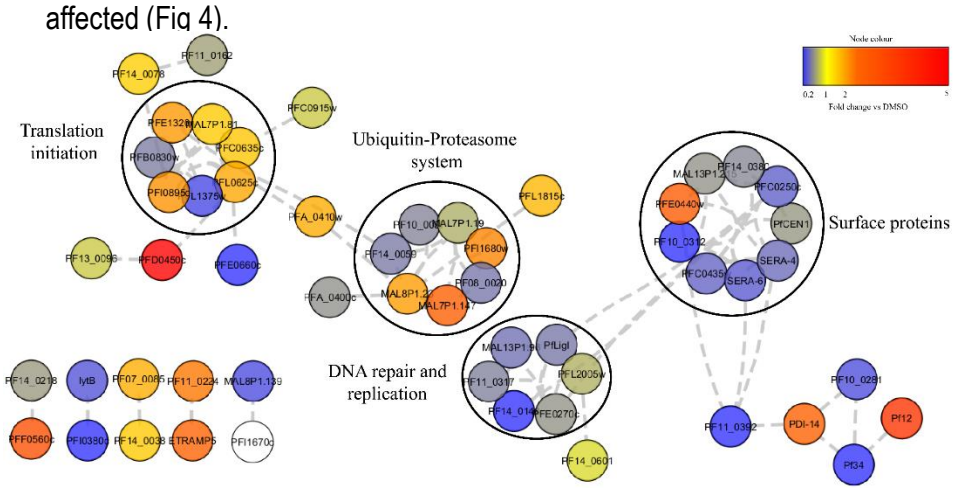


Fig 4: Network analysis of significantly perturbed trophozoite-stage proteins following treatment with 1 µM MIPS-2862. The network analysis was built using the STRINGdb interaction network analysis output (minimum connectivity interaction score of 0.7) in Cytoscape 3.7.2 with the ClusterONE algorithm. Node colour represents fold change vs DMSO control.

Conclusions

- Metabolomics and peptidomics results are suggestive that ABI14 could disrupt Hb digestion, with a decrease in both short and long chain Hb derived peptides observed.
- Further research using *in vitro* resistance generationmethods such as CETSA proteomics and chemoproteomic approaches is required to identify the biological target of ABIs and to better understand their MOA.

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References

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