Hundreds of dual-stage antimalarial molecules discovered by a functional gametocyte screen

Celia Miguel-Blanco1,2, Irene Molina1, Ana I. Bardera3, Beatriz Díaz1, Laura de las Heras1, Sonia Lozano1, Carolina González1, Janneth Rodrigues1, Michael J. Delves2, Andrea Ruecker2,†, Gonzalo Colmenarejo3, Sara Viera1, María S. Martínez-Martínez1, Esther Fernández1, Jake Baum2, Robert E. Sinden2 & Esperanza Herreros1

Plasmodium falciparum stage V gametocytes are responsible for parasite transmission, and drugs targeting this stage are needed to support malaria elimination. We here screen the Tres Cantos Antimalarial Set (TCAMS) using the previously developed P. falciparum female gametocyte activation assay (Pf FGAA), which assesses stage V female gametocyte viability and functionality using Pf s25 expression. We identify over 400 compounds with activities <2 μM, chemically classified into 57 clusters and 33 singletons. Up to 68% of the hits are chemotypes described for the first time as late-stage gametocyte-targeting molecules. In addition, the biological profile of 90 compounds representing the chemical diversity is assessed. We confirm in vitro transmission-blocking activity of four of the six selected molecules belonging to three distinct scaffold clusters. Overall, this TCAMS gametocyte screen provides 276 promising antimalarial molecules with dual asexual/sexual activity, representing starting points for target identification and candidate selection.
During the last 10 years, there has been a substantial intensification of malaria control and prevention with the implementation of both new vector control strategies and chemotherapies. Nevertheless, the disease still remains a worldwide public health challenge with *Plasmodium falciparum* causing more than 214 million cases and 438,000 deaths per year. Malaria elimination is now being discussed as a realistic goal, but new tools will be needed to achieve this aim, particularly in areas of high transmission. It is unlikely that malaria elimination can be achieved in all contexts unless drugs and/or vaccines that interrupt malaria transmission can be discovered, developed and deployed. *Plasmodium* stage V gametocytes are solely responsible for malaria transmission. Thus, they constitute an important target to block the parasite lifecycle through drug administration to infected patients or asymptomatic carriers.

Since 2001, artemisinin-based combination therapies (ACTs) have been recommended as first-line treatment for uncomplicated *falciparum* malaria. Although ACTs rapidly remove asexual blood stages and early gametocytes from the patient, their effectiveness against transmissible stage V gametocytes remains unclear. The only chemotherapeutic intervention available for transmission interruption is low-dose (0.25 mg kg⁻¹) primaquine, recommended for use in areas of low transmission as a single administration following ACT in patients with *P. falciparum* malaria. Higher doses may be more efficacious, but the use of primaquine for transmission reduction is limited because of the potential for haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, a relatively common genetic variation found in malaria endemic areas. When considered with the recently reported cases of artemisinin resistance in the Greater Mekong subregion that are threatening the effectiveness of ACTs, the discovery of safer drugs with new modes of action for malaria treatment, prevention and transmission interruption is more urgent than ever.

Several assays have been developed in recent years with the objective of identifying drugs with transmission-blocking potential. Drugs with gametocytocidal activity have been detected using diverse readouts, such as metabolic parameters (ATP, pLDH, oxidoreduction) or mitochondrial damage and luciferase reporters to track different gametocyte stages. Most of these assays are amenable to high throughput screening (HTS) of large compound libraries. Alternatively, a new generation of gametocyte assays assess male and/or female gamete formation as a broader metabolic framework within which to determine drug activities modulating stage V gametocyte functionality and viability. The idea was first reported by Delves and colleagues, and subsequently developed into a single assay, the *P. falciparum* Dual Gamete Formation Assay (PF DGFA). To date, a limited but diverse group of studies suggest that results obtained in the PF DGFA usefully correlate with those of the ex vivo standard membrane feeding assay (SMFA), but we remain alert to the need to confirm in vitro activities with those in vivo. The *P. falciparum* female gametocyte activation assay (PF FGAA) was the first to be scaled to 384-well format and validated for HTS.

The Tres Cantos Antimalarial Set (TCAMS) is the largest published collection of compounds active against *P. falciparum* asexual blood stages. In this study, the 13.5 K compounds in the TCAMS are screened against stage V gametocytes in the *Pf* FGAA to identify molecules effective against female gamete formation. As both female and male gametes are required for the development of mosquito stages, the parasite lifecycle may be interrupted by solely targeting one of them. The objective of this study is the discovery of new chemical diversity with activity against both asexual blood stages and stage V (female) gametocytes that may not only treat clinical symptoms but also block malaria transmission.

### Results

**Hit identification.** The TCAMS was screened in the *Pf* FGAA following the progression cascade described in Fig. 1. The 13,533 compounds were tested once at 2 µM single concentration using 48 h exposure, and 755 hits were identified using a mean plus 3 s.d.’s statistical cut-off, corresponding to 53% inhibition for this screen. The hits were then re-tested in triplicate at the same concentration resulting in 405 compounds with confirmed activity (3% final hit rate). These compounds were then evaluated in dose–response to determine their 50% inhibitory concentration (IC₅₀) (Supplementary Data 1). A good correlation was found between the confirmed hits and dose–response assay with more than 80% of compounds possessing an IC₅₀ < 2 µM. Additionally, the cytotoxicity of these molecules was evaluated in mammalian cells (HepG2) to determine their specificity for the parasite. Up to 120 compounds showed 50% inhibitory concentration (Tox₅₀) values above 10 µM (Supplementary Data 1).

In parallel, the set of confirmed hits from the *Pf* FGAA screen was cross-compared in the gametocyte ATP-depletion assay, at 2 µM single concentration (Supplementary Data 1). This assay monitors ATP levels as surrogate of gametocyte viability. We observed that of the 405 compounds which were identified by the
Pf FGAA, only 129 compounds were also identified in the ATP-depletion assay (40% inhibition cut-off) (Fig. 2).

To assess the chemical diversity provided by the Pf FGAA screen, all confirmed hits (405 compounds) were subjected to computational analysis by applying a complete-linkage clustering algorithm, using a Tanimoto similarity threshold of 0.55. This analysis identified 82 compound clusters and 63 singletons. After chemical reviewing, they were reorganized based on structure similarity into 57 clusters and 33 singletons. Initial triage was based on simple and relaxed physicochemical properties (molecular weight, lipophilicity, number of aromatic rings) and selectivity index. Seven clusters comprising 97 compounds with molecular weights above 600 g mol\(^{-1}\), clogP above 6 and HepG2 cytotoxicity <10 \(\mu\)M were flagged and deprioritized from further biological profiling. This left a total of 308 compounds classified in 50 clusters, plus 33 singletons for consideration (Supplementary Data 2). Clusters with three or less representatives comprised more than 70% of the set, which is indicative of the large chemical diversity identified in this screen.

A subset of 90 compounds representative of the new chemical diversity was selected for further characterization of the parasitological profile (Supplementary Data 3). Some of these chemical series are depicted in Fig. 3 and Supplementary Fig. 1. Compound selection was performed taking into consideration the top potency (IC\(_{50}\)) and cytotoxicity (Tox\(_{50}\)) values of compounds in each cluster.

**Hit profiling.** The parasitological properties of the selected molecules were further investigated to assess their speed of action and activity against female gametocytes or gametes (Fig. 4 and Supplementary Data 3).

To determine their speed of action, gametocytes were preincubated with the compounds for only 24 h before inducing gamete formation and their IC\(_{50}\) values compared to the ones obtained in the 48 h preincubation described above (Supplementary Data 3). There were 49 compounds that were only active with a 48 h exposure (designated as slow-acting), while 29 compounds additionally showed activity with only 24 h exposure (designated as fast-acting) (Fig. 4).

Moreover, when the compounds were added 30 min after triggering gametocyte activation, 12 compounds prevented cell surface expression of Pfs25 (designated as gamete-targeting; Fig. 4). This suggests that these molecules target female gametes directly, although this does not discount the possibility that they are also active against stage V gametocytes.

**Validation of the in vitro transmission-blocking activity.** To validate the transmission-blocking potential of the TCAMS hits and assess the predictive value of the Pf FGAA as an in vitro high throughput surrogate of full mosquito feeding, the ‘gold-standard’ SMFA was performed. Six compounds showing different biological profiles, belonging to four distinct chemical clusters, were selected based on their potencies in the Pf FGAA (Supplementary Data 1, Table 1). For each compound, inhibition of exflagellation of male gametocytes was measured. Five compounds showed more than 50% inhibition while TCMDC-124559 displayed 30% inhibition (Fig. 5, Table 1). SMFAs were then performed with a single dose at a concentration equivalent to the respective Pf FGAA 90% inhibitory concentration (IC\(_{90}\)) at the

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![Figure 2](image-url) **Figure 2 | Correlation graph of hits identified in the Pf FGAA screen compared to their activities in the ATP-depletion assay.** 276 compounds (green circles and all stars) out of the 405 hits were exclusively identified in the Pf FGAA while 129 compounds (grey circles) were active in both assays. Stars represent those compounds further profiled: slow-acting (blue stars), fast-acting (yellow starts) and gamete-targeting (magenta stars). Compounds tested in the SMFA are marked as big stars.
48 h incubation time (Table 1). Compounds were tested in duplicate in 2–3 independent experiments in the indirect format of the assay, that is, gametocytes were exposed to the drug for 48 h before mosquito feeding to replicate a drug exposure time equivalent to that used in the Pf FGAA. Five of these selected compounds showed a reduction in oocyst prevalence of 58–100% (Fig. 6a), and a reduction in oocyst intensity of 480% (Fig. 6b).

By contrast, TCMDC-124559 reduced prevalence and intensity by o20% (Fig. 6a, b, respectively).

**In vivo efficacy and pharmacokinetics of selected molecules.**

Two out of the six compounds progressed to SMFA, TCMDC-123767 (cluster 30) and TCMDC-141154 (cluster 4), were...
evaluated in vivo in the Plasmodium berghei murine model. Selection criteria was based on the compound potencies in both asexual stages and gametocytes in the Pf FGAA, as well as on their transmission-blocking activity observed in the SMFA (Table 1). Owing to compound availability, TCMDC-141154 had to be replaced by a close analogue from cluster 4: TCMDC-140549 (Supplementary Data 3). Compounds were tested at 50 mg kg \(^{-1}\) single dose 2-day oral treatment and efficacy was measured as a reduction of asexual blood stages parasitaemia greater than 40% compared with the vehicle-treated mice. P. berghei growth was not reduced above the threshold after a single dose 2-day oral treatment. TCMDC-140549 was estimated to be a compound with moderate permeability through artificial membranes (170 nm s \(^{-1}\)), which may drive a low oral bioavailability.

Discussion

To the best of our knowledge, this paper reports the largest screening effort performed to date using an assay that targets P. falciparum gamete formation as the endpoint. The TCAMS library was screened in the Pf FGAA and 405 chemical-starting points for antimalarial transmission-blocking drug discovery were identified. Further analysis of these molecules revealed that 276 compounds (25% with selectivity index > 10) belonged to chemotypes never described before as gametocyte-targeting molecules. Concerning the chemical diversity identified, a few of these compounds showed similarity with classical antimalarial scaffolds, for example, 4-aminoquinoline (TCMDC-138933, cluster 7, IC\(_{50}\) = 0.76 μM) and diaminopyrimidine (TCMDC-137820, cluster 25, IC\(_{50}\) = 1.06 μM) (Supplementary Data 3). The majority comprises novel chemotypes with good physicochemical properties. This TCAMS set of hits includes several scaffolds that have been previously identified by GSK as promising antimalarial starting points for drugs against parasite asexual stages, and that are or have been part of internal medicinal chemistry programmes (Supplementary Fig. 1). These potential drugs can now be prioritized with the added value of being gametocyte-targeting. Interestingly, a preliminary analysis of hit structure revealed that some of the compounds are closely related to the GSK Published Kinase Inhibitor Set (PKIS) (Supplementary Fig. 1), suggesting Plasmodium kinases as potential targets for these molecules.

The original literature annotation for TCAMS was also investigated. TCMDC-141611 is a compound patented by SmithKline Beecham as an inhibitor of the Tie-2 tyrosine kinase receptor, which is involved in angiogenesis. Despite the lack of a classical tyrosine kinase family in the P. falciparum kinome, there is evidence for tyrosine phosphorylation being involved in regulatory functions in the parasite. TCMDC-142257 is an antagonist of the Dopamine 2 receptor, a kind of G-protein-coupled receptor, for which there is some bioinformatic evidence in the P. falciparum genome. Further investigations will be necessary to understand the mechanisms of action of these compounds and by publishing these structures we intend to stimulate research in this area.

From the point of view of malaria drug discovery, the most practical way to interrupt the Plasmodium lifecycle would be through the clearance of transmissible stages present in the peripheral bloodstream of infected patients. In this context, those compounds with a fast-acting profile would be preferred to those requiring longer exposure times. Overall, almost 90% of the compounds tested in this study exerted their effect in stage V gametocytes (and asexual blood stages) but not in female gametes, which revealed their potential utility for transmission-blocking strategies. Further research would be required to determine if the 12 compounds active against female gametes also target stage V gametocytes and so might have an added value as multi-stage antimalarial drugs.

Six selected compounds with diverse biological and chemical profiles were tested in the SMFA (Table 1). Four out of the six tested compounds showed more than 80% block in transmission (Fig. 6, Table 1), while TCMDC-124559 and TCMDC-125345 had...
lower efficacy, even though all compounds had IC\textsubscript{50} values between 0.16 and 0.5 \textmu M in the Pf FGAA. However, there was a good correlation between inhibition of exflagellation, reduction in oocyst intensity and block in transmission (Figs 5 and 6). This suggests that compounds affecting both male and female gamete formation may lead to a more efficacious blockade of malaria transmission.

Further \textit{in vivo} evaluation of two selected molecules, TCMDC-123767 and TCMDC-140549, in the \textit{P. berghei} murine model\textsuperscript{31} showed an ED\textsubscript{50} >50 mg kg\textsuperscript{-1} after a single dose 2-day treatment (Fig. 7). Subsequent pharmacokinetic analysis revealed very low exposure levels in blood for TCMDC-123767, which might explain the lack of effect of this molecule in the \textit{in vivo} model. Given that both compounds showed a good

| TCAMS ID | Chemical structure | Cluster number | Pf FGAA IC\textsubscript{50} (\textmu M) | SMFA concentration (\textmu M) | Inhibition of exflagellation (%) | Block in transmission (%) | Tox\textsubscript{50} (\textmu M) | Biological profile |
|----------|--------------------|---------------|---------------------------------|--------------------------------|-------------------------------|---------------------------|-----------------|------------------|
| TCMDC-123767 | ![Chemical structure](image1) | 30 | 0.16 | 1 | 88 | 83 | 100 | Slow-acting |
| TCMDC-125345 | ![Chemical structure](image2) | 18 | 0.36 | 1 | 50 | 60 | 39.82 | Fast-acting |
| TCMDC-141698 | ![Chemical structure](image3) | 11 | 0.44 | 1 | 84 | 82 | 4.79 | Gamete-targeting |
| TCMDC-141070 | ![Chemical structure](image4) | 4 | 0.53 | 2 | 99 | 88 | 28.96 | Gamete-targeting |
| TCMDC-141154 | ![Chemical structure](image5) | 4 | 0.21 | 1 | 97 | 93 | 11.9 | Gamete-targeting |
| TCMDC-124559 | ![Chemical structure](image6) | 18 | 0.5 | 1 | 30 | 15 | >100 | Fast-acting |
parasitological profile in vitro, further studies to improve stability and bioavailability will be performed to address their potential use as antimalarial drugs. Clearance of TCMDC-12376 and TCMDC-140549 may be reduced by blockade of labile positions, whereas solubility of TCMDC-123767 may be increased by incorporation of polar and/or ionizable groups.

In conclusion, the Pf FGAA can identify potential transmission-blocking chemotypes which, due to the biological content covered in this assay, were undetected by previous gametocyte-based assays. The TCAMS library screen yielded 276 new gametocyte-targeting molecules belonging to 57 diverse chemical entities and 3 biological profiles. Nearly 25% of the hits identified are within the appropriate physicochemical space (clogP < 5, number of aromatic rings < 3) and will therefore be considered for further progression, while other hits represent valuable starting points as tool compounds to identify new modes of action involved in the biology of Plasmodium transmission.

**Methods**

**Gametocyte production.** *P. falciparum* NF54 (originally isolated from an imported malaria case in the Netherlands in the 1980s; BEI Resources, cat. no. MRA-1000) asexual-stage culture was performed as described by Trager and Jensen[22]. Cultures synchronized at the ring stage were used to start gametocyte cultures (day 0) at 1% parasitaemia and 4% hematocrit in 200 ml final volume using culture bottles (Nalgene 3110-42, Thermo Scientific). Complete culture medium (RPMI 1640 supplemented with 25 mM HEPES, 50 μg ml⁻¹ hypoxanthine, 2 g l⁻¹ NaHCO₃ and 10% pooled human male type A serum) was obtained from Interstate Blood Bank, Aþ serotype; no aspirin 2 h before drawing and no antimalarials 2 weeks before drawing. To ensure a stable temperature at 37°C, which is crucial for gametocyte production and maturation, pre-warmed medium and a slide warmer (XH-2001, Premiere) were used. Under these conditions, parasitaemia reaches a peak of asexual stages on days 4–5, and the first gametocytes are differentiated in culture on day 6. Sexual-stage development was monitored microscopically by Giemsa-stained thin blood smears at day 7 (mainly asexual stages and stages I to III gametocytes) and day 14 (stages IV and V gametocytes).

**Female gametocyte activation assay (Pf FGAA).** Cultures showing mainly stage V gametocytes were purified by differential sedimentation as previously reported[14]. The gametocyte concentration was adjusted to plate 8,000 gametocytes per well (100 μl per well) in 384-well poly-d-lysine coated plates (781946, Greiner Bio-One) containing the compounds to test. Gametocytes were incubated with drugs for

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**Figure 5 | Exflagellation inhibition by six TCAMS compounds.** Stage V gametocyte cultures were exposed to 2 μM of TCMDC-141070 and 1 μM of the five remaining compounds for 48 h. The percentage inhibition was determined compared to DMSO-treated controls. Compounds were then progressed into SMFA to further determine the transmission blocking in mosquitoes. Each bar represents mean value of two (TCMDC-125345, TCMDC-141698, TCMDC-123767, TCMDC-141154) or three (TCMDC-141070, TCMDC-124559) independent replicates with s.d.

**Figure 6 | Effect of selected TCAMS compounds in the SMFA.** The following parameters were measured: (a) Transmission-blocking activity (prevalence reduction) and (b) reduction in mean *P. falciparum* oocyst intensity. Each bar represents mean value of two (TCMDC-125345, TCMDC-141698, TCMDC-123767, TCMDC-141154) or three (TCMDC-141070, TCMDC-124559) independent replicates with s.d.

**Figure 7 | Effect of two TCAMS hits in the *P. berghei* in vivo mouse model.** Each experiment included a control group treated with vehicle (green circle) used as a reference to calculate the percentage of inhibition of parasitaemia in peripheral blood (dotted red line). The response of standard antimalarials in the same assay is also presented (blue, magenta and grey circles). Represented data are the mean ± s.e.m. log₁₀ [% asexual blood stages at day 4] of two mice per point.
either 24 h or 48 h at 37 °C (3% O₂, 5% CO₂ and 92% N₂). Female gametocyte activation was then triggered by temperature drop and addition of xanthurenic acid (D120804, Sigma) as previously described.²⁴ To detect female gametocytes, monoclonal anti-Pf55 antibody²⁵ 4B7 (BEL Resources (formerly MR4), cat. no. MRA-315) conjugated to the Cy3 fluorochrome (GE Healthcare) was added to ookinetes medium (RPMI 1640 supplemented with 25 mM HEPES, 50 μM γ-l hypoxanthine, 2 g l⁻¹ NaHCO₃, 100 μM xanthurenic acid and 20% human serum) at a final concentration of 0.5 μg ml⁻¹. Activated cultures were then kept at 26 °C for 24 h protected from light, and image acquisition was performed in the Opera High-Content Screening System (PerkinElmer). Using × 10 objective, five images per well were taken using 532 nm excitation. Images were analysed with a Columbus image data storage and analysis system (PerkinElmer) based on a script specifically designed for this assay that considers size, roundness and intensity of fluorescence of the female activated gametocytes.

### Gametocytoidal ATP-depletion assay

Viability of mature gametocytes was determined using the gametocyte ATP bioluminescence assay.²⁵ Briefly, stages IV–V gametocyte cultures were double-purified by differential sedimentation followed by magnetic isolation.²⁶ Then, parasites (50 μl per well containing 12,500 gametocytes) were added to compound pre-dispersed 384-well plates (781091, Greiner Bio-One) and incubated at 37 °C for 48 h (3% O₂, 5% CO₂ and 92% N₂). BacTiter-Glo kit (G8231, Promega) was used to determine the ATP levels of live parasites according to the manufacturer’s instructions. After reagent addition (50 μl per well), luminescence of the plates was measured using a microplate reader (HTS counter Victor, Wallac).

### Exflagellation assay

Exflagellation was measured as previously reported with minor modifications. Using 5 μl of day-14 gametocyte cultures, parasites were incubated in the corresponding concentration for each compound in 384-well plates at 37 °C (3% O₂, 5% CO₂ and 92% N₂). After 48 h incubation, a 200 μl sample was spun down in a microfuge, the supernatant was carefully removed and the pellet resuspended in 15 μl of pre-warmed ookinite medium. Parasites were placed in Fast-Read disposable haemocytometer slides and, after 15 min at room temperature (22 °C), exflagellation centres per field were counted under × 10 objective.

### Standard membrane feeding assay (SMFA)

Day-14 gametocyte cultures were plated in pre-warmed six-well-plates (5 ml per well) and were exposed to the compounds (1 μM or 2 μM final concentration) for 48 h at 37 °C (3% O₂, 5% CO₂ and 92% N₂). Within this incubation time, 3 ml of media were removed after the first 24 h and replenished with the same volume of fresh media with compound added to obtain the required final concentration. After 48 h of total exposure, cultures were centrifuged at 2,500 x g for 3 min at 37 °C, diluted 1:1 with 100% packed volume of fresh erythrocytes and finally formulated as artificial mosquito blood meals at 50% haematocrit with pre-warmed human serum. All steps were performed at 37 °C to avoid gametocyte activation. Four- to six-day old female Anopheles stephensi mosquitoes (GlaxoSmithKline Insectary) were fed for 30 min by Parafilm membrane attached to glass feeders (12831283, Fisher Scientific) connected to a 37 °C circulating water bath. Fed mosquitoes were maintained in an incubator at 27 °C and 75% relative humidity with 12 h light/dark cycles. Seven to eight days post-feeding, mosquitoes with fully developed ovaries were dissected for midguts, which were then dissected out of stomach and bottom of the plate (S5EJ046I08, MilliPore Corp.). The lipids are allowed to form bilayers across the small holes in the filter and compound concentration is measured 3 h later in both donor and the acceptor compartments by HPLC (Agilent 1100 LC). Permeability (P (nm s⁻¹)) is calculated using the following formulas:

\[
\frac{X_D - X_R}{X_D} = \frac{D_{xy}}{X_D} - \frac{D_{yx}}{X_R}
\]

where \(X_D\) and \(X_R\) are final concentrations in the donor and receiver side, respectively; \(C_Q\) and \(C_R\) are final concentrations in the receiver and donor side, respectively; \(V_D\) and \(V_R\) are volumes in the receiver and donor compartment, respectively; \(A\) is the area (cm²) and \(t\) is the incubation time (s). The considered cut-off values are as follows: High: P > 200 nm s⁻¹; Medium: 10 nm s⁻¹ < P < 200 nm s⁻¹; Low: P < 10 nm s⁻¹.

### Ethics statement

All the experiments were ethically reviewed and approved by the GlaxoSmithKline Diseases of the Developing World (DDW) Group Ethical Committee on Animal Research and were conducted according to Spanish legislation, European Directive 2010/63/EU and GlaxoSmithKline policy on the Care, Welfare and Treatment of Laboratory animals.

### Compounds and plates

Compounds were dissolved in 100% DMSO and dispensed in the 384-well plates using an Echo-CRS liquid handler. Compounds were tested in 2–3 independent experiments. For each assay, both positive (a drug highly effective against the biological process) and negative (the vehicle consisting in 0.5% DMSO) controls were added and a minimum of ten wells per plate were analysed. Based on that, Z’ factor was calculated for each plate and only those ones with values higher than 0.4 were considered acceptable for data analysis.

### Data analysis

Data were normalized to percentage of inhibition of the biological response by using positive (that is, highest inhibition achieved by a drug, ICtrl2) or negative (that is, lowest inhibition achieved in the absence of drug but in presence of...
of the vehicle 0.5% DMSO, Ctrl1) controls following the equation described below:

\[
\text{% Inhibition} = 100 - \left[ \frac{X - Ctr12}{Ctr1 - Ctr12} \times 100 \right]
\]

where X is the inhibition of measured process for the compound X. Ctrl1 and Ctr12 are calculated as the average of replicates in the same microtiter plate where compound X is tested.

Assay performance statistics, such as signal to background ratio, Z’ and robust 3 s.d. activity cut-off were calculated using templates in ActivityBase XE (IDBS, Guilford, Surrey, UK). Hit population analysis and visualization were conducted using Spotfire DecisionSite (Spotfire, Inc., Somerville, MA, USA). The pIC\textsubscript{50} (– log IC\textsubscript{50}) values were obtained using the ActivityBase XE nonlinear regression function in the full curve analysis bundle.

Data availability. The GSK TCAMS dataset for \textit{P. falciparum} whole cell screening was deposited in ChEMBL-NTD, www.ebi.ac.uk/chemblntd. Additional chemical structures of compounds described in this study can be found in https://www.ebi.ac.uk/chembl/index.php/

The authors declare that all relevant data supporting the findings of this study are available within the article and its Supplementary Information files or are available from the authors on request.

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Author contributions

C.M.-B., I.M. and E.H. planned, designed and supervised the work. C.M.-B. and I.M. performed the screening assays, biological profiling and contributed to data analysis. A.I.B. performed data analysis of primary screening, dose-responses and further profiling. G.C. performed the chemoinformatic analysis after each screening step. B.D., L.d.l.H. and E.F. performed the computational and chemical clustering. S.L., C.G. and I.R. performed the SMFA experiments and data analysis. A.R. and M.I.D. contributed to define the different biological profiles of hits. S.V. performed the in vivo experiments. M.S.M.-M. performed pharmacokinetic studies. E.H., R.E.S. and J.R. managed the project. C.M.-B., I.M., B.D., L.d.l.H., J.R., S.V., M.S.M.-M. and G.C. wrote different sections of the manuscript and/or prepared figures and tables. C.M.-B. integrated contributions and issued the final manuscript. All authors reviewed the manuscript and accept responsibility for its publication.
