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Chemical genomic profiling for antimalarial therapies, response signatures and molecular targets

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Abstract

Malaria remains a devastating disease largely because of widespread drug resistance. New drugs and a better understanding of the mechanisms of drug action and resistance are essential for fulfilling the promise of eradicating malaria. Using high-throughput chemical screening and genome-wide association analysis, we identified 32 highly active compounds and genetic loci and genes associated with differential chemical phenotypes (DCPs), defined as 5-fold differences in half-maximum inhibitor concentration (IC₅₀) between parasite lines. Chromosomal loci associated with 49 DCPs were confirmed by linkage analysis and tests of genetically modified parasites, including three genes that were linked to 96% of the DCPs. Drugs whose responses mapped to wild type or mutant *pfcr* alleles were tested in combination *in vitro* and *in vivo*, yielding promising new leads for antimalarial treatments.

Keywords

Plasmodium falciparum; high-throughput screening; genetic mapping; chemical genomics; phenotype

The deployment of artemisinin (ART) and its derivatives against *Plasmodium falciparum* malaria parasites has been effective, and ART-based combination therapies (ACTs) are currently the recommended treatment in most endemic regions (1). The choice of partner drug is critical; an ideal partner drug should have pharmacokinetic and pharmacodynamic properties compatible with ART, employ a mode of action different from that of ART, retain

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Supporting Online Material

Materials and Methods

Figs S1 to S3

Table S1 to S16

efficacy against existing populations of drug-resistant parasites, and have no adverse pharmacologic interactions or additional toxicity (2). Unfortunately, parasites resistant to ART and its current partner drugs have been reported (3-5). New drugs or combinations are therefore urgently needed. Indeed, some promising leads have recently been identified through large-scale screening (6-12). Combinations of new or existing drugs that are synergistic or act on variant forms of parasite targets may mitigate the emergence of drug resistance. Here we have used quantitative high-throughput screening (qHTS) and genome-wide association and linkage analyses to identify candidate new antimalarial drugs with complementary or distinct response signatures for effective combination therapies. We show that many of the responses to a diverse collection of compounds are determined by a surprisingly limited number of genes, a finding that has broad implications for antimalarial drug development.

Chemical library screens for inhibitors and DCPs

Sixty-one parasite lines (Table S1) were screened against the NIH Chemical Genomics Center Pharmaceutical Collection containing 2816 compounds registered or approved for human or animal use (13). The compounds were tested at eight 5-fold serial dilutions (from 29 μ M to 0.5 nM) using a parasite growth inhibition assay (9, 14) to obtain dose-response curves and IC₅₀ determinations for each compound. From 171,776 drug assays that generated ~1.4 million data points, we identified 32 highly active compounds that inhibited the growth of at least 45 parasite lines with IC₅₀ values $\leq$ 1 μ M (Table 1). Among these pan-active compounds, seven (ecteinascidin 743, gramicidin, arteminol, decoquinatone, epothilone B, atovaquone, and actinomycin D) yielded mean IC₅₀ values lower than that of ART (IC₅₀ < 10 nM) and, to our knowledge, 10 have not been reported to have antimalarial activity. Pairwise comparison of the IC₅₀ values among 61 parasite lines identified 72,538 DCPs from 689 compounds, including 161 compounds that elicited DCPs between the parents of three *P. falciparum* laboratory crosses (7G8 $\times$ GB4, Dd2 $\times$ HB3, and 3D7 $\times$ HB3) (Table S2). As most of these compounds have been approved for human use, their ability to inhibit parasite growth at nanomolar levels make them promising candidates for developing new antimalarial drugs or drug combinations. These DCPs can be investigated using genome-wide association studies (GWAS) or linkage analysis to identify genetic determinants of susceptibility and study mechanisms of differential drug sensitivity.

Compounds with correlated responses and response signature groups

Compounds with positively correlated response patterns may act on common pathways or targets within the parasite. To search for compounds with correlated response patterns, we performed pairwise comparisons of 492 compounds (Table S3) that were active (see SOM for active compound definition) against at least one-third of the 61 lines profiled and identified 2082 pairs of compounds with highly correlated responses (correlation coefficient, [CC] > 0.7) among these lines using methods described (21). For example, responses to 52 compounds were highly correlated with that of ART, 40 with that of mefloquine, and 25 with both of ART and mefloquine. The 25 compounds included halofantrine, lumefantrine, dihydroergocristine, ergotamine, rifapentine, and bromocriptine. Correlations of parasite responses to ART, mefloquine, halofantrine, and lumefantrine have been documented in studies of culture-adapted parasites and *in vivo* patient isolates (15-17). The observations suggest that the compounds with highly correlated responses may share common features of drug action and/or resistance.

To investigate this idea further, we clustered the compounds into groups of common chemical response signatures using K-means and Dunn's index algorithms (18, 19). The 492 compounds were grouped into 44 clusters, with some having relatively high activity indices

(AIs, ranging from 0 to 1 with 1 being 100% correlation) that suggest potential common mechanisms of response (19) (Fig. 1A and Table S4). Although the compound library was screened against 61 parasite lines, the numbers of clusters reached a plateau with just 10 lines (Fig. 1A). This result suggests that parasite responses to the compounds are restricted to a few common pathways. Based on their response patterns, parasite lines generally clustered according to their geographic origins (Fig. 1B). Distinct groups included one from South America, two from Cambodia, and a cluster of Africa/Central America/Thailand lines that could represent a recent parasite expansion from Africa. One of the Cambodian clusters exhibited high IC_{50} values to most of the compounds, reminiscent of an earlier description of SE Asian parasites with an accelerated resistance to multiple drugs phenotype (20), and might represent an emerging ART-resistant population. These patterns of chemical response may reflect the population separation of Cambodian parasites that has been proposed on the basis of genome-wide microsatellite (MS) and single-nucleotide polymorphism (SNP) data (17, 21) and suggest that antimalarial drugs have played an important role in the recent evolution and population structure of *P. falciparum*.

The compounds within most clusters generally showed strongly correlated responses and, in some cases, had similar structures as measured by a structure index (SI; values ranging from 0 to 1, with higher values having more similar structures) (22). For example, cluster 26 (AI = 0.55 and SI = 0.4) comprises ten compounds, eight of which contain a quinoline core, including chloroquine (CQ), cinchonine, quinacrine, quinidine, and quinocidum (Fig. S1 and Table S4). *P. falciparum* responses to various quinolines have been associated with the *P. falciparum* CQ resistance transporter (PfCRT) and multidrug resistance 1 protein (PfMDR1, also named P-glycoprotein homolog 1), both of which are proposed to function as transporters (23-25). Clusters 28 and 40 were characterized by high AI values (0.73) but relatively low SI values (0.15 and 0.2, respectively), suggesting highly correlated responses among compounds of diverse structures. Further investigation of these clusters and their targets may reveal how parasites respond to structurally diverse compounds.

Mutations enabling resistance to one compound may render parasites more sensitive to other compounds; in cases of negative correlation, pairs of such compounds may be good candidates for drug combination therapy, particularly if they are complementary in their actions on wild type and mutant forms of a parasite target. Pairwise comparison of the 492 active compounds identified 1,250 pairs that were negatively correlated, with CC ranging from -0.26 to -0.90 (Table S5). In particular, 43 compounds were negatively correlated with CQ, including ciprofloxacin and clindamycin, each of which has been reported to have antimalarial activity alone (26, 27). The combination of clindamycin and CQ (CC = -0.27) substantially improves patient cure rate as compared with CQ alone (94% compared to 32%) (26), and ciprofloxacin (CC = -0.30) significantly enhances CQ activity *in vitro* (27). Tylosin, a veterinary drug used to treat bacterial infections, is negatively correlated with CQ as well (CC = -0.43) and synergizes with it to inhibit *P. falciparum in vitro* (28). Further investigation of the compounds negatively correlated with CQ response could lead to new drug combinations for treating resistant parasites (also see below).

Tocainide and etonogestrel showed marked negative correlations with responses to ART or mefloquine (CC = -0.52 to -0.67) (Table S5), warranting further evaluation as candidates for new classes of ACTs. These compounds were also negatively correlated with quinine, although to a somewhat lesser extent (CC = -0.34 and -0.42, respectively), suggesting potential shared mechanisms in response to quinine, ART, and mefloquine.

Impact of antimalarial drugs on parasite populations

To investigate how antimalarial drugs influence parasite evolution and population structure, we analyzed parasite responses to 134 compounds that produced an IC_{50} value with a good quality dose-response curve (curve class.1.1, 1.2, and 2.1, see SOM) in each of the 61 parasite lines. Consistent with the geographic clustering, principal component analysis (PCA) separated the American parasites from the African and Asian parasites by component 2 and the Asian parasites from the African parasites by component 5 (Fig. 1C). Compounds contributing most positively to component 5 were predominantly the quinoline antimalarial drugs in cluster 26, while compounds contributing negatively to this component included mibefradil, vinorelbine, and homoharringtonine (Table S6). These results suggest that CQ and the other quinoline drugs have played a substantial role in the evolution of these two parasite populations. Similarly, the compounds contributing most negatively to component 2 that separates the New and Old World parasites included dihydroergotamine, dihydroergocristine, and reserpine; responses to these drugs were mapped to *pfmdr1* in the progeny of the *P. falciparum* crosses (see below). Compounds contributing most positively to this component included docetaxel and clobetasone. Although these compounds have not been used to treat malaria, they could be linked to (mutant) alleles of parasite molecules (possibly *pfmdr1*) selected by other antimalarial drugs. These data suggest that *pfmdr1* played a significant role in shaping the *P. falciparum* populations in America. The two groups of compounds showing positive or negative contributions to component 2 (eigenvector values < 0.14 and those > 0.14) were themselves positively correlated in response patterns within each group, but were largely negatively correlated between the groups (Fig. 1D). Similar correlations were observed for the compounds in component 5, for example, CQ and mibefradil (Fig. 1E). The compounds with negatively correlated responses provide additional starting points for novel combination therapies.

GWAS of parasite responses with SNPs

To identify genes that may associate with the differences in responses to the active compounds, we performed GWAS using 3,354 SNPs collected previously to search for SNPs associated with differential drug responses (17). First, we investigated whether known resistance determinants could be identified using this method. Indeed, mutations in *pfcr* were significantly (corrected $P < 0.01$) associated with responses to more than 200 compounds, including the expected quinolines such as hydroxychloroquine, CQ, quinine, and quinacrine (Table S7). Similarly, responses to dihydrofolate reductase (DHFR) inhibitors such as trimethoprim, trimetrexate, and triamterene were significantly (corrected $P < 0.005$) associated with mutations in *pfdhfr* (Table S8). Compounds associated with mutations in the *pfmdr1* included dihydroergotamine and lumefantrine, consistent with previous reports (9, 29) (Table S9). Additionally, the major compounds contributing to PCA component 2 separating the South American population from those of Asia and Africa were also associated with polymorphisms of *pfmdr1* (Table S9). These results show that known mutations strongly influencing parasite drug responses can be identified using GWAS.

Fifteen genes were significantly (corrected P -values < 0.005) associated with responses to both ART and mefloquine, consistent with the presence of these drugs in cluster 23 (Tables S4, Table S10, and Table S11). The top six genes associated with responses to ART and its derivatives were MAL13P1.268 (*Plasmodium* conserved protein), PF11_0188 (heat shock protein 90), PFE0565w (conserved *Plasmodium* protein), PF08_0130 (rRNA processing WD-repeat protein), PFA0655w (SURFIN), and PFI0355c (ATP-dependent heat shock protein). Additionally, the majority of the genes associated with ART response were also associated with responses to derivatives such as artemisinin, artemimol, and artemetero (Table S10). Parasite response to primaquine was also significantly associated with

MAL13P1.268 (corrected P -value = $1.74\text{E-}04$) (Table S12). Since MAL13P1.268 was associated with responses to primaquine, ART, mefloquine, dihydroergotamine, and dihydroergocristine, the role of this gene in drug resistance should be studied further. Association analysis of 26 PCA-corrected parasites from the Thai-Cambodian border linked many SNPs with different drug responses; however, the genes associated with responses to CQ, mefloquine, ART, and antifolate drugs were not evident in this analysis because these parasites were all resistant to these drugs (Table S13). Although some of the associated genes identified in this study could be false positives, these studies provide a starting point for further functional investigations of the genes and their roles in antimalarial drug resistance.

Three genetic loci linked to the majority of DCPs

To identify the genetic determinants that contribute to the DCPs and to further investigate some of the compounds associated with mutations in *pfprt* and *pfmdr1*, we tested 128 DCP compounds on 33 recombinant progeny of the *P. falciparum* 7G8×GB4 cross and 98 DCP compounds on 34 recombinant progeny of the Dd2×HB3 cross (Table S1, and S14). Using quantitative trait loci (QTL) analysis, we mapped 49 DCPs to 57 chromosomal loci with a logarithm (base 10) of odds (LOD) score ≥ 3.0 . Remarkably, 47 of the 49 (96%) DCPs were mapped to three loci containing *pfdhfr* (chromosome 4), *pfmdr1* (chromosome 5), or *pfprt* (chromosome 7) (Fig.2 and Table S14). These results indicate that *pfdhfr*, *pfmdr1*, and *pfprt* dominate the parasite's differential response to many drugs. Additionally, eight DCPs were mapped to loci on chromosomes 3, 7, 8, 12 and 14, with DNA segments ranging from 55 kb to 193 kb (Table S14 and S15 and SOM text). Further studies are needed to identify the mutations conferring these DCPs.

The linkage analyses also confirmed many differential compound sensitivities associated with *pfprt*, *pfmdr1* or *pfdhfr* by GWAS. Responses to 11 compounds associated with *pfprt*, three antifolate drugs with *pfdhfr*, and 15 compounds with *pfmdr1* were confirmed by linkage analysis and/or testing of parasites with genetically modified *pfprt* or *pfmdr1* (Tables S7-9 and Table S14) (also see below). These results demonstrate the usefulness of GWAS for detecting mutations mediating drug resistance in malaria parasites.

Confirmation of PfDHFR as the target of trimethoprim and triamterene using parasites with different mutant *pfdhfr* alleles has been reported (9). To determine whether *pfprt* and *pfmdr1* are indeed responsible for the DCPs that map to their respective loci, we tested the DCP compounds against parasites with genetically modified *pfmdr1* or *pfprt* genes (Table S14 and S16). There are five common amino acid polymorphisms in PfMDR1 (30), and changes at positions S1034C, N1042D, and D1246Y have been shown to confer differential sensitivity to some drugs (9). In two progeny of the Dd2×HB3 cross, the CQ-sensitive GC03 parasite and the CQ-resistant 3BA6 parasite, conversion of the SDD *pfmdr1* allele (encoding amino acids S1034, D1042, and D1246) to a SND allele decreased sensitivity to some antimalarial agents, while conversion to a CDY allele resulted in increased sensitivity. For the eight DCP responses mapped to *pfmdr1* in Dd2×HB3 and 7G8×GB4 crosses, the IC_{50} values were 3- to 23- fold higher for parasites carrying the SND allele *versus* those carrying the CDY allele. As expected, control parasites in which the allelic exchange produced no change from *pfmdr1* SDD resulted in less than two-fold changes in IC_{50} values for all DCPs mapped to the *pfmdr1* locus (Table S14). Comparison of IC_{50} ratios from a genetically modified parasite receiving the parental SND *pfmdr1* allele over the one receiving the CDY allele showed significantly higher mean ratios (unpaired t-test, $P<0.001$) for the DCP compounds that mapped to *pfmdr1* than those that did not (Table S14). These particular amino acid substitutions therefore mediate the DCPs that map to this locus.

Among the 23 DCPs linked to chromosome 7, two-fold or greater changes in IC₅₀ value were seen for 19 DCPs in the GC03 parasites engineered to carry the mutant *pfcr*t alleles of Dd2 or 7G8 (GC03^{Dd2}, GC03^{7G8}) (Table S14). Similarly, the mean IC₅₀ ratios for compounds mapped to *pfcr*t were significantly higher than those not mapped to *pfcr*t ($P=0.002$) in the recombinant GC03^{Dd2} parasites. In the genetically modified parasites, IC₅₀ values were elevated for four compounds and decreased for 15, which suggests specific interactions with wild type or mutant *pfcr*t alleles, respectively (Table S14). Responses to many compounds such as perhexiline, lopinavir, lumefantrine, carbetapentane, memantine, and duloxetine that did not map to any loci were also altered in the *pfcr*t- or *pfmdr*1-modified parasites, suggesting that these compounds target or are transported by PfCRT or PfMDR1, both of which reside on the membrane of the intraerythrocytic parasite's digestive vacuole, the site of hemoglobin degradation (23, 31).

The compounds generating the DCPs linked to *pfcr*t have amine groups that could become protonated at physiologic or lower pH (Fig. S2). A plot of the parasite response patterns showed two groups of compounds with positive correlation in responses within each group, but negative correlation between groups (Fig. 3A). Except for the presence of a basic amine, however, there was no consistent structural basis for separating the two groups of compounds whose responses correlated positively or negatively with that of CQ (Fig. S2). Although agents with structures similar to some of these compounds have been tested for 'reversal' of CQ resistance (32-35), our data provide genetic evidence showing that the mechanism of this reversal is specific to mutant PfCRT, consistent with results from a recent study (36). These compounds may act as blockers of the PfCRT drug transport pore, as they all carry a basic nitrogen group (Fig. S2) that may impede the mutant PfCRT-mediated flux of CQ out of the digestive vacuole (37). As expected, the DCPs linked to *pfmdr*1 also included compounds with diverse structures, including dihydroergotamine, dihydroergocristine, miconazole, and rifampin in cluster 40; ergotamine and zeaxanthin in cluster 41; and reserpine, loe 908, and sorafenib in cluster 36.

Drug combinations targeting PfCRT

CQ is no longer effective in treating *P. falciparum* infections because of mutations in *pfcr*t (38). Identification of compounds that interact with either wild type or mutant forms of PfCRT may allow the development of novel combination therapies to help overcome CQ resistance. Mibefradil, NNC55-0396, and other compounds whose response profiles negatively correlated with CQ (Fig. 3A; Tables S5, S6, and S7) could be combined with CQ for treating both CQ-resistant and sensitive parasites. Indeed, when tested with a low concentration of CQ (IC₁₅) *in vitro*, these combinations proved to be highly potent against the CQ resistant line Dd2 (Fig. 3B and Table S14). Among the 23 compounds that mapped to the *pfcr*t locus, 17 showed reduced IC₅₀ values (21 to 229 fold) in the presence of a low dose of CQ (IC₁₅), including six with IC₅₀ values that dropped to below 100 nM. Such combinations offer a promising approach for treating CQ-resistant parasites and could prevent new resistance mutations because of structural and functional constraints on PfCRT. Similar strategies can be used to develop combinations targeting PfMDR1.

To further investigate the interactions of compounds negatively associated with CQ, we performed isobologram analysis of the combinations of CQ with either mibefradil or NNC55-0396 against the CQ-resistant line Dd2 and the CQ-sensitive line HB3. The actions of mibefradil and NNC55-0396 (both T- and L-type Ca⁺⁺ channel blockers) were synergistic with that of CQ against Dd2 (Fig. 3C). Verapamil, a L-type Ca⁺⁺ channel blocker and a known CQ resistance reversal agent (36), is synergistic with CQ in Dd2, but antagonistic in HB3 (Fig. S3). However, the IC₅₀ of verapamil against Dd2 is 3-4 fold higher than of mibefradil and NNC55-0396 (1.3 μ M for verapamil vs. ~200 nM for NNC55-0396 and

~300 nM for mibefradil). These results suggest that either mibefradil or NNC55-0396 may be more effective than verapamil in combination with CQ in treating the drug resistant parasites.

We also tested these combinations against a CQ resistant strain of *Plasmodium chabaudi* *in vivo* and showed that inclusion of a low dose of CQ (3mg/kg) with NNC55-0396 or mibefradil greatly increased the effect of these drugs, leading to ~15 and ~40 fold reduction in the treatment concentrations of NNC55-0396 and mibefradil, respectively (Fig. 3D-3F). Mibefradil is rapidly absorbed *in vivo*, reaching peak plasma concentration within 1-2 hours, and has an elimination half-life of 17-25 hours (39)(SOM), indicating that this drug may have a suitable pharmacokinetic profile for combination treatments. The results are consistent with the *in vitro* data from *P. falciparum* and suggest that the *P. chabaudi* homolog of *pfcr* may be involved in CQ resistance in this rodent model, but requires further investigation.

Our analyses show that the majority of differential sensitivity of current *P. falciparum* populations to many compounds is linked to mutations in *pfdhfr*, *pfmdr1*, or *pfcr*, suggesting that the number of parasite genes that can contribute to drug responses may be limited. Analyses of DCPs and genome-wide MS and SNP genotypes from the progeny of two genetic crosses and field isolates identified many compounds interacting with wild type or mutant *pfcr* or *pfmdr1*, which were confirmed using genetically modified parasites and drug combinations. In addition to the 32 highly potent pan-active compounds, the compounds active against wild type or mutant forms of PfCRT or PfMDR1 offer novel strategies for antimalarial therapies, and the loci and genes associated with drug responses provide a genetic basis to better delineate the nature of drug resistance in malaria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Clusters of chemical response patterns and major compounds in the components separating parasite populations

A, Plot of chemical clusters with common response signatures and the number of *Plasmodium falciparum* lines; **B**, clustering of parasites based on patterns of parasite responses (IC_{50} values). Cam1, parasites from Cambodia; SA, South America; AF/Thai/CA, Africa, Thailand, and Central America; Thai/Cam2, Thailand and Cambodia; * indicates two parasites from Africa. **C**, principal component analysis (PCA) of parasite response patterns showing separation of parasite continental populations; **D**, correlation plot of the top compounds (eigenvector values <-0.14 or >0.14) positively and negatively contributing to PCA component 2 separating American population from Asian and African populations. **E**, correlation plot of the top compounds (eigenvector values <-0.14 or >0.14) positively and negatively contributing to PCA component 5 separating African and Southeast Asian populations. For the full names of the compounds, please see Table S6.

Fig. 2. Genetic loci linked to differential chemical phenotypes determined using progeny from two genetic crosses

A, plots of quantitative trait loci from compounds with a LOD score >3.0 in the Dd2×HB3 cross; **B**, plot of quantitative trait loci from compounds with a LOD score >3.0 in the 7G8×GB4 cross.

Fig. 3. Correlated responses among the progeny of the Dd2×HB3 cross and changes in parasite sensitivity against compounds mapped to *pfcr1* in the presence of low-dose chloroquine *in vitro* and *in vivo*

For all sub-figures, CQ, chloroquine; Mib, mibefradil; NNC, NNC55-0396. **A**, positive and negative correlations of compounds mapped to *pfcr1*; the color bar indicates correlation coefficient values from 1(100% positive correlation, red) to -1 (100% negative correlation, blue). **B**, Ratios of IC₅₀ values from drug treatments with or without IC₁₅ level of CQ for the Dd2 line. Red and blue bars indicate ratios for compounds whose responses are negatively and positively correlated with CQ, respectively. * compounds with IC₅₀<100 nM in the presence of IC₁₅ levels of CQ. **C**, Isobolograms showing synergistic interactions of CQ with NNC (blue dots) or Mib (red dots) in the CQ-resistant Dd2, but slightly antagonistic in the CQ-sensitive HB3 parasite (red circles, Mib; blue circles, NNC); FIC IC₅₀, fractional inhibitory concentration. Shown are representative plots of two independent experiments for each combination. **D and E**, Mean parasitemia and standard deviations from groups of 3-6 mice treated with various doses of Mib (**D**) or NNC (**E**) in the presence (blue bars) or absence (red bars) of 3 mg/kg CQ. CQ3, 3 mg/kg CQ. The numbers after Mib (NNC) are Mib (NNC) doses in mg/kg, and the numbers after CM (CN) are Mib (NNC) doses plus 3

mg/kg CQ. **F**, Plots of mean growth inhibition with standard errors from D and E to estimate IC_{50} values; blue lines, NNC; red lines, Mib; dash lines, without CQ; solid lines, with 3 mg/kg CQ.

Table 1

Compounds highly active against multiple *Plasmodium falciparum* isolates

Name	CN	Lines active	Mean IC ₅₀ (M)	Annotations	CC	NP/Syn
Ecteinascidin 743	0	46	7.15E-10	Antineoplastic, alkylating	yes	NP
Gramicidin **	0	51	1.52E-09	Anti-infective; antibiotics	yes	NP
Decoquinat	9	47	2.40E-09	Anti-infective; antiparasitic, anti-coccidian	no	Syn
Epothilone B *	11	61	3.22E-09	Antineoplastic; tubulin modulators	yes	NP
Actinomycin D **	NA	57	4.89E-09	Antineoplastic; nucleic acid synthesis inhibitors	yes	NP
Homoharringtonine **	22	61	2.27E-08	Antineoplastic; phytogetic; angiogenesis inhibitors	yes	NP
Monensin sodium salt **	5	61	3.29E-08	Anti-infective; antiparasitic	yes	NP
Docetaxel **	11	58	5.18E-08	Anti-infective; antiparasitic	yes	NP [†]
Buquinolate	5	59	1.03E-07	Antimicrobials; anti-coccidiosis	no	Syn
Narasin **	1	61	1.12E-07	Anti-infective; antibiotics	yes	NP [†]
Bortezomib **	37	61	1.50E-07	Antineoplastic; protease inhibitors	yes	Syn
Mupirocin **	6	58	1.88E-07	Anti-bacterial; anti-infective; antibiotics;	yes	NP
Fumagillin **	38	45	2.03E-07	Anti-infective; antibiotics; growth inhibitors	yes	NP
Alazanine triclofenate	38	61	2.36E-07	Antiparasitic	no	Syn
Aclarubicin hydrochloride **	38	61	3.00E-07	Antineoplastic; topoisomerase II inhibitor	yes	NP
Emetine **	20	60	3.28E-07	Protein synthesis inhibitors; anti-infective	yes	NP
Lestauritinib	23	60	3.41E-07	FLT3-selective tyrosine kinase inhibitor	yes	NP [†]
Deserpidine	39	61	3.50E-07	Cardiovascular; antihypertensive	yes	NP [†]
Dextroamphetamine saccharate	43	60	3.52E-07	Peripheral nervous system; sympathomimetics;	yes	Syn
Plitumycin **	38	51	3.52E-07	Antineoplastic; nucleic acid synthesis inhibitors	yes	NP [†]
Demecarium bromide **	11	59	3.90E-07	Parasympathomimetic; acetylcholinesterase inhibitor	no	Syn
Paclitaxel **	11	59	5.29E-07	Antineoplastic; antineoplastic, phytogetic	yes	NP
Acriflavinium Hydrochloride **	20	60	6.72E-07	Antiseptic; dye	no	Syn
Zinc pyrithione *	18	60	6.82E-07	Antifungal and antibacterial	no	Syn
Suberoylamide **	31	58	7.30E-07	Anti-inflammatory, non-steroida	no	Syn

Name	CN	Lines active	Mean IC ₅₀ (M)	Annotations	CC	NP/Syn
Orlistat	30	46	7.43E-07	Anti-obesity; lipoprotein lipase (LPL) inhibitor	yes	NP [‡]
Lasalocid sodium ^{**}	23	60	8.18E-07	Anti-infective; antibiotics	yes	NP
Clotrimazole ^{**}	29	54	8.20E-07	Ca ²⁺ -activated K ⁺ channel inhibitors	no	Syn
Salinomycin ^{**}	1	57	8.83E-07	Anti-infective; antibiotics	yes	NP
Puromycin hydrochloride ^{**}	NA	60	8.92E-07	Anti-infective; protein synthesis inhibitors	yes	NP
Lauryl isoquinolinium bromide	11	57	9.26E-07	Detergent	no	Syn
Ciclesonide	30	50	9.36E-07	Anti-allergic	yes	NP

CN, the cluster numbers in Table S4; Lines active, numbers of parasite lines a compound is active with an IC₅₀ value < 1μM; Mean IC₅₀, mean half-maximum inhibitory concentrations determined from the numbers of parasite lines that produced good response data (indicated in the 'Lines active' column); CC, compounds with chiral centers; NP/Syn, natural product or synthetic. Some of the compounds may contain mixtures of chiral isomers, but specific chiral information for each compound is not available.

[‡] Natural product derivative. Nineteen known antimalarial drugs that meet the activity cutoff criteria but are not listed in the table include artemetero, ART, artemisinin, artemimol, amodiaquine, atovaquone, cinchonine, clindamycin, ethidium bromide, halofantrine, lumefantrine, mefloquine, methylene blue, methyl violet, quinacrine, quinidine gluconate, quinaldine blue, quinine sorbate, and trimetrexate glucuronate.

* compounds with antimalarial activity identified previously, but IC₅₀ values were not determined;

** compounds with IC₅₀ values reported previously (See SOM for additional explanations). NA, not applicable.