Sudarshan Publications

Available online at https://s-epub.in/index.php/trp/index

Pharma Research

An International Peer Reviewed, Indexed Journal

10.62655/s-epub.2023.v15.i01.pp65-72

SYNTHESIS AND ANTIMALARIAL SCREENING OF 4-AMINOQUINOLINE-PYRIMIDINE HYBRIDS

MARTA VALDIVIESO[1]*, RUI CORREIA[2], ANNIKA KESSELRING[3]

Affiliation

[1]Department of Pharmaceutical Chemistry, Navarre Plain Institute of Pharmacy, Pamplona, Spain
[2]Department of Organic Chemistry, Douro Valley Institute of Pharmaceutical Sciences, Vila Real, Portugal
[3]Department of Organic Chemistry, Neckar Valley Institute of Pharmaceutical Sciences, Heilbronn, Germany

*Corresponding author: Marta Valdivieso, m.valdivieso@npip.es
ORCID iD: Marta Valdivieso 0000-0001-4122-4893; Rui Correia 0000-0001-1936-4945; Annika Kesselring 0000-0001-0173-5349

Article info

Received date:14-08-2022
Accepted date:20-12-2022
Published date:25-04-2023
ISSN;0975-8216

Keywords:

Antimalarial, 4-Aminoquinoline, Pyrimidine, Hybrid molecules, Plasmodium falciparum

Abstract

Resistance to chloroquine and, more recently, to artemisinin has renewed interest in new chemical entities for malaria. One strategy is to link a 4-aminoquinoline with a pyrimidine into a single hybrid molecule. This narrative review summarises the rationale for such hybrids, the synthetic routes used to prepare them, the structure-activity relationships reported by research groups, and the in vitro and in vivo screening methods applied to them. Many hybrids retain activity against chloroquine-resistant parasites in culture, and a few have shown efficacy in rodent models. The review draws on published papers and reports no new data. Limitations include small compound series, varied assay protocols and sparse pharmacokinetic and safety information.

1. INTRODUCTION

Malaria still kills several hundred thousand people each year, most of them young children in sub-Saharan Africa, and progress has stalled in recent years [1]. Treatment now rests on artemisinin-based combinations, but the gains of the past two decades are threatened by parasites that clear slowly after artemisinin treatment. Mutations in the kelch13 gene have been linked to this delayed clearance in South-East Asia, and cases have begun to be reported elsewhere [2, 3]. Chloroquine, once the cheap and safe backbone of therapy, lost most of its value in the second half of the twentieth century as resistant Plasmodium falciparum spread from a few foci across the world [4, 5]. Disease burden is concentrated in places with limited health budgets, so any new treatment has to be cheap to manufacture as well as effective. That economic constraint is one reason why

chemists keep returning to scaffolds such as the quinoline ring, which can be assembled from simple, inexpensive starting materials [1, 6].

The 4-aminoquinoline scaffold nonetheless remains attractive. Chloroquine is inexpensive, well tolerated in short courses, and has a long record of use in pregnancy and in infants. Its mechanism is reasonably well understood: it accumulates in the parasite's acidic digestive vacuole and interferes with detoxification of haem released from digested haemoglobin [7, 8]. Resistance arises mainly through mutations in the transporter PfCRT, which allow the drug to be pumped out of the vacuole [5, 9]. Because resistance depends on the side chain and not on the quinoline ring alone, chemists have long tried to modify the side chain to overcome efflux [10, 11]. Clinical experience reinforces the point. Chloroquine remains in use for Plasmodium vivax in some regions and the drug's safety margin at standard doses is wide, so a successor that reproduces its benefits without the resistance liability would be welcome [4, 6].

Molecular hybridisation offers a different route. Two pharmacophores are joined covalently, in the hope that the resulting molecule will act on two targets, or on one target in two ways, and that resistance to both at once will be slow to develop [12, 13]. Pairing a 4-aminoquinoline with a pyrimidine, a ring found in antifolates such as pyrimethamine, is one of the most widely explored combinations [14, 15, 16]. This review describes the design rationale, synthesis, structure-activity relationships and screening of these hybrids, drawing on published work. The main findings are summarised in table 1. No new experimental data are reported. Throughout the text, the word hybrid means a single molecule in which two distinct structural units are joined by a covalent linker, as opposed to a fixed-dose combination of two separate drugs in one tablet [12, 13].

2. RATIONALE FOR HYBRID DESIGN

The case for hybrids rests on three arguments. First, a single molecule with two pharmacophores avoids the pharmacokinetic mismatch that can occur when two separate drugs with different half-lives are combined. Second, the hybrid might bind two targets and so need two independent mutations for resistance. Third, linking a known scaffold to a new fragment can restore activity against resistant parasites, as when the second fragment blocks efflux or adds a new interaction [12, 13]. Not all of these ideas have been proven for the 4-aminoquinoline-pyrimidine class, and some are best seen as working hypotheses. The history of fixed combinations offers a cautionary note. Combinations such as sulfadoxine with pyrimethamine were widely used and lost effectiveness as resistance mutations accumulated in both targets, so the benefit of a dual-action design depends on how the two actions are chosen and balanced [6, 17].

Dual-target claims should be read with care. A hybrid can only act on two targets if both fragments retain the interactions they have on their own after being linked, and this is by no means guaranteed. Linking often changes conformation, charge distribution and size, so that a fragment which bound its target as a free molecule may no longer fit. The most convincing demonstrations come from direct assays on each target and from resistance selection experiments, neither of which is common in the published quinoline-pyrimidine work [13, 15, 18]. Until such evidence accumulates, hybridisation is better regarded as a productive source of new active structures than as a proven route to preventing resistance [12, 13]. A further consideration is that very different linkers can place the pyrimidine in a different spatial relationship to the quinoline, and this geometry may matter for target binding as much as for accumulation [13, 16].

The pyrimidine partner is chosen for good reasons. Pyrimidine rings are present in nucleic acids and in many drugs, they tolerate a wide variety of substituents, and 2,4-diaminopyrimidines inhibit dihydrofolate reductase, an established antimalarial target [17, 18]. Resistance to pyrimethamine arises through point mutations in the parasite enzyme, and work on flexible inhibitors has shown that the target can still be exploited if

the molecule is designed to cope with the mutations [17, 18]. In hybrids, however, the pyrimidine is often unsubstituted at the positions needed for strong folate pathway inhibition, so activity may come from the quinoline portion, from a new mode of action, or from both [15, 16]. Interest in the pyrimidine also reflects the experience that the ring is synthetically convenient. Halogenated pyrimidines react readily with amines under mild conditions, which makes them natural partners for an amine-bearing quinoline and allows many analogues to be prepared quickly [14, 19].

Table 1: Published findings on 4-aminoquinoline-pyrimidine hybrids and related antimalarial work

Topic|focus	Main reported finding or feature	Ref.
Chloroquine resistance PfCRT mutations	Mutant transporter pumps drug from the digestive vacuole, reducing accumulation.	[5, 9]
Artemisinin resistance kelch13	Delayed parasite clearance linked to mutations in kelch13.	[2, 3]
Hybrid drug concept dual mode of action	Covalent linking of two pharmacophores to address resistance and pharmacokinetic mismatch.	[12, 13]
Quinoline-pyrimidine hybrids SAR and mode of action	Active against sensitive and resistant strains; haematin interaction reported for several series.	[15, 16]
Synthetic route SNAr from 4,7-dichloroquinoline	Two-step substitution with diamine linker and chloropyrimidine; microwave heating shortens reaction time.	[14, 19]
7-Chloro group aminoquinoline SAR	Electron-withdrawing group important for haematin binding and potency.	[20, 21]
Side chain modification chloroquine analogues	Altered chain length can retain activity against resistant parasites.	[10, 11, 22]
In vitro assays 3D7 versus K1 or W2	Resistance index from resistant and sensitive strains; SYBR Green, LDH or radiolabel readout.	[23, 24, 25, 26]
Cytotoxicity MTT assay	Selectivity index from mammalian cell viability relative to antiplasmodial activity.	[27]
In vivo model P. berghei four-day test	Suppression of parasitaemia in mice as early efficacy indicator; limited translation to humans.	[28, 29]

Evidence on mode of action in the published hybrids is mixed. Studies that measured binding to haematin, inhibition of beta-haematin formation and interaction with DNA reported effects on the first two in several series, with the quinoline ring responsible for the haem interaction [15, 20]. Some hybrids also showed weak binding to parasite DNA, though the biological meaning is unclear. Inhibition of the folate enzyme was rarely demonstrated directly in the quinoline-pyrimidine hybrids, which is a gap in the evidence. A reasonable summary is that the quinoline half probably drives the activity, with the pyrimidine modulating uptake, accumulation and resistance profile [8, 15]. Some groups have argued that a hybrid might behave more like a prodrug or a carrier, releasing two active fragments after metabolism, but evidence for that in this class is scant and most authors treat the intact molecule as the active species [13, 15].

3. SYNTHETIC APPROACHES

Most syntheses start from 4,7-dichloroquinoline, an inexpensive commercial building block. Nucleophilic aromatic substitution at the 4-position with a diamine, commonly an alkyl diamine or piperazine, gives a 4-aminoquinoline with a free amine at the end of a linker of chosen length [14, 15]. A second nucleophilic substitution then joins this amine to a chloropyrimidine, such as a 2,4-dichloropyrimidine or a 4,6-dichloropyrimidine, usually in the presence of a base and a polar solvent at elevated temperature [15, 16]. The two-step sequence is straightforward and tolerant of variation in linker, so that libraries of analogues can be prepared from a modest set of reagents. Yields reported for the two steps are usually moderate to good, and the quinoline intermediates can often be used without extensive purification, which keeps cost low for academic groups [14, 16]. Reaction progress is usually followed by

thin-layer chromatography, and the products are characterised by melting point, infrared and NMR spectroscopy, and mass spectrometry before biological testing [14, 15].

Reaction conditions have been refined over time. Conventional heating in alcohol or dimethylformamide can take many hours, while microwave irradiation shortens reaction times to minutes and often improves yields and purity [19]. Regioselectivity needs attention with dichloropyrimidines, because the 4-position is more reactive than the 2-position toward amines, so the order of substitution determines which isomer forms. Further elaboration of the remaining chlorine, for example with a second amine, adds diversity to the pyrimidine ring and allows tuning of basicity and polarity [15, 16]. Purification is normally by column chromatography or crystallisation, and structures are confirmed by NMR and mass spectrometry. Protecting groups are seldom needed when a symmetrical diamine is used in excess, because the mono-substituted product precipitates or can be separated easily; with unsymmetrical linkers, one amine may be protected first, which adds steps [15, 19].

4. STRUCTURE-ACTIVITY RELATIONSHIPS

A long body of work on aminoquinolines gives context. The 7-chloro substituent is important for haematin binding and for activity, and replacing it with groups that are less electron-withdrawing reduces potency [20, 21]. The basicity of the side-chain amine influences accumulation in the vacuole, and a side chain of moderate length retains activity against chloroquine-resistant strains, with shortened or lengthened chains in several series being less affected by the resistance transporter than chloroquine itself [10, 11, 22]. These principles carry across into the hybrids, where the quinoline core is generally kept unchanged and the optimisation work centres on the linker and the pyrimidine. The principle that has emerged is that small changes in the quinoline ring tend to cost activity, whereas changes in the part joined to the side chain are better tolerated, which is why the pyrimidine end is where most of the variation is explored [20, 21, 22].

Within the published hybrid series, linker length and nature emerge as significant variables. Short, flexible alkyl diamines and piperazine linkers produced active compounds in several reports, while very long or rigid linkers tended to be less active [15, 16]. The substitution pattern on the pyrimidine mattered too: additional amino groups and moderate-sized lipophilic substituents often favoured activity, whereas bulky or strongly polar groups were less welcome. A number of compounds were more potent against a chloroquine-resistant strain than against a sensitive one, a pattern attributed to avoidance of efflux, though it requires confirmation by uptake studies [11, 15, 16]. Reports from different groups should be compared with caution, because the same linker can give different results with different pyrimidine substituents, and no single study has examined all combinations systematically [14, 15].

The role of the second basic centre needs a brief comment. In chloroquine, the protonation of the side-chain tertiary amine and the quinoline nitrogen in the acidic vacuole traps the drug, and more strongly basic compounds are concentrated to a greater degree [8, 22]. Hybrids that carry an additional basic nitrogen in a piperazine or alkyl linker can reproduce this effect, whereas those where the pyrimidine absorbs the basicity may show reduced accumulation. Measured uptake studies in parasitised red cells are the proper test, and the correlation between calculated pKa and measured activity is encouraging but imperfect [8, 22]. In practice this means that a hybrid with an unusually good potency figure should prompt questions about the assay as well as the chemistry, and repeat testing in an independent laboratory is the sensible next step [23, 29].

The findings from the main series are collected in table 1, and their distribution across categories is shown in fig. 1. Several patterns are worth stressing. First, hybrids as a class show activity at low concentrations in culture and little cross-resistance with chloroquine. Second, the best compounds were generally those that kept two basic

centres in the linker. Third, cytotoxicity against mammalian cells was usually moderate to low, giving selectivity indices that were acceptable for early leads but not always striking [15, 16, 27]. These points hold for the series examined, and generalising beyond them would be unwise. The picture is therefore one of consistent trends rather than firm rules, which is common for early-stage medicinal chemistry [15, 16, 30]. Such work has been valuable when the descriptors are chosen with a chemical rationale, and unhelpful when they are simply the largest correlation in a small data set [22].

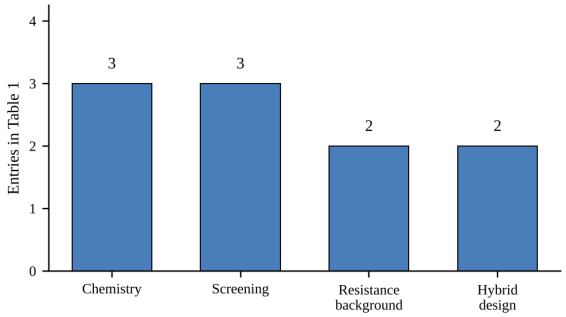


Fig. 1: Number of entries in table 1 by topic group

Computational work often supports the experimental findings. Quantitative structure-activity relationships built on aminoquinolines highlight lipophilicity, side-chain basicity and the electronic character of the 7-substituent as descriptors of potency against resistant and sensitive parasites [22]. Docking of hybrids into parasite dihydrofolate reductase has also been described, and gives plausible binding poses, but docking scores do not by themselves prove inhibition and should be viewed as hypothesis generators [18]. Without enzymatic confirmation, a computed interaction with the folate enzyme remains speculative. Predictive models of this kind are only as good as the dataset behind them, and series with few compounds give wide uncertainty [22].

5. IN VITRO AND IN VIVO SCREENING

Screening begins with cultured P. falciparum, grown in human red cells by the method of Trager and Jensen [31]. Chloroquine-sensitive laboratory lines such as 3D7 are tested alongside resistant lines such as K1, Dd2 or W2 to estimate a resistance index, the ratio of the concentration inhibiting growth by half in the resistant strain to that in the sensitive one [23, 24, 29]. Growth is measured by incorporation of radiolabelled hypoxanthine, by parasite lactate dehydrogenase activity, or by fluorescent DNA dyes such as SYBR Green I, which are cheap, safe and well suited to plates [24, 25, 26]. Reference antimalarials such as chloroquine, artemisinin derivatives and pyrimethamine are included on each plate so that results can be normalised and the assay's performance monitored from run to run [24, 29].

Selectivity is checked using mammalian cell lines, typically in a colorimetric viability assay such as the MTT method, and the ratio of cytotoxic concentration to antiplasmodial concentration gives a selectivity index [27]. Hits are expected to have potency in the nanomolar to low micromolar range and selectivity well above ten in order to be pursued, although exact thresholds differ among programmes [29, 32]. Early assays should also screen for pan-assay interference, since reactive or aggregating compounds can look potent for the wrong reasons, and some chemical classes are well known for this behaviour [33]. Many programmes add a counter-screen against another cell line and, later, microsomal stability and hERG-related cardiac assays, as these are common reasons for late failure in the quinoline class [27, 32, 34]. Clinical experience with the older 4-aminoquinolines, including amodiaquine, shows that side-chain design can also affect toxicity, which makes the choice of substituent a safety issue as well as a potency one [6, 10].

Interpreting potency figures calls for some statistical care. The half-maximal inhibitory concentration is obtained by fitting a sigmoidal curve to a series of dilutions, and its reliability depends on the number of replicates, the range of concentrations and the stability of the parasite culture [24, 25, 29]. Values quoted to several decimal places often hide an uncertainty of two- or threefold between repeat experiments. When two hybrids differ by less than this, ranking them is unjustified. Good reports give the mean and spread from independent experiments, list the

reference drugs and state the assay conditions, so that others can judge the numbers fairly [23, 24]. Reporting the raw dose-response data in supplementary files would let readers reanalyse the curves with their own methods.

In vivo testing mostly uses the four-day suppressive test in mice infected with Plasmodium berghei, where the compound is given orally or by injection for four days and parasitaemia is compared with untreated controls [28, 29]. This model is cheap and quick, and it reports on absorption, metabolism and efficacy together, but it does not reproduce human falciparum infection, and rodent pharmacokinetics differ from human. Several quinoline-pyrimidine hybrids reduced parasitaemia in this model, and at least one series gave a survival benefit at doses that did not cause obvious toxicity [16]. Even so, such results are early indicators rather than predictions of clinical response. Group sizes in mouse experiments are typically small, and the route and vehicle of dosing influence exposure considerably, so results from different laboratories are not easily compared [28, 29].

6. LIMITATIONS AND FUTURE DIRECTIONS

The published evidence has several limits. Series are small, often a few dozen compounds from a single laboratory, and the assay protocols differ in strain, incubation time, readout and serum content, which makes comparison of potency across papers uncertain [23, 24, 25]. Activity is usually reported against laboratory strains, not recent field isolates. Stage specificity, speed of kill, activity against gametocytes and liver stages, and the potential for resistance selection in vitro are infrequently measured, though these properties decide whether a compound could join a combination therapy [29, 32]. Funding and access constraints partly explain this, since academic laboratories seldom have the resources to run parallel assays in several strains or to profile properties in vivo [29, 32]. Journals could help by requiring a minimum set of reference compounds and strains.

Pharmacokinetic and safety information is the largest gap. Hybrids with two or three basic centres and high molecular weight may show poor permeability, strong tissue binding and long half-lives, and cardiotoxicity linked to potassium channel blockade is a recognised concern for the 4-aminoquinoline class [6, 34]. Few hybrid papers present metabolic stability, plasma protein binding or cardiac channel data. Without this information, it is impossible to judge the clinical potential, and promising potency numbers can mislead. Early measurement of these properties would help teams stop weak series sooner and put resources behind better ones [32, 34]. Open sharing of structures, raw potency data and negative results would let others build on each series rather than repeat it, a practice that large screening collaborations have already shown to be productive [30, 35]. Such testing is not expensive when done early and avoids much wasted effort later.

Priorities for the future are fairly clear. Agreed reference strains and assay formats would let results be compared directly. Mechanism-of-action studies should include direct enzyme assays and uptake measurements, so that claims of dual action rest on evidence [13, 18]. Testing against recent clinical isolates, including those carrying kelch13 mutations, would show whether the hybrids add value in the setting where new drugs are most needed [2, 3]. Finally, early absorption, distribution, metabolism and safety profiling should become routine in hybrid papers, rather than being left to a later stage that is rarely reached. Collaboration with groups that have access to clinical isolates and to pharmacokinetic expertise would speed progress considerably [29, 32].

7. CONCLUSION

The 4-aminoquinoline-pyrimidine hybrids are an interesting but still early class of antimalarial leads. They are easy to make, retain much of the haem-directed activity of chloroquine, and in several published series maintain potency against chloroquine-resistant parasites with acceptable selectivity in cell culture [15, 16, 20]. Rodent studies add some encouragement, though they are limited in number and in depth [16, 28]. Whether any of them can be developed depends on information that has

not yet been generated, in particular on pharmacokinetics and cardiac safety.

What the field now needs is rigour more than novelty. Standard assays, activity against recent field isolates, direct evidence for any second target, and early pharmacokinetic and cardiac safety data would turn a collection of promising potency figures into a coherent case for development [29, 32, 34]. Resistance to artemisinin makes the need for new partners in combination therapy more pressing, and well-characterised hybrids may yet contribute, provided they are judged against the demanding profile of an effective, safe and affordable medicine [1, 2, 6]. Honest reporting of failures would help the field as much as new compounds.

8. ABBREVIATIONS

WHO-World Health Organization, CQ-Chloroquine, PfCRT-Plasmodium falciparum chloroquine resistance transporter, DHFR-Dihydrofolate reductase, SAR-Structure-activity relationship, SNAr-Nucleophilic aromatic substitution, NMR-Nuclear magnetic resonance, MTT-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, LDH-Lactate dehydrogenase, MMV-Medicines for Malaria Venture

9. FUNDING

Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

1. World Health Organization. World malaria report 2022. Geneva: WHO; 2022.
2. Dondorp AM, Nosten F, Yi P, *et al.* Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med 2009;361:455–67.
3. Ariey F, Witkowski B, Amaratunga C, *et al.* A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature 2014;505:50–5.
4. Fidock DA, Eastman RT, Ward SA, Meshnick SR. Recent highlights in antimalarial drug resistance and chemotherapy research. Trends Parasitol 2008;24:537–44.
5. Sidhu ABS, Verdier-Pinard D, Fidock DA. Chloroquine resistance in Plasmodium falciparum malaria parasites conferred by pfcrt mutations. Science 2002;298:210–3.
6. World Health Organization. Guidelines for the treatment of malaria. 3rd ed. Geneva: WHO; 2015.
7. Egan TJ, Marques HM. The role of haem in the activity of chloroquine and related antimalarial drugs. Coord Chem Rev 1999;190–192:493–517.
8. Hawley SR, Bray PG, O'Neill PM, Park BK, Ward SA. The role of drug accumulation in 4-aminoquinoline antimalarial potency. Biochem Pharmacol 1996;52:723–33.
9. Fidock DA, Nomura T, Talley AK, *et al.* Mutations in the P. falciparum digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell 2000;6:861–71.
10. Ridley RG, Hofheinz W, Matile H, *et al.* 4-Aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant Plasmodium falciparum. Antimicrob Agents Chemother 1996;40:1846–54.
11. De D, Krogstad FM, Cogswell FB, Krogstad DJ. Aminoquinolines that circumvent resistance in Plasmodium falciparum in vitro. Am J Trop Med Hyg 1996;55:579–83.
12. Meunier B. Hybrid molecules with a dual mode of action: dream or reality? Acc Chem Res 2008;41:69–77.
13. Muregi FW, Ishih A. Next-generation antimalarial drugs: hybrid molecules as a new strategy in drug design. Drug Dev Res 2010;71:20–32.
14. Vandekerckhove S, D'hooghe M. Quinoline-based antimalarial hybrid compounds. Bioorg Med Chem 2015;23:5098–119.
15. Singh K, Kaur H, Smith P, de Kock C, Chibale K, Balzarini J. Quinoline-pyrimidine hybrids: synthesis, antiplasmodial activity, SAR, and mode of action studies. J Med Chem 2014;57:435–48.
16. Manohar S, Rajesh UC, Khan SI, Tekwani BL, Rawat DS. Novel 4-aminoquinoline-pyrimidine based hybrids with improved in vitro and in vivo antimalarial activity. ACS Med Chem Lett 2012;3:555–9.
17. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to antifolates. Pharmacol Rev 2005;57:117–45.

18. Yuthavong Y, Tarnchompoo B, Vilaivan T, *et al.* Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target. Proc Natl Acad Sci USA 2012;109:16823–8.

19. Kappe CO, Stadler A, Dallinger D. Microwaves in organic and medicinal chemistry. 2nd ed. Weinheim: Wiley-VCH; 2012.

20. Egan TJ, Hunter R, Kaschula CH, *et al.* Structure-function relationships in aminoquinolines: effect of amino and chloro groups on quinoline-hematin complex formation, inhibition of beta-hematin formation, and antiplasmodial activity. J Med Chem 2000;43:283–91.

21. Kaschula CH, Egan TJ, Hunter R, *et al.* Structure-activity relationships in 4-aminoquinoline antiplasmodials: the role of the group at the 7-position. J Med Chem 2002;45:3531–9.

22. Hocart SJ, Liu H, Deng H, *et al.* 4-Aminoquinolines active against chloroquine-resistant Plasmodium falciparum: basis of antiparasite activity and quantitative structure-activity relationship analyses. Antimicrob Agents Chemother 2011;55:2233–44.

23. Rieckmann KH, Sax LJ, Campbell GH, Mrema JE. Drug sensitivity of Plasmodium falciparum: an in-vitro microtechnique. Lancet 1978;1:22–3.

24. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob Agents Chemother 1979;16:710–8.

25. Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug testing. Antimicrob Agents Chemother 2004;48:1803–6.

26. Makler MT, Ries JM, Williams JA, *et al.* Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. Am J Trop Med Hyg 1993;48:739–41.

27. Weyermann J, Lochmann D, Zimmer A. A practical note on the use of cytotoxicity assays. Int J Pharm 2005;288:369–76.

28. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of P. berghei in screening for blood schizontocidal activity. Ann Trop Med Parasitol 1975;69:155–71.

29. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. Nat Rev Drug Discov 2004;3:509–20.

30. Gamo FJ, Sanz LM, Vidal J, *et al.* Thousands of chemical starting points for antimalarial lead identification. Nature 2010;465:305–10.

31. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science 1976;193:673–5.

32. Burrows JN, Duparc S, Gutteridge WE, *et al.* New developments in anti-malarial target candidate and product profiles. Malar J 2017;16:26.

33. Baell JB, Walters MA. Chemistry: chemical con artists foil drug discovery. Nature 2014;513:481–3.

34. Hann MM, Oprea TI. Pursuing the leadlikeness concept in pharmaceutical research. Curr Opin Chem Biol 2004;8:255–63.

35. Guiguemde WA, Shelat AA, Bouck D, *et al.* Chemical genetics of Plasmodium falciparum. Nature 2010;465:311–5.