

A clinical drug library screen identifies astemizole as an antimalarial agent

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The high cost and protracted time line of new drug discovery are major roadblocks to creating therapies for neglected diseases. To accelerate drug discovery we created a library of 2,687 existing drugs and screened for inhibitors of the human malaria parasite *Plasmodium falciparum*. The antihistamine astemizole and its principal human metabolite are promising new inhibitors of chloroquine-sensitive and multidrug-resistant parasites, and they show efficacy in two mouse models of malaria.

Only recently has a systematic high-throughput approach been used to screen existing drugs for previously unknown activities, and these screens have focused primarily on diseases with relatively low prevalence in the developing world¹. Of the existing drug libraries reported, the largest contains less than 25% of the 3,400 drugs approved by the US Food and Drug Administration (FDA) and less than 10% of the approximately 11,500 drugs ever used in medicine (Supplementary Fig. 1 online). We assembled a library of 1,937 FDA-approved drugs and 750 drugs that were either approved for use abroad or undergoing phase 2 clinical trials, and we screened this collection, called the Johns Hopkins Clinical Compound Library (JHCCL), for inhibition of *P. falciparum* growth (Supplementary Methods online). A preliminary screen using a concentration of 10 μM revealed 189 existing drugs, distributed across many drug classes, that resulted in >50% inhibition (Fig. 1a and Supplementary Fig. 2 online). After eliminating topical drugs, known antimalarials, cytotoxic drugs and compounds previously reported to inhibit the malaria parasite, we determined half-maximal inhibitory concentration (IC_{50}) values for the 87 remaining drugs (Supplementary Table 1 online). Some inhibitors, such as pyrvinium pamoate, have no absorption with oral dosing. Other weak *P. falciparum* inhibitors that we identified, such as paroxetine, could be improved upon by screening related analogs that have not been developed to phase 2 drug trials as antidepressants. The unique ability of individual drugs within a class to inhibit *P. falciparum* supports building a comprehensive library of existing drugs rather than selecting representative members of each mechanistic class.

One of the more promising drugs identified is the non-sedating antihistamine astemizole (1; Fig. 1b), which inhibits (at submicromolar

concentrations) the proliferation of three *P. falciparum* parasite strains that differ in chloroquine sensitivity (Table 1). After oral ingestion in humans, astemizole is rapidly converted primarily to desmethylastemizole (2), which is ten-fold more abundant in plasma than astemizole and has a half-life of 7–9 d (ref. 2). Notably, desmethylastemizole had an IC_{50} of approximately 100 nM and was 2- to 12-fold more potent than astemizole in inhibiting *P. falciparum*, whereas the minor metabolite norastemizole (3) weakly inhibited the parasite. Astemizole and desmethylastemizole showed only an additive effect in combination with chloroquine (4), quinidine (5) and artemisinin (6) on the 3D7 and Dd2 *P. falciparum* strains (data not shown). During intraerythrocytic infection, *P. falciparum* parasites crystallize heme released from hemoglobin catabolism within the food vacuole, and quinoline antimalarials such as chloroquine inhibit this reaction³. Astemizole and desmethylastemizole (like the quinoline antimalarials) inhibit heme crystallization, concentrate within the *P. falciparum* food vacuole and co-purify with hemozoin in chloroquine-sensitive and multidrug-resistant parasites (Supplementary Fig. 3 online).

To determine whether astemizole has *in vivo* antimalarial activity, we tested it in two mouse models using the 4-d parasite suppression test. We used intraperitoneal dosing of desmethylastemizole in mice because in humans this is the principal active metabolite, and the parent compound has an oral bioavailability of 95% (ref. 2). Vehicle-treated mice that were infected with the lethal, chloroquine-sensitive *P. vinckei* strain developed parasitemias of approximately 64% on day 5 (Fig. 1c). In contrast, mice treated with astemizole at 30 $\text{mg m}^{-2} \text{d}^{-1}$ or desmethylastemizole at 15 $\text{mg m}^{-2} \text{d}^{-1}$ had an 80% or 81% reduction in parasitemia, respectively. Mice infected with chloroquine-resistant *P. yoelii*, then treated with astemizole or desmethylastemizole at 15 $\text{mg m}^{-2} \text{d}^{-1}$, showed a 44% or 40% reduction in parasitemia, respectively (Fig. 1d). Recrudescence occurred for both strains if we stopped treatment after 4 d at these doses, although high doses of astemizole (300 mg m^{-2}) delivered *per os* for 4 d cured infection. Doses as high as 18.6 mg m^{-2} have been used in humans to treat seasonal allergic rhinitis⁴.

Astemizole was introduced in 1983 under the brand name Hisminal as a non-sedating selective H_1 -histamine receptor antagonist for treating allergic rhinitis and was sold in 106 countries and also over the counter². The use patent for astemizole has expired. Although astemizole was voluntarily withdrawn in 1999 from the United States and Europe after decreased sales due to warnings about its safety and to the availability of antihistamines with fewer side effects⁵, it is currently sold in generic form in over 30 countries, including Cambodia, Thailand and Vietnam, which are malaria endemic (Dr. Reddy's Laboratories, personal communication). Astemizole and desmethylastemizole potently inhibit the ether-a-gogo (HERG)

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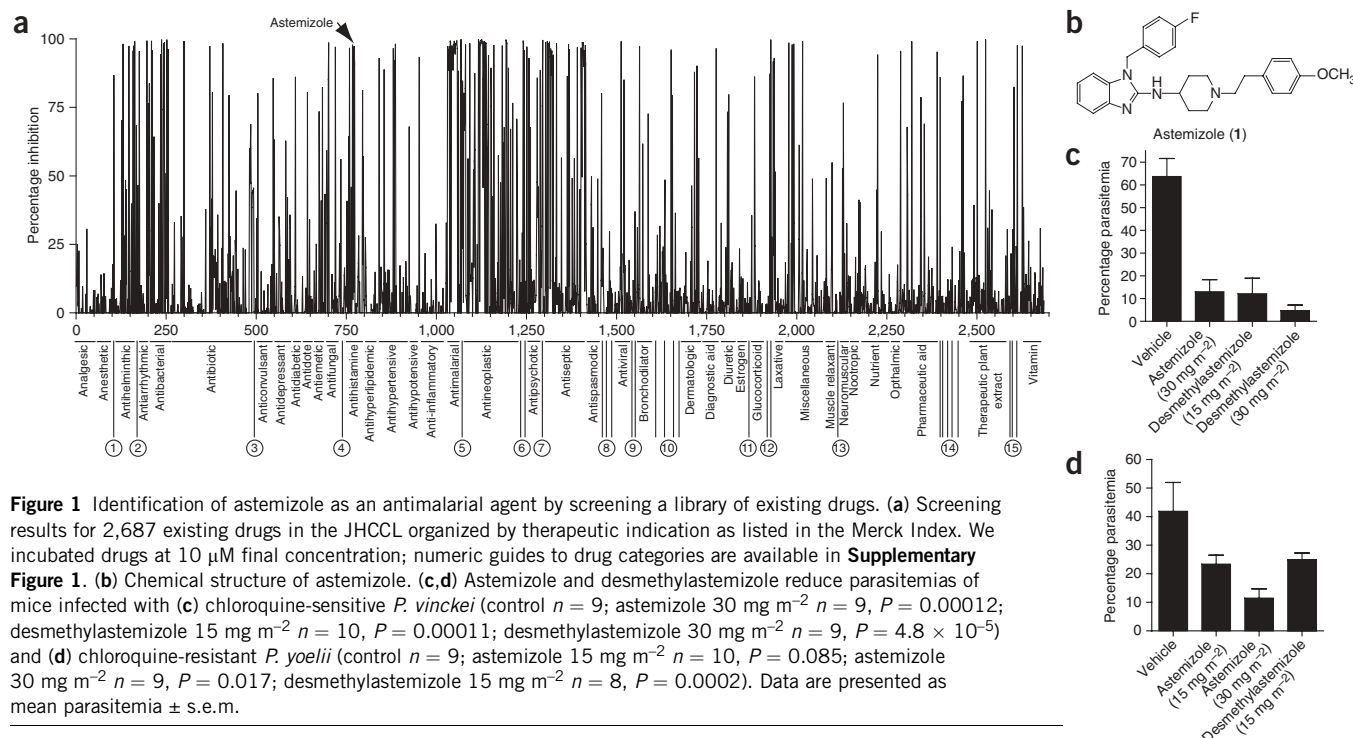


Figure 1 Identification of astemizole as an antimalarial agent by screening a library of existing drugs. **(a)** Screening results for 2,687 existing drugs in the JHCCL organized by therapeutic indication as listed in the Merck Index. We incubated drugs at 10 μ M final concentration; numeric guides to drug categories are available in **Supplementary Figure 1**. **(b)** Chemical structure of astemizole. **(c,d)** Astemizole and desmethylastemizole reduce parasitemias of mice infected with **(c)** chloroquine-sensitive *P. vinckei* (control $n = 9$; astemizole 30 mg m^{-2} $n = 9$, $P = 0.00012$; desmethylastemizole 15 mg m^{-2} $n = 10$, $P = 0.00011$; desmethylastemizole 30 mg m^{-2} $n = 9$, $P = 4.8 \times 10^{-5}$) and **(d)** chloroquine-resistant *P. yoelii* (control $n = 9$; astemizole 15 mg m^{-2} $n = 10$, $P = 0.085$; astemizole 30 mg m^{-2} $n = 9$, $P = 0.017$; desmethylastemizole 15 mg m^{-2} $n = 8$, $P = 0.0002$). Data are presented as mean parasitemia \pm s.e.m.

potassium channel at nanomolar concentrations⁶. Life-threatening cardiac arrhythmias can occur after astemizole overdose or when it is taken with drugs that block its metabolism via cytochrome P450 3A5 (CYP 3A4)⁷. Surveillance data from 17 countries over a decade revealed one cardiac rate or rhythm disorder per 8 million doses of astemizole and less than one cardiac fatality per 100 million doses⁸. Considerations relating to potential astemizole side effects in the treatment of malaria include the following: (i) antimalarial use is likely to be acute, in contrast to chronic administration as an antihistamine; (ii) malaria patients in resource-poor settings may be less likely to take interacting medications than were patients treated with astemizole in the past; and (iii) established quinoline antimalarials that less potently inhibit the HERG channel also have known cardiotoxicity⁹. Hundreds of astemizole analogs have been synthesized, and re-examination of this pharmacophore class may improve antimalarial activity and reduce HERG-related and other side effects¹⁰.

Given the economic challenges of *de novo* drug development for neglected diseases, screening existing drugs for new activities may be helpful. Even though many leads lack the potency to immediately enter the clinic or have unacceptable toxicity, the pharmacophore we have identified is a starting point for further development. Currently, the JHCCL is undergoing expansion to include every available drug ever used in the clinic via phase 2 clinical trials or approval by the FDA or its foreign counterparts. When complete, the JHCCL will be

available to any researcher interested in screening for existing drugs that may be useful as economically viable new therapies for diseases of the developing world.

Note: Supplementary information is available on the Nature Chemical Biology website.

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AUTHOR CONTRIBUTIONS

C.C., J.L. and D.S. contributed to library design, construction and screening as well as manuscript preparation. X.C. assisted with the mouse malaria model. L.S. performed the *P. falciparum* *in vitro* screen.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Table 1 Astemizole inhibition of three *P. falciparum* strains of different chloroquine sensitivity

<i>Plasmodium falciparum</i> strain	Astemizole IC ₅₀ (nM)	Norastemizole IC ₅₀ (nM)	Desmethylastemizole IC ₅₀ (nM)	Chloroquine IC ₅₀ (nM)
3D7	227 \pm 6.4	4,477 \pm 15	117 \pm 1.4	31.8 \pm 3.5
Dd2	457 \pm 12.3	3,590 \pm 16	106.2 \pm 10.3	79.3 \pm 6.8
ItG	734 \pm 2.2	2,230 \pm 934	56.8 \pm 27	107.3 \pm 13.8

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