

neous heparin and oral anticoagulation for 4 to 6 weeks after surgery, postpartum, and during long-term immobilization. It is unlikely that less prophylaxis would be given to those with a history of a provoked thrombotic event. Although not advised, patients with idiopathic thrombosis, particularly those with prothrombotic defects, may receive more frequent or long-term anticoagulation.

Our main finding was no excess risk of recurrent thrombosis in those patients with prothrombotic defects. This finding did not change when we adjusted for anticoagulant use or even excluded all periods of increased risk, as well as all periods of anticoagulant use. Therefore, our main finding cannot be explained by more frequent anticoagulant use in these patients. Nevertheless, as our study shows the recurrence risks given the current standard of care, it is possible that in the complete absence of thromboprophylaxis the estimates would have been different.

Suzanne C. Cannegieter, MD
Frits R. Rosendaal, MD
f.r.rosendaal@lumc.nl
Department of Clinical Epidemiology
Leiden University Medical Center
Leiden, the Netherlands

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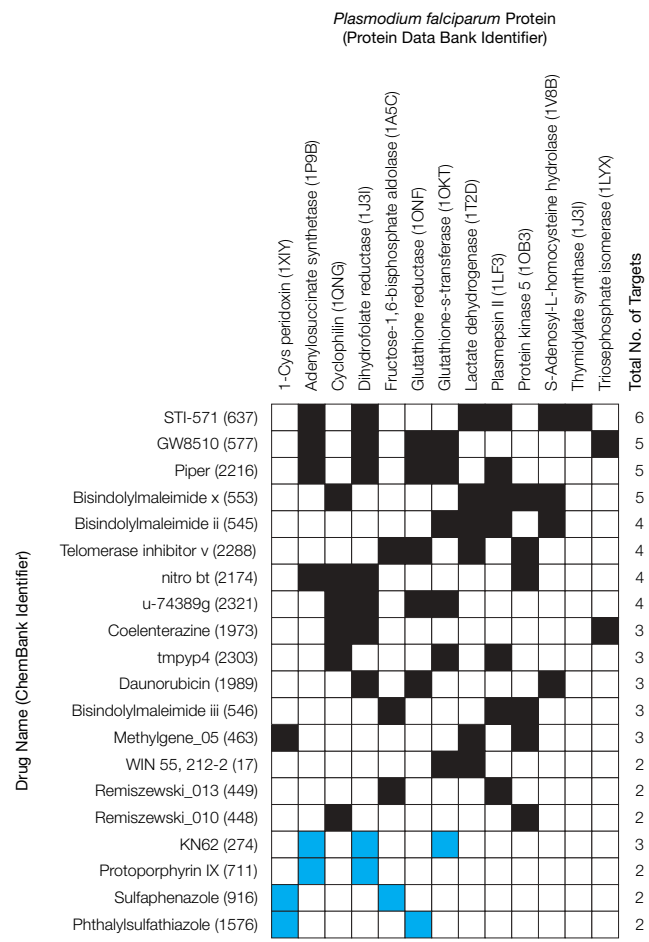
RESEARCH LETTER

Identification of Potential Multitarget Antimalarial Drugs

To the Editor: Malaria is one of the deadliest tropical diseases, causing more than 300 million infections yearly.¹ Successful clearance of the malarial parasites, *Plasmodium* species, from a patient's body by antimalarial drugs is impeded by the emergence of drug-resistant strains. Drugs that effectively eliminate *Plasmodium* with short treatment duration reduce risk of treatment failure and emergence of drug-resistant strains.¹

Antimalarial drugs currently target single *Plasmodium* proteins. Effective therapeutic regimens require a combination of drugs that have different mechanisms of action during the same stage of the parasite's life cycle.¹ However, malaria is a disease that occurs mostly in tropical and subtropical areas, where patients have limited access to drugs, and combination drug regimens may not succeed due to poor adherence.² Multitarget drugs are currently being used extensively to treat both infectious and inherited diseases.³ New antimalarial therapies that include multitarget drugs may have higher efficacy than single-target drugs and provide a simpler regimen for antimalarial therapy.⁴ Our purpose in this study was to predict a list of drugs that will bind to the active site of multiple *Plasmodium falciparum* proteins with high affinity.

Figure. Binding Patterns of 4 Approved (Blue) and 16 Experimental (Black) Multitarget Drugs to 13 *Plasmodium falciparum* Proteins



These drugs target the active site of 2-6 proteins with high affinity.

Methods. We used a computational protein-inhibitor docking with dynamics protocol³ to calculate the binding affinities of 1105 approved and 1239 experimental drugs (obtained from ChemBank⁶) against 13 *Plasmodium* proteins whose structures have been determined by x-ray crystallography. Binding affinity calculations were carried out using AutoDock version 3.0.5 with a Lamarckian genetic algorithm (The Scripps Research Institute, La Jolla, Calif). We first placed each drug into the active site of the protein to find the most stable binding mode. The protein-drug complexes were consequently solvated in a water shell with sodium and chloride ions. We applied 100 steps of energy minimization followed by 0.1 ps of molecular dynamics simulation to each complex using XPLOR version 3.851 (Yale University, New Haven, Conn). The conformations at 0.1 ps were used for the protein-drug binding affinity calculations.

For each protein, a given drug was docked into the active site and allowed to move in an exhaustive manner to find the most stable binding conformation. The protein-

Table. Comparison of the Inhibitory Activities of 3 Antimalarial Drugs and the Drugs Predicted to Have the Highest Binding Affinity*

Drug	Targeted Protein	Calculated K_i (Rank)	Experimental Inhibitory Activity		Drug Predicted to Have Highest Affinity	
			Activity, mol/L	Source	Drug	Calculated K_i
Pyrimethamine	Dihydrofolate reductase	2.80×10^{-8} (102)	$K_i = 2.00 \times 10^{-10}$	Yuvaniyama et al ⁷	Nitro bt	7.16×10^{-12}
WR99210	Dihydrofolate reductase	1.10×10^{-8} (48)	$K_i = 1.10 \times 10^{-11}$	Yuvaniyama et al ⁷	Nitro bt	7.16×10^{-12}
EH58	Plasmeprin II	1.58×10^{-9} (7)	$K_i = 1.00 \times 10^{-7}$	Asojo et al ⁸	Remiszewski_013	1.01×10^{-10}

Abbreviation: K_i , inhibitory constant.

*The calculated K_i values for these known drugs correlate with the experimentally determined inhibitory activities and usually rank within the top 5th percentile compared with the entire set of drugs.

drug binding affinity in terms of the inhibitory constant (K_i) was calculated every time the drug molecule was moved. After repeating this procedure for all of the drugs for each protein, the 20 drugs with the lowest K_i values were considered high-affinity drug candidates. Further details of the molecular dynamics simulation and docking protocols are available elsewhere.⁵

Results. We predicted 20 multitarget drugs that showed high affinity across 2 or more proteins (FIGURE). Four are drugs approved by the US Food and Drug Administration for treatment of diseases other than malaria: KN62 (targeting 3 proteins), protoporphyrin IX, phthalylsulfathiazole, and sulfaphenazole (targeting 2 proteins each). The other 16 are experimental, each targeting up to 6 proteins. The best drugs in terms of multitarget functionality were STI-571 (targeting 6 proteins), bisindolylmaleimide x, GW8510, and Piper (targeting 5 proteins each). The best combination of 2 drugs was bisindolylmaleimide x and GW8510, which together target 10 *Plasmodium* proteins. An analysis of 3 known single-target antimalarial drugs against these proteins showed that our calculated K_i s for these drugs compared well with experimentally determined values (when available) and that the inhibitory activity usually ranked within the top 5th percentile compared with our entire set of drugs (TABLE).

Conclusions. Promising vaccines targeting multiple *Plasmodium* proteins have been evaluated.^{9,10} In a similar fashion, we propose designing new antimalarial drugs that simultaneously target multiple *Plasmodium* proteins. Our computational drug screening protocol provides evidence for 20 approved or experimental drugs that bind strongly to 13 *Plasmodium* proteins. We recommend that these drug candidates be experimentally tested for inhibition of *Plasmodium* growth and used as a starting point for further design of a high-efficacy multitarget antimalarial drug.

Ekachai Jenwitheesuk, DVM, PhD
 Ram Samudrala, PhD
 ram@compbio.washington.edu
 Department of Microbiology
 University of Washington School of Medicine
 Seattle

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Study concept and design; analysis and interpretation of data: Jenwitheesuk, Samudrala.

Drafting of the manuscript: Jenwitheesuk.

Critical revision of the manuscript for important intellectual content; obtained funding; study supervision: Samudrala.

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CORRECTIONS

Omitted Author: In the Letter to the Editor entitled "BRCA Mutations and Ductal Carcinoma In Situ" published in the August 3, 2005, issue of *JAMA* (2005;294:553-554), there was an author omitted from the author affiliations. On page 553, after "Kathleen Klein Oros, BSc" and before "Department of Human Genetics," it should have read "Patricia N. Tonin, PhD."

Numbers Transposed: In the Original Contribution entitled "Effect of Orlistat on Weight and Body Composition in Obese Adolescents: A Randomized Controlled Trial" published in the June 15, 2005, issue of *JAMA* (2005;293:2873-2883), 2 numbers were transposed. On page 2879, the second to the last sentence in column 3 should be "Participants in the orlistat group (+2116 g) gained a similar amount of fat-free body mass as those in the placebo group (+2312 g)." Also, on page 2873, the author affiliation for Dr Jensen should be "Department of Pediatrics, Baylor College of Medicine, Houston, Tex."