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Antimalarial Asexual Stage-Specific and Gametocytocidal Activities of HIV Protease Inhibitors[▽]

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The stage-specific antimalarial activities of a panel of antiretroviral protease inhibitors (PIs), including two nonpeptidic PIs (tipranavir and darunavir), were tested *in vitro* against *Plasmodium falciparum*. While darunavir demonstrated limited antimalarial activity (effective concentration [EC_{50}], >50 μM), tipranavir was active at clinically relevant concentrations (EC_{50}, 12 to 21 μM). Saquinavir, lopinavir, and tipranavir preferentially inhibited the growth of mature asexual-stage parasites (24 h postinvasion). While all of the PIs tested inhibited gametocytogenesis, tipranavir was the only one to exhibit gametocytocidal activity.

The global distributions of HIV and malaria overlap in many regions of the world (reviewed in reference 17). Although data on the number of individuals with both diseases are unavailable, rates of coinfection are likely to be high (7). Furthermore, coinfection often leads to severe disease (4, 12, 19, 20). While the effects of antiretroviral therapy on the outcome of malaria infection are not understood, defining these interactions is important (11, 13, 16, 18). Understanding the antimalarial activities of the antiretroviral protease inhibitors (PIs) (reviewed in reference 17), for example, may lead to treatment recommendations that improve clinical outcomes and may also result in the identification of a new antimalarial drug target.

Current data suggest that PIs kill malaria parasites by inhibiting one or more of the six nondigestive vacuole plasmepsins (reviewed in reference 17). In the present study we investigated the stage-specific effects of the PIs on asexual- and sexual-stage *Plasmodium falciparum* parasites in order to help define the antimalarial target(s) of these drugs and to help guide partner drug choices in the field. To gain additional structure-activity data and information that may be relevant for coinfected individuals we also examined the activities of the nonpeptidic PIs tipranavir (Aptivus) and darunavir (Prezista), new-generation PIs that are active against HIV-1 strains resistant to first generation PIs (9).

The antimalarial activities of saquinavir, lopinavir, ritonavir, tipranavir, darunavir, and chloroquine (diphosphate salt; Sigma) were determined as described previously (18). Concentrations required to achieve 10, 50, and 90% growth inhibition (± the standard error [SE]) were determined by nonlinear regression curve fitting. Each assay was performed in triplicate on at least two separate occasions. Stage-specific growth inhibition assays were performed on synchronized parasite cultures (8) at 0 (ring), 24 (trophozoite), and 36 h (schizont) postsynchronization. Cultures were washed post-drug exposure, resuspended in drug-free medium, and seeded into tissue culture plates containing 0.5 μCi/well [^{3}H]hypoxanthine for 40 h. Incorporation of [^{3}H]hypoxanthine was compared to that in vehicle controls.

Drug-induced effects on gametocytogenesis were examined using Pfs16-GFP parasites (3) as previously described (14). Assays were performed in triplicate on three separate occasions. The antigametocyte activities of selective PIs were also determined using Pfs16-GFP parasites (3). In these assays gametocytes were sorted from parasite cultures, seeded into microtiter plates (1,000 gametocytes and 5% hematocrit), and exposed to drugs or controls for 48 h. Hydroethidine was used to assess viability. The number of viable gametocytes in test cultures after treatment was compared to controls, and results were analyzed by one-way analysis of variance. Assays were performed in triplicate on two separate occasions.

Tipranavir was active against all parasite lines tested, including the chloroquine-resistant line Dd2 (50% effective concentration [EC_{50}], 21±2 μM) and three chloroquine-sensitive lines (3D7, EC_{50} of 20±2 μM; D10, EC_{50} of 12±2 μM; Pfs16-GFP, EC_{50} of 18±4 μM). These EC_{50}s are all below the C_{min}

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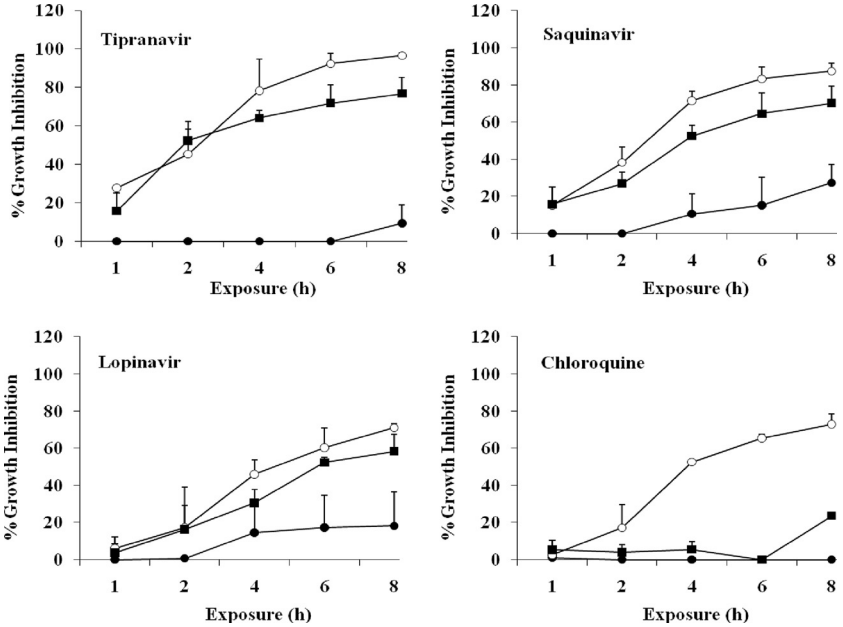

FIG. 1. *In vitro* stage-specific activities of PIs against *P. falciparum* asexual parasites. Erythrocytes infected with *P. falciparum* line D10 at ring (filled circles) trophozoite (open circles), or schizont (filled squares) stages were exposed to saquinavir (SQV; 40 μM), tipranavir (TPV; 150 μM), lopinavir (LPV; 20 μM), and chloroquine (CHQ; 50 nM) for 1, 2, 4, 6, or 8 h, as described in the text. Data are presented as percent growth inhibition (+SE) compared to vehicle controls (taken as 100% growth). Each assay was repeated twice in triplicate.

to C_{max} range (60 to 185 μM) for this drug in humans (6). Darunavir also inhibited the growth of *P. falciparum*. However, the EC_{50} (Dd2 EC_{50} of 70 μM) (data not shown) for this drug was well above clinically achievable levels (C_{min} to C_{max}, 0.7 to 12.4 μM) (5). The EC_{50}s of saquinavir (D10 EC_{50}, 3 ± 1 μM; 3D7 EC_{50}, 3 ± 3 μM; Pfs16-GFP EC_{50}, 5 ± 3 μM), lopinavir (D10 EC_{50}, 2 ± 1 μM; 3D7 EC_{50}, 2 ± 3 μM; Pfs16-GFP EC_{50}, 3 ± 1 μM), ritonavir (D10 EC_{50}, 3 ± 1 μM; 3D7 EC_{50}, 3 ± 1 μM; Pfs16-GFP EC_{50}, 5 ± 1 μM) and chloroquine (D10 EC_{50}, 23 ± 1 nM; 3D7 EC_{50}, 25 ± 6 nM; Pfs16-GFP EC_{50}, 23 ± 3 nM) were comparable between parasite lines and similar to previously published values for 3D7 (1, 2).

Saquinavir, lopinavir, and tipranavir demonstrated significantly greater growth inhibition ($P < 0.05$) against trophozoite and schizont stages in comparison to ring stages (Fig. 1). Similar results were obtained for the drug-resistant Dd2 *P. falciparum* line (data not shown). Chloroquine was used as a control and, as expected, trophozoite stages were more sensitive to this drug than either ring or schizont stages (Fig. 1). Each of the four PIs tested also reduced the number of gametocytes produced *in vitro* (Fig. 2A). The reduction in gametocytes was dose dependent and statistically significant ($P < 0.01$) when cultures were exposed to EC_{90} levels of ritonavir and all concentrations of tipranavir (Fig. 2A). Tipranavir was also able to directly kill gametocytes (Fig. 2B) ($P < 0.01$). While saquinavir, ritonavir, and lopinavir reduced the numbers of live gametocytes in a dose-dependent fashion, these data did not reach statistical significance (Fig. 2B) ($P > 0.05$).

Evidence suggesting that PIs may be beneficial to HIV and malaria parasite-coinfected individuals is mounting. In addition to possessing antiretroviral activities these drugs also inhibit the growth of malaria parasites (1, 13, 15, 18). In the present study we have extended these data by demonstrating that tipranavir can inhibit the growth of malaria parasites at clinically relevant concentrations (6). Although additional studies, including those examining pharmacokinetic drug interactions and the effects of increased plasma proteins on the activity of tipranavir, are needed (6), these data further indicate that PIs are likely to be beneficial during HIV-malaria coinfection. The antigametocyte activity demonstrated by tipranavir adds an additional dimension to this observation, suggesting that this drug may also have an impact on malaria transmission. These data are novel in that very few antimalarial drugs have antigametocyte activity. Indeed most induce gametocytogenesis *in vitro* (14) and as a result can perpetuate transmission and the spread of drug-resistant parasites. The observation that none of the PIs induced gametocytogenesis *in vitro* is significant, given recent malaria eradication goals and the need for tools to achieve this (10).

To gain an understanding of how HIV PIs kill *P. falciparum* we investigated their effects on individual stages of asexual development. Data from these studies indicated that the trophozoite and schizont stages are significantly more sensitive to PIs than ring-stage parasites (Fig. 1). Taken together with gametocyte inhibition data these results suggest that the primary target of the PIs is likely to be expressed in both gametocytes and intraerythrocytic parasites. Although expression data (http://plasmodb.org/) require confirmation and the possibility that the PIs might target different proteases in the different parasite stages cannot be ruled out, plasmepsins V, IX, and X appear to be the best candidate targets of these drugs. Future studies investigating these enzymes may identify the antimalarial target of the PIs and help explain the poor antimalarial activity of darunavir. Interestingly, darunavir has a

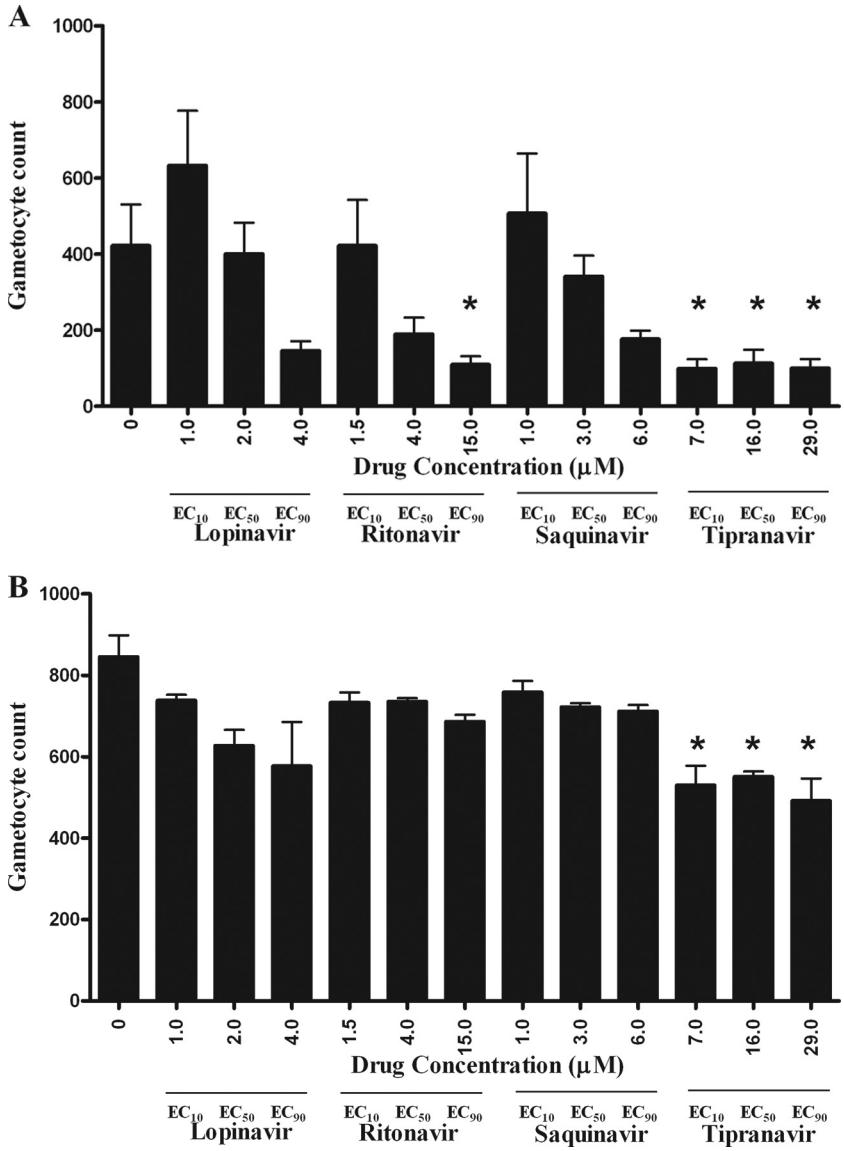

FIG. 2. Activities of selected PIs in gametocyte and gametocyte induction inhibition assays. (A) *In vitro* antigametocytogenesis activities of selected PIs as determined using transgenic Pfs16-GFP *P. falciparum* parasites. (B) Activities of PIs against Pfs16-GFP *P. falciparum* gametocytes. All drugs were assessed at their EC_{10}, EC_{50}, and EC_{90} values as determined by [^{3}H]hypoxanthine incorporation against asexually replicating parasites. Bars with * indicate significant changes compared to vehicle control wells ($P < 0.01$). Pfs16-GFP gametocytes, unlike Pfs16-GFP asexual-stage parasites, express GFP-tagged Pfs16 (3).

similar structure to amprenavir, another PI with weak antimalarial activity (5, 18).

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