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Short communication

Differential drug binding by the highly conserved *Plasmodium falciparum* thymidylate synthase

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Active sites that are highly conserved between host and parasite enzymes are generally expected to display uniform binding of inhibitors at the catalytic site. Crystal structures of human and *Plasmodium* thymidylate synthase (TS) reveal that all amino acids within 5 Å of the folate-binding site are conserved [1,2]. Indeed, human and malarial TS were previously shown to have identical kinetic properties [3]. Furthermore, low nanomolar level inhibitors such as 5-fluoro-2′-deoxyuridylate and full-length TS antifolates (1843U89 and polyglutamylated D1694; Fig. 1A) inhibited host and parasite TS equally well [3,4]. Surprisingly, here we show that small, lipophilic folate analogs AG337 and AG331 (Fig. 1A), optimized to bind human TS at low nanomolar concentrations, bound *Plasmodium* TS 130-fold more weakly. We conclude that, based on thermodynamic principles, it should be possible to identify reciprocal small molecules that bind *Plasmodium* TS better than human TS (Fig. 1B).

Dihydrofolate reductase-thymidylate synthase (DHFR-TS) is a well-established drug target in malaria chemotherapy. In many cell types, partial inhibition of either the DHFR or the TS domain leads to nucleotide imbalance and cell death [5,6]. The early clinical success of pyrimethamine and cycloguanil was followed by a demonstration of host-parasite differences in binding of these drugs to the DHFR domain [7]. However, other mechanisms may also contribute to selective drug action [8–12]. At least in part, selectivity arises because human cells start with high levels of DHFR and are able to further increase protein translation in the presence of DHFR inhibitors, while malarial parasites cannot [11,12].

The TS domain may also be considered as a drug target [3,4], given the complex mechanisms underlying selectivity of drugs against *Plasmodium* DHFR-TS. Potent inhibitors, originally developed for cancer chemotherapy, are available [13]. Indeed, full-length, folate-based inhibitors of human TS, D1694 pentaglutamate and 1843U89 (Fig. 1), inhibited malaria TS at low nanomolar concentrations [3,4]. Human cells treated with TS inhibitors, as with DHFR inhibitors, increase translation of target protein thereby decreasing toxicity [10,11]. Due to the lack of a pyrimidine salvage pathway in malaria, human cells but not malaria parasites can be rescued by thymidine [4]. Thus, in principal, species-specific TS inhibitors may not be required to get selective activity against malaria. In practice, however, thymidine rescue would require co-administration of the inhibitor and thymidine, which could be problematic. A selective inhibitor of malarial TS could decrease the need to provide thymidine. Given the large differences in amino acid residues away from the TS active site and the well-known TS conformational changes during catalysis [14,15], some inhibitors could bind TS molecules of different species with different affinity.

Abbreviations: DHFR, dihydrofolate reductase; TS, thymidylate synthase; mTHF, 5,10-methylenetetrahydrofolate; dUMP, 2′-deoxyuridine monophosphate

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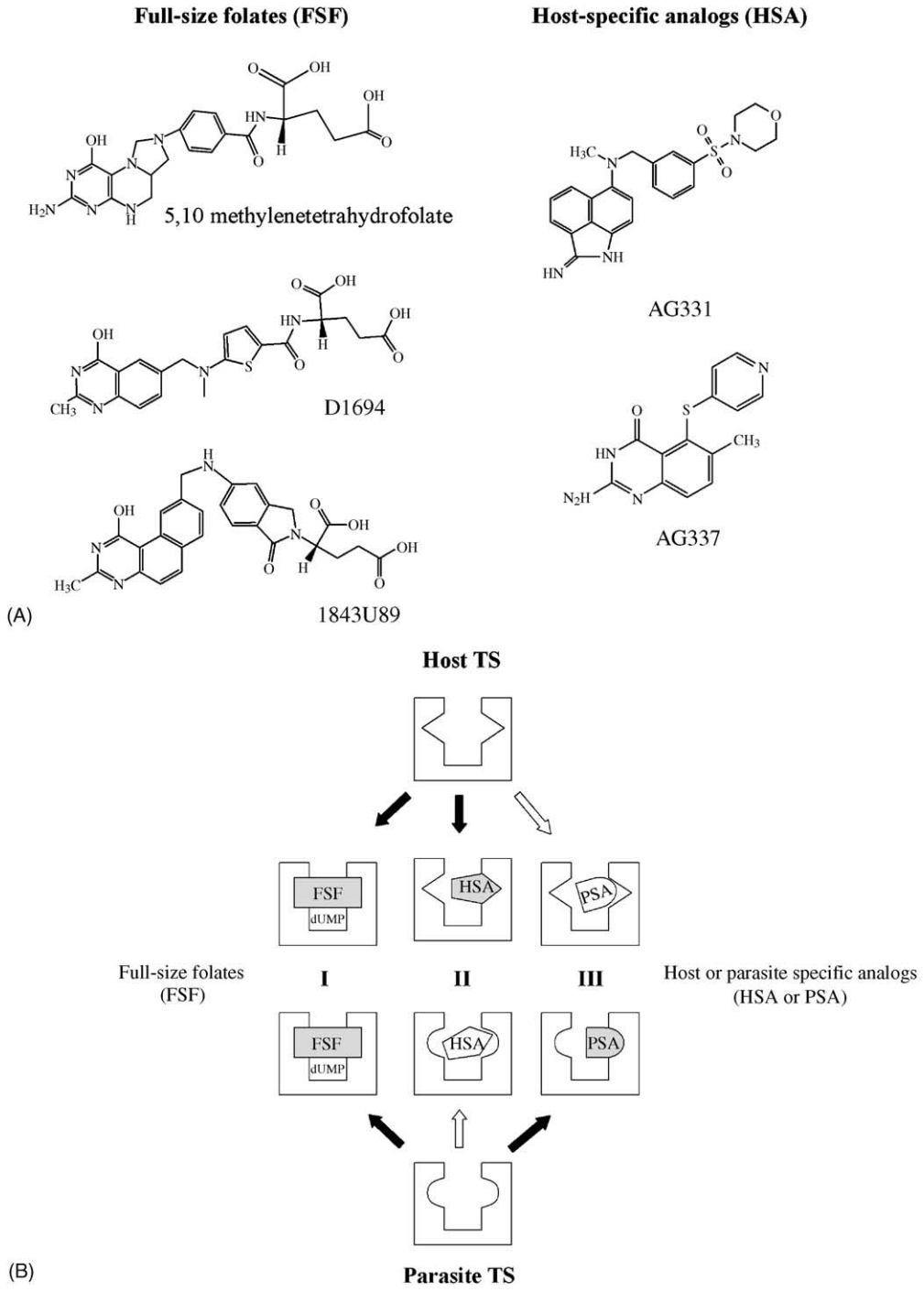

Fig. 1. (A) Comparisons of the structures of the TS substrate mTHF, full-size folate analogs (D1694 and 1843U89), and smaller, lipophilic folate analogs (AG331 and AG337). (B) A conformation dynamics model to explain the differential binding of small antifolates to host but not parasite TS. Abbreviations: full size folates (FSF), host specific analog (HSA), and parasite specific analog (PSA). Details of the hypothesis are presented in the last paragraph of the text.

AG331 and AG337 were prepared as previously described [16,17] and confirmed by NMR and MS (data not shown). In addition, human TS [18,19] helped verify the biological activity of the synthetic antifolates. Recombinant malarial DHFR-TS was purified to homogeneity as previously described [20]. Following methotrexate affinity column chromatography, the protein was further purified on a Superdex S200 (Amersham-Pharmacia) column. Fractions containing pure malarial DHFR-TS were concentrated and desalted on an Amicon YM-10 centrifugal device. TS activity was measured under initial rate conditions by tracking the release of tritium from 5-^{3}H-deoxyuridylate [3,4,21]. Details of reaction conditions are presented in the legend to Fig. 2. Cell proliferation of drug-treated *Plasmodium falciparum* was