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Antimalarial Activities of Polyhydroxyphenyl and Hydroxamic Acid Derivatives

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Several known mammalian ribonucleotide reductase inhibitors featuring a polyhydroxyphenyl and/or hydroxamate moiety as the active group were screened for potency in inhibiting growth of the malaria parasite *Plasmodium falciparum*. Compounds containing a 2,3- or 3,4-dihydroxyphenyl group as well as benzohydroxamate appear to be the most effective inhibitors of the malaria parasite.

There is an urgent need to develop antimalarials targeted against new metabolic targets as the drugs available to fight the disease are rapidly losing their efficacy. Ribonucleotide reductase (RNR) catalyzes the reduction of ribonucleotides to deoxyribonucleotides, the first and rate-limiting step for de novo synthesis of 2′-deoxyribonucleoside 5′-triphosphates (16). RNR activity has been shown to be closely correlated to the rate of tumor growth (5, 19); consequently, inhibitors directed against RNR have been used for many years in cancer chemotherapy (7, 11, 12). The central role of the ubiquitous enzyme RNR in DNA metabolism also makes this enzyme an excellent target for chemotherapy of malaria. We have previously reported that an oligodeoxynucleotide phosphorothioate complementary to RNR small subunit sequences showed antimalarial activity (2). We have now initiated a systematic investigation to test known RNR inhibitors for possible antimalarial activity. Several antitumor and antiviral compounds exist which are specific inhibitors of RNR (15). The hydroxamate groups of RNR inhibitors such as hydroxyurea are electron reductants and destroy the tyrosyl radical (6). This paper reports evaluation of hydroxamic acid and polyhydroxyphenyl derivatives as potential antimalarials.

We evaluated three known RNR inhibitors, hydroxyurea, acetohydroxamate, and benzohydroxamate, for potential antimalarial activities in synchronous *Plasmodium falciparum* Dd2-infected erythrocyte culture according to the method of Desjardins et al. (3). Ring-stage cultures were incubated with [^{3}H] hypoxanthine (1 μCi/μl; 1 μCi = 37 kBq) and various concentrations of inhibitors for 24 h at 37°C. The radiolabeled genomic DNA was isolated by sodium dodecyl sulfate-proteinase K treatment (14) and counted with a liquid scintillation spectrometer. The data were analyzed by logistic regression (11) since experimental variance is not constant for all drug concentrations, which makes the standard least squares technique inappropriate. This type of analysis was conducted for each drug evaluated to produce curves from which the drug concentration inhibiting 50% of the parasite growth (IC_{50}) was calculated.

As can be seen in Table 1, both hydroxyurea and acetohydroxamate were weak inhibitors of malaria parasite growth. However, the IC_{50} for benzohydroxamic acid was about 50-fold lower than that for either hydroxyurea or acetohydroxamate. Some trends emerge from these data which encourage us in thinking that RNR could be a promising antimalarial target. Hydroxyurea and benzohydroxamate inhibit mammalian RNR to similar extents with IC_{50}s of 500 and 400 μM, respectively (7). We found benzohydroxamate to be a much more effective inhibitor of malaria parasite growth than hydroxyurea, requiring a 20-fold lower concentration to effect the same level of inhibition as that of the human enzyme. The fact that benzohydroxamic acid proved to be a much more potent inhibitor of *P. falciparum* than the human system is noteworthy for two reasons. Not only does this identify benzohydroxamate as a potential antimalarial, but in addition and perhaps more importantly, it provides evidence of a difference between *P. falciparum* and human RNRs. This evidence supports that of Klayman et al. (9, 10), who cured *Plasmodium berghei* malaria in mice with RNR inhibitors, suggesting a possible difference between the *P. berghei* and mouse forms of reductase. Unlike the *P. berghei* data, the *P. falciparum* result presented here could not be explained by a difference in drug permeability, since benzohydroxamate was more effective in inhibiting the *P. falciparum* reductase enclosed within the parasite than the human enzyme, which was free in solution.

The results suggesting a difference between *P. falciparum* and human RNRs prompted us to test several substituted or modified forms of benzohydroxamate, such as vicinal polyhydroxyphenyl-containing compounds. This family of compounds has demonstrated antitumor activity, presumably due to inhibition of RNR activity (6–8, 17). We focused on vicinal di- and trihydroxyphenyls both with and without a hydroxamate moiety. The positions of the hydroxyl groups were varied; in addition, one drug (VF268) had nonadjacent hydroxyls and on another (VF282) the hydroxyl hydrogens were replaced with a methyl group.

Polyhydroxyphenyl and hydroxamic acid compounds are effective metal chelators. Since a ferric iron center plays a key role in RNR activity, the metal-chelating capacity of these compounds could explain their ability to inhibit RNR. Although it has been shown that changing hydroxyl group positions on the benzene ring has little effect on Fe^{3+}-chelating activity if hydroxamic acid is present (7), such changes have large and correlative effects on RNR inhibition and free rad-

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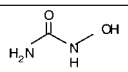
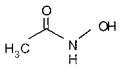
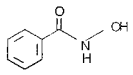
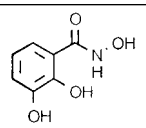
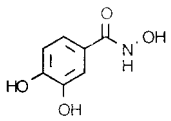
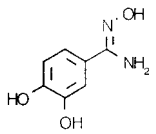
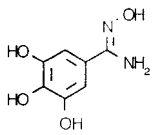
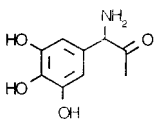
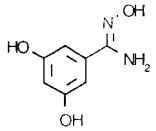
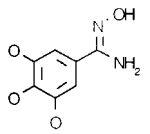
TABLE 1. Antimalarial activities of hydroxamic acids[a]

Compound and structure		IC_{50} (μM)	95% upper bound (μM)	95% lower bound (μM)	No. of tests
Aminohydroxamate (hydroxyurea)		792	>10,000	97	58
Acetohydroxamate		1,032	3,912	26.4	60
Benzohydroxamate		17.1	78.5	2.2	76

[a] Hydroxyurea (Sigma), acetohydroxamate (National Cancer Institute), and benzohydroxamate (National Cancer Institute) were tested in an in vitro culture system for potential antimalarial activities according to the method of Desjardins et al. (3).

ical quenching potency (4). Hence, the mechanism by which polyhydroxyphenyls inhibit RNR is now believed to be free radical scavenging. Table 2 provides the structure and a summary of test results for each drug tested. VF149 and VF147, the two vicinal dihydroxybenzohydroxamates tested, outperformed the other drugs as inhibitors of *P. falciparum* growth. Trihydroxyphenyl-containing compounds are more effective mammalian RNR inhibitors than are compounds which con-

TABLE 2. Antimalarial activities of substituted benzohydroxamic acids[a]

Compound	Structure	IC_{50} (μM)	95% upper bound (μM)	95% lower bound (μM)	No. of tests
VF 149		6.4	25.7	<1	76
VF 147		15.5	35.5	3.0	68
VF 236		23	80	3.5	52
VF 233		38	185	9.2	51
VF 368		301	>2,000	46	51
VF 268		589	>2,000	91	51
VF 282		2,010	>10,000	520	52

[a] RNR inhibitors synthesized in one of our laboratories (H.L.E.) were tested on *P. falciparum* cultures.

tain one fewer hydroxyl group but are otherwise identical (4, 7, 8, 17). Yet we found the reverse to be true when these drugs were tested as antimalarials. It could be argued that the trihydroxyphenyls were at a disadvantage in our test program since they are not associated with the hydroxamate group. But testing on mammalian systems demonstrated that the hydroxamate functional group is relatively unimportant for antitumor activity and that the polyhydroxyphenyl group is the primary source of activity (6). This appears to be further evidence of a difference between mammalian and malarial forms of RNR, since hydroxamate-containing agents were the best antimalarials. Of the drugs tested, vicinal dihydroxyphenyl-substituted hydroxamic acids are the most effective antimalarials. The inhibitory effect though was reversible at the IC_{50}. At four times the IC_{50} the effects of these inhibitors were found to be irreversible (data not shown). Didox (VF147, 3,4-dihydroxybenzohydroxamate) has been in clinical trials as an anticancer agent since 1988 (18). It exhibits low toxicity to the extent that steady-state concentrations in plasma during treatment (involving 36 h of infusion) are typically near the malaria parasite IC_{50} of 15 μM (1). Didox also stimulated hemoglobin F production in an anemic baboon and transgenic mice (13), an added benefit for malaria patients with anemia. Efforts are under way to characterize the mechanisms of action of these inhibitors in in vitro assays with recombinant malarial RNR.

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