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Comparative Complement Selection in Bacteria Enables Screening for Lead Compounds Targeted to a Purine Salvage Enzyme of Parasites

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Expression plasmids encoding the hypoxanthine phosphoribosyltransferases (HPRTs) of *Plasmodium falciparum*, *Schistosoma mansoni*, *Tritrichomonas foetus*, and *Homo sapiens* were subcloned into genetically deficient *Escherichia coli* that requires complementation by the activity of a recombinant HPRT for growth on semidefined medium. Fifty-nine purine analogs were screened for their abilities to inhibit the growth of these bacteria. Several compounds that selectively altered the growth of the bacteria complemented by the malarial, schistosomal, or tritrichomonal HPRT compared with the growth of bacteria expressing the human enzyme were identified. These results demonstrate that the recombinant approach to screening compounds by complement selection in a comparative manner provides a rapid and efficient method for the identification of new lead compounds selectively targeted to the purine salvage enzymes of parasites.

The traditional method of discovering drugs for the treatment of parasitic diseases involves the random screening of thousands of naturally occurring substances or synthetically produced chemicals for their activities against the parasite. Alternatively, a specific enzyme essential for survival of the parasite may be identified as an appropriate target for antiparasitic chemotherapy. Because of their pivotal roles for the survival of parasites, purine salvage enzymes were proposed more than 25 years ago as potential targets for the chemotherapeutic treatment of malaria (24, 25). During the past two decades, the purine salvage enzymes of a number of other parasites have been investigated. These studies include enzymes from the etiologic agents for human leishmaniasis (15), giardiasis (26), Chagas' disease (2), and schistosomiasis (22), as well as bovine tritrichomoniasis (27). The long-range objective of the majority of those investigations was to discover or design a compound that selectively inhibits the activity of a pivotal purine salvage enzyme and that thus could be developed as a lead compound or drug for the treatment of the parasitic disease. Limited progress has been made toward the identification of lead compounds selectively targeted to the purine salvage enzymes of parasites. Exceptions include a report that mercaptopurine and thioguanine selectively inhibit a purine salvage enzyme of the parasite responsible for human malaria (19).

Hypoxanthine phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase [EC 2.4.2.8]; also hypoxanthine-guanine phosphoribosyltransferase or hypoxanthine-guanine-xanthine phosphoribosyltransferase) is a key purine salvage enzyme that has been studied extensively, and cDNAs encoding the human, schistosomal, malarial, and tritrichomonal HPRTs have been cloned and sequenced (3, 4, 11, 13). In addition, active recombinant enzymes from humans, schistosomes, and tritrichomonads have previously been expressed in bacteria (3, 5, 6). The human HPRT has been the subject of extensive investigation because defects in this enzyme are known to be responsible for genetically inherited gout and Lesch-Nyhan syndrome (12, 21). Thus, in an effort to minimize potential side effects because of interactions with enzymes of the human host, drugs targeted to an HPRT will need to be quite selective in their affinities for the enzymes of parasites.

Herein we describe the development of a method, referred to as comparative complement selection, for the rapid screening in bacteria of purine analogs for their abilities to selectively inhibit recombinant HPRTs of parasites compared with their abilities to inhibit the human enzyme. The procedure is based on traditional complementation techniques which use the activity of a recombinant HPRT to complement the genetic deficiencies of the host bacteria. Subsequently, the effects of compounds on the growth of these bacteria can readily be screened by using sterile, blank antibiotic testing disks and methods similar to those used for standard antibiotic susceptibility assays. In this manner, complement selection can be used to identify compounds that affect the growth of bacteria expressing a particular enzyme. However, direct comparisons with the effects on bacteria expressing the recombinant human enzyme enable the possible identification of compounds that selectively target the enzymes of the parasites. Thus, the comparative aspect of this simple and rapid screening method provides the means of identifying selective substrates or inhibitors of HPRTs. Those compounds that demonstrate selective preference for a parasite HPRT rather than that of the human host represent initial lead compounds and may provide information which will be useful in the design of new drugs for the treatment of these parasitic diseases.

MATERIALS AND METHODS

Source of purines and purine analogs. Guanine, hypoxanthine, xanthine, caffeine (Table 1, compound 37) and mercaptopurine (Table 2, compound 51), were from Sigma Chemical Co., St. Louis, Mo. All other purine analogs (listed in Tables 1 and 2) were generously provided by Burroughs Wellcome Co., Research Triangle Park, N.C.

Plasmids and bacterial strains. The recombinant HPRTs were inserted into pBAce plasmids (6) possessing an ampicillin-selectable marker, and expression was induced by phosphate starvation in a *recA* mutant strain of *Escherichia coli*

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SØ606 (Δ*pro-gpt-lac hpt ara thi*) (10) to verify the levels of enzyme production (see below). For complement selection and screening assays, the same constructs were expressed in *E. coli* SØ609 (Δ*pro-gpt-lac hpt purHJ thi pup ara strA*) (10).

Expression of recombinant HPRTs. Complementary DNAs encoding the HPRTs of *Plasmodium falciparum* and *Homo sapiens* were amplified in PCRs, inserted into pBAce expression plasmids (6), and subcloned into SØ606 cells. The sequences of the PCR-amplified DNAs were confirmed with ^{35}S-labeled dATP by the dideoxy chain termination method as described previously (4). In addition, two other constructs of the pBAce expression plasmid containing cDNAs encoding the schistosomal (6) or tritrichomonal (3) HPRTs were subcloned into SØ606 cells. Recombinant HPRT expression was induced in low-phosphate medium (30) for 20 h, and whole-cell extracts were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) as described previously (6). Bacteria transformed with pBAce plasmids without cDNA inserts were used as negative controls.

Soluble enzyme was extracted and partially purified from the bacteria for all four expression constructs as described previously (6, 30). The amino-terminal sequences for amino acids in the recombinant tritrichomonal (3), schistosomal (6), and human HPRTs have been determined and are consistent with those reported for the predicted open reading frame of *Tritrichomonas foetus* (3) or the amino-terminal sequences of the native human and schistosomal enzymes (29, 30), with the exception that the amino-terminal alanine for the recombinant human enzyme is unmodified (data not shown). Also, the most prominent protein bands from the bacteria expressing the four recombinant enzymes (see Fig. 1) possess electrophoretic mobilities that are consistent with the predicted molecular weights of their subunits (3, 4, 11, 13).

The specific activities of all four recombinant enzymes were determined by using the assay conditions described previously (30). One advantage of using the SØ606 strain of *E. coli* as a host for the expression of recombinant HPRTs is that this strain is totally deficient in its ability to salvage detectable levels of guanine, hypoxanthine, and xanthine (10). Thus, in the absence of detectable host activities, the specific activities of all four recombinant enzymes (with guanine, hypoxanthine, and xanthine as substrates) can be easily estimated. For these studies, the specific activity of enzyme that was ≥95% pure was determined. The specific activity of the recombinant malarial enzyme was determined to be approximately 10-fold higher than the value reported for the partially purified native enzyme (18). The specific activities of the other three recombinant enzymes were essentially identical to the activities reported for the enzymes purified from their native sources (3, 17, 30).

Bacterial plating and complementation. Plasmids identical to those used for protein expression were transformed into *E. coli* SØ609 (10). These cells were grown from frozen stocks in nutrient medium (LB or NZCYM) to an optical density at 600 nm of 1.0. The cells were pelleted and washed three times with semidefined medium and were finally resuspended at a density of 10^{10} cells per ml. The semidefined medium was modified from that described previously (6, 30) and contained 20 μg of guanine per ml, 100 μg of ampicillin per ml, 25 μg of streptomycin per ml, and 150 μM P_i. The washed cells were plated in molten top agarose onto agar plates containing the semidefined medium at a density of 5×10^9 cells per plate using standard disposable plastic petri dishes (100 by 15 mm). The plates were incubated at 37°C for 18 to 20 h to allow for the growth of lawns of bacteria whose genetic deficiencies were complemented by the expression of the recombinant enzymes.

Comparative screening assay. Fifty-nine purine analogs were tested for their abilities to inhibit or alter the growth of the SØ609 bacteria complemented by expression of the recombinant HPRTs. The compounds were dissolved in dimethyl sulfoxide (at 1 mg/ml), and 13-μl aliquots were adsorbed onto sterile antibiotic testing disks (Difco Laboratories, Detroit, Mich.). The disks were distributed on preseeded lawns of either control bacteria (transformed with the pBAce plasmid and grown on nutrient agar plates) or those complemented by the human, malarial, schistosomal, or tritrichomonal HPRTs on plates containing semidefined medium. The plates were incubated at 37°C for 18 to 20 h to allow for the growth of the bacterial lawns. Dimethyl sulfoxide alone had no effect on the growth of the bacterial lawns. Zones where the growth of the lawns was inhibited or affected were observed, and the widths of the zones (starting from the edge of the disk) were measured and tabulated (see Table 2) in order to compare the results for bacteria complemented by each of the recombinant enzymes.

RESULTS AND DISCUSSION

Figure 1 shows a Coomassie-stained SDS-polyacrylamide gel demonstrating that each of the recombinant HPRTs is induced by phosphate starvation and is expressed in SØ606 bacteria at comparable mass levels when the pBAce expression plasmid is used. The pBAce plasmid uses the *phoA* promoter to regulate expression of the recombinant HPRTs when phosphate is depleted from the medium. This plasmid has previously been demonstrated to work well as a vector for expressing HPRT in *E. coli* (to 60% of the total soluble protein [5, 6]).

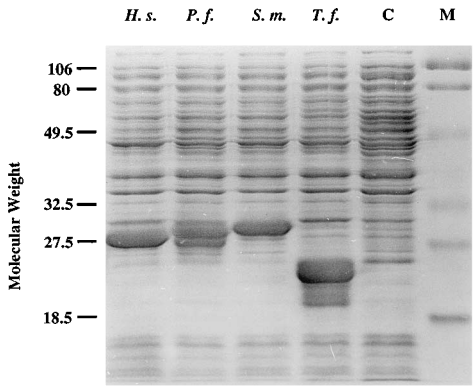


FIG. 1. Expression of recombinant HPRTs in *E. coli* SØ606. cDNAs encoding the recombinant HPRTs were ligated into pBAce plasmids (6) and were cloned into a *recA* mutant derivative of strain SØ606. Expression of the cDNAs was induced in low-phosphate medium, and total protein from cell lysates was analyzed by Coomassie-stained SDS-PAGE. The recombinant HPRTs appear as prominent bands with electrophoretic mobilities between the 18.5- and 27.5-kDa molecular size markers. The labels above the lanes indicate that the protein extract is from bacteria transformed with cDNA encoding HPRT from *H. sapiens* (*H. s.*), *P. falciparum* (*P. f.*), *Schistosoma mansoni* (*S. m.*), *T. foetus* (*T. f.*), or bacteria transformed with control plasmids (C). The sizes of the molecular mass standards (M; in kilodaltons) are shown to the left of the gel.

For the complementation experiments, the expression constructs were transformed into *E. coli* SØ609. This bacterial strain is deficient in its ability to synthesize purine nucleotides de novo and in its ability to convert hypoxanthine, guanine, or xanthine to the corresponding nucleotide (10). The *phoA* promoter of the pBAce plasmid has been reported to be "leaky," with expression levels for alkaline phosphatase during phosphate starvation being 430- to 1,000-fold higher than the levels in the presence of excess phosphate (28). Thus, during early growth of the recombinant bacteria on selective medium, before phosphate levels become limiting, the levels of HPRT expression are predicted to be in the range of 0.005 to 0.01% of the total protein level (1/1,000 to 1/430 × 60%). The level of recombinant enzyme resulting from this leaky expression was adequate to complement the genetic deficiencies of *E. coli* SØ609 cells and enabled their growth as low-density confluent lawns on the semidefined medium supplemented with guanine as the sole purine source (Fig. 2). However, the use of higher as well as lower concentrations of P_i in the semidefined medium resulted in bacterial lawns with reduced densities.

As a negative control, SØ609 cells were transformed with the pBAce plasmid without an HPRT-encoding cDNA. The isolated colonies apparent on these control plates (Fig. 2C) probably result from reverse mutations in either the *hpt* locus

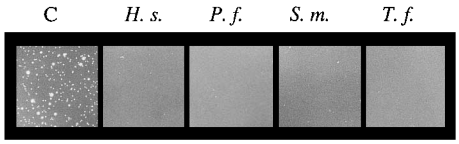


FIG. 2. Growth of *E. coli* SØ609 on semidefined medium requires complementation by a recombinant HPRT. Agar plates containing semidefined medium were seeded with bacteria transformed with pBAce control plasmids (C) or those containing cDNA encoding the human (*H. sapiens* [*H. s.*]), malarial (*P. falciparum* [*P. f.*]), schistosomal (*S. mansoni* [*S. m.*]), or tritrichomonal (*T. foetus* [*T. f.*]) HPRT. The bacteria complemented by expression of recombinant HPRTs (*H. sapiens*, *P. falciparum*, *S. mansoni*, and *T. foetus*) grew as confluent lawns, whereas bacteria containing the control pBAce plasmid with no cDNA insert (C) grew as isolated colonies which likely resulted from reversion of one of the genetic deficiencies of the SØ609 bacteria.

or the *purHJ* loci of SØ609 cells, which were plated at a density of 5×10^9 cells per plate. The growth of isolated bacteria from these colonies in semidefined medium supports this hypothesis. Their occurrence does not allow the complement selection assays to be conducted in liquid medium. However, on solid medium the isolated colonies are clearly distinct from the confluent lawns generated by the bacteria complemented with a recombinant HPRT (Fig. 2). Previously, expression of the malarial HPRT, under control of the bacteriophage lambda P_L and P_R promoters, has been used to complement genetic deficiencies of bacteria (23).

An important aspect in screening compounds for leads that show specificity for HPRTs has been access to an assortment of potential substrate analogs. In these screens, 59 purine analogs were tested. The screens were performed by adsorbing dissolved compounds onto sterile blank disks that were then distributed onto preseeded lawns of bacteria which are able to grow only if there is expression of enzymatically active recombinant human, malarial, schistosomal, or tritrichomonal HPRT.

Since all of the tested compounds are potential substrate analogs, many were expected to be competitive inhibitors of the recombinant HPRTs. The majority of the compounds tested (Table 1) had little or no effect on the growth of the bacterial lawns. For example, caffeine, a relatively inexpensive and abundant analog of xanthine, showed no activity against the recombinant HPRTs at the concentration tested. However, several compounds generated zones of inhibited or altered bacterial growth around the impregnated disks (Table 2 and Fig. 3).

Inhibition of bacterial growth could result either from direct inhibition of the recombinant HPRT or from the toxicities of the substrate analogs that are salvaged by the recombinant enzyme and incorporated into the nucleotide pools of the bacteria. However, for both possibilities the compound must interact directly with the recombinant HPRT as either an inhibitor or a substrate.

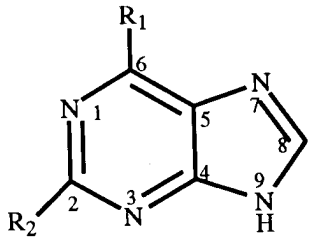
In a few cases (Table 2) the radii of the zones of inhibition varied depending on whether the bacteria expressed the human, malarial, schistosomal, or tritrichomonal HPRT. For compounds judged to selectively target the enzyme of a parasite, the zone of altered growth around a disk was significantly greater for the bacteria complemented by a parasite HPRT than for bacteria complemented by the human HPRT. For controls, bacteria containing negative control pBAce plasmids were grown on nonselective nutrient medium to assay for bactericidal effects unrelated to the HPRT salvage pathway (Table 2). These effects were nominal in comparison with the results for compounds yielding positive results.

Bacteria expressing the human, schistosomal, and tritrichomonal HPRTs seemed to be similarly and strongly affected by 2-amino-6-chloropurine (Table 2 and Fig. 3, compound 39). However, the schistosomal HPRT seems to be more selectively affected by 6-(bromomethyl)purine (compound 44), and the tritrichomonal enzyme is slightly more affected by 2-amino-6-cyanopurine (compound 48; Table 2). Also, several compounds were judged to selectively target the malarial HPRT (Fig. 3 and Table 2). Among these compounds, the analog 6-mercaptopurine-3*N*-oxide (compound 56) was the most selective compound for the malarial HPRT.

For some compounds, zones of enhanced growth were seen around the disks, sometimes in addition to the zones of inhibition (Table 2). These zones of enhanced growth were similar to the zones observed when natural substrates (hypoxanthine, guanine, or xanthine) were tested (Fig. 3 and Table 2). In some cases the additional zones of enhanced growth might result from contamination of the analogs with guanine or hypoxanthine, from which they were synthesized. Even slight contamination could increase the level of available purines that can be salvaged and could allow for enhanced growth of the bacteria. In this regard, the majority of the compounds tested in the screening assay are not available commercially, and many were synthesized a number of years ago. However, they are of the highest purity available.

Several of the compounds that could be potential leads for drugs targeted to the malarial HPRT possess a sulfur-containing moiety at the 6 position of the purine ring (Table 2). This observation is consistent with the conclusion that the 6 position

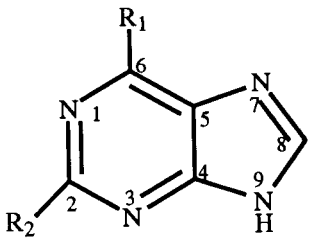
TABLE 1. Compounds with no effect on bacterial growth



Compound no.	Compound substitution		
	R_1	R_2	X[a]
1	—OH	—H	1-Deaza
2	—OH	—H	3-Deaza
3	—OH	—NH_2	7-Deaza
4	—OH	—H	7-Deaza
5	—OH	—NH_2	7-Deaza, 8-Aza
6	—OH	—H	8-Aza
7	—OH	—NH_2	8-Aza
8	—OH	—CH_3	
9	—OH	—H	8-CH_3
10	—OH	—NH_2	8-CH_3
11	—OH	—H	8-SH
12	—OH	—NH_2	8-SH
13	—OH	—H	8-C_6H_5
14	—OH	—NH_2	8-C_6H_5
15	—OH	—H	8-OH
16	—OH	—NH_2	8-OH
17	—OC_6H_5	—H	
18	—$OCH_2C_6H_5$	—H	
19	—$O(CH_2)_2C_6H_5$	—H	
20	—OH	—$NHCH_3$	
21	—OH	—$NHCH_2CH_3$	
22	—OH	—NHC_6H_5	
23	—OCH_3	2-Cl	
24	—OCH_3	2-CF_3	
25	—CH_3	—NH_2	
26	—CO_2	—H	
27	—$CSNH_2$	—H	
28	—$CSNH_2$	—NH_2	
29	—$CONH_2$	—H	
30	—$CH_2CH_2CH_3$	—H	
31	—$C(NH_2)N$	—H	
32	—$CONHCH_2CH_3$	—H	
33	—(*p*-NO_2)—C_6H_5	—H	
34	—CH═NO	—H	
35	—OCH_3	—H	
36	Azathioprine	—NH_2[b]	
37	Caffeine[c]		

[a] X, other substitutions.
[b] 2-Amino-6-[(1-methyl-4-nitroimidazol-5-yl)-thio]purine.
[c] 1,3,7-Trimethyl-2,6-dioxopurine.

TABLE 2. Inhibition or enhancement of bacterial growth



Compound	Compound substitution			Zone of inhibition[a]				
	R_1	R_2	X[b]	Control	Human	Malarial	Schistosomal	*T. foetus*
H	—OH	—H		−	†	†	†	†
G	—OH	—NH_2		−	†	†	†	†
X	—OH	—OH		−	†	†	†	†
38	—Cl	—H		−	+	−	−	+
39	—Cl	—NH_2		+	+++†	−	+++†	+++†
40	—I	—H		+	+	+	−	+†
41	—I	—NH_2		†	++†	†	++†	+++†
42	—Br	—H		+	†	†	†	†
43	—CH_2Cl	—H		+†	++	†	++†	+†
44	—CH_2Br	—H		+†	+	++	+++	++
45	—OH	—NH_2	8-NH_2	−	†	−	†	†
46	—$SOCH_3$	—H		−	+†	−	+	+
47	—CN	—H		−	−	†	−	+
48	—CN	—NH_2		−	†	++	+	+++
49	—SCN	—H		+†	++	++†	−	++
50	—SCN	—NH_2		+†	++	++†	++†	−
51	—SH	—H		+	++†	+++†	−	++†
52	—SH	—NH_2		+	+++	+++†	†	−
53	—SCH_3	—H		+	++	+++†	+++	−
54	—SCH_3	—NH_2		−	−	+†	−	+
55	Azathioprine[c]			−	−	+†	+	−
56	—SH	—H	N_3-oxide	+	+	+++†	†	+†
57	—OH	—H	9-Deaza, 8-aza[d]	+	+	+†	+†	++
58	—OH	—H	7-Deaza, 8-aza[e]	+	+†	−	+	++
59	—OCH_3	—NH_2		−	−	−	−	+

[a] +, zones of inhibition with radii that average 0 to 1 mm; ++, zones of 2 to 4 mm; +++, zones of >4 mm; †, a zone of enhanced growth; −, no observed effect on bacterial growth.
[b] X, other substitutions.
[c] 6-[(1-Methyl-4-nitroimidazol-5-yl)-thio]purine.
[d] 7-Hydroxypyrazolo(4,3-*d*)pyrimidine.
[e] 4-Hydroxypyrazolo(3,4-*d*)pyrimidine.

of purine bases plays a major role in substrate specificity for the HPRT of a related malarial parasite, *Plasmodium lophurae* (20). Furthermore, two of the compounds identified in the recombinant screens as selective for the malarial HPRT (6-mercaptopurine [compound 51] and 6-thioguanine [compound 52] had previously been identified as potent competitive inhibitors of the malarial enzyme (19). Previous kinetic analyses of these compounds as inhibitors showed that the K_is (at 0.42 μM for thioguanine and 0.55 μM for 6-mercaptopurine) were about 1 order of magnitude lower than the reported K_is (at 2.4 μM for thioguanine and 3.9 μM for 6-mercaptopurine) of the corresponding enzyme from human erythrocytes (14, 19). The previous studies of these two compounds permit them to be used as standards for this recombinant screening method. In this regard, the observed effects of these compounds on bacteria expressing the malarial or human HPRT are consistent with the kinetic data reported in the literature.

In addition to the enzymological data, an independent screen tested the effects of 52 of the purine analogs for their in vitro activities (at 10 μg/ml) against *P. falciparum* in cultures of infected human erythrocytes (16). Although conclusions for that study await the results of tests of the cytotoxicities of the compounds against the human host cells, the preliminary data correlate well with the results of our bacterial screening study. Of the 10 most active compounds for inhibiting the growth of *P. falciparum* (yielding >79% inhibition), five (compounds 49, 50, 55, 56, and 57) were shown to inhibit the growth of bacteria expressing the malarial HPRT. Of seven additional compounds that we would predict to have at least some antimalarial activity (compounds 40, 44, 48, and 51 to 54) on the basis of their effects on the growth of bacteria expressing the recombinant *P. falciparum* HPRT, four (compounds 40, 48, 53, and 54) were included in the antimalarial screens (16) and were found to inhibit the growth of *P. falciparum* (in the range of 25 to 36%). Of the remaining three compounds, 6-mercaptopurine (compound 51) and 6-thioguanine (compound 52) were previously tested for their antimalarial activities in a separate study (19) and were reported to have 50% inhibitory doses for *P. falciparum* of approximately 6.2 and 18 μM, respectively. With the exception of untested compound 44, all of the compounds identified by the recombinant screens as inhibiting the malarial HPRT have been shown to inhibit the growth of *P. falciparum*

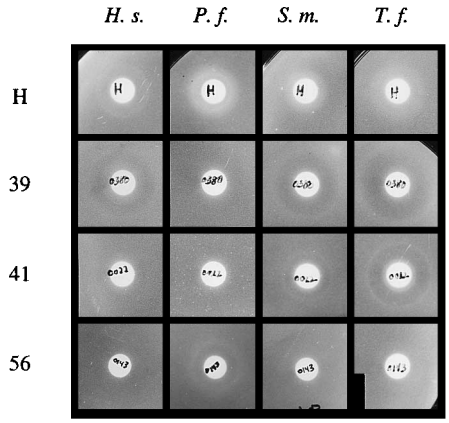


FIG. 3. Selective inhibition or alteration of bacterial growth by purine analogs targeted to recombinant HPRTs. The results from tests of three purine analogs (compounds 39, 41, and 56) for their abilities to inhibit or alter the growth of the recombinant bacteria are shown. Also shown are the effects of hypoxanthine (H) on the growth of the bacteria complemented by recombinant HPRTs. The data for the complete set of 59 compounds appear in Tables 1 and 2. Compounds were adsorbed to sterile antibiotic testing disks (13 μg in dimethyl sulfoxide) and were distributed onto preseeded lawns of bacteria complemented by the human, malarial, schistosomal, or tritrichomonal HPRTs on semidefined medium. Each column is labeled according to the source of the HPRT that complements the genotype of the host, resulting in a confluent lawn of bacteria (*H. sapiens* [*H. s.*], *P. falciparum* [*P. f.*], *S. mansoni* [*S. m.*], *T. foetus* [*T. f.*]). The code numbers provided for the compounds shown to the left of the photos are identified in Table 2. The testing disks appear as white circles in the center of each panel. The numbers inscribed on each disk are codes used only for experimental purposes and are unrelated to those used in Table 2 for identification.

or host cells in vitro. Thus, the results from independent tests of the purine analogs for their toxicities against malarial parasites are consistent with the results from the bacterial complement selection screening assays.

Comparative complement selection with bacteria complemented by the activity of a recombinant enzyme identifies compounds that are either selective inhibitors or substrates of recombinant HPRTs. These compounds are likely to be good leads for the initiation of enzyme structure-based drug design. Structural analysis of the enzymes cocrystallized with the leads should provide information useful for the redesign of the lead compounds. In support of this effort, the high levels of expression of recombinant HPRTs have already enabled the purification of large quantities of these enzymes and the initiation of X-ray crystallographic analyses (5, 9).

Recently, the structure of the human HPRT with bound GMP was solved to a resolution of 2.5 Å (7). This structure enables identification of the amino acids that participate in the binding of GMP to the human HPRT. Initial inspection of possible differences in the amino acids within the substrate-binding site of the human and parasite HPRTs provides no obvious explanations for the natural differences in substrate specificity or in the observed selectivity of the lead compounds identified by our recombinant screening method. However, the availability of the three-dimensional coordinates for the human HPRT will facilitate solution of the three-dimensional structures for the enzymes of the parasites. These three-dimensional structures, plus the structures of the enzymes cocrystallized with the lead compounds identified by the screening method reported herein, should provide details of the atomic interactions needed for compound design and the iterative process of enzyme structure-based drug design (1, 8). Subsequent improvements to the initial lead compounds may further enhance their selectivities for the parasite enzymes and may result in the development of new drugs for the treatment of parasitic diseases.

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