Characterization of Potential Drug Targets Farnesyl Diphosphate Synthase and Geranylgeranyl Diphosphate Synthase in Schistosoma mansoni

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Schistosomiasis affects over 200 million people worldwide, with over 200,000 deaths annually. Currently, praziquantel is the only drug available against schistosomiasis. We report here that Schistosoma mansoni farnesyl diphosphate synthase (SmFPPS) and geranylgeranyl diphosphate synthase (SmGGPPS) are potential drug targets for the treatment of schistosomiasis. We expressed active, recombinant SmFPPS and SmGGPPS for subsequent kinetic characterization and testing against a variety of bisphosphonate inhibitors. Recombinant SmFPPS was found to be a soluble 44.2-kDa protein, while SmGGPPS was a soluble 38.3-kDa protein. Characterization of the substrate utilization of the two enzymes indicates that they have overlapping substrate specificities. Against SmFPPS, several bisphosphonates had 50% inhibitory concentrations (IC50s) in the low micromolar to nanomolar range; these inhibitors had significantly less activity against SmGGPPS. Several lipophilic bisphosphonates were active against ex vivo adult worms, with worm death occurring over 4 to 6 days. These results indicate that FPPS and GGPPS could be of interest in the context of the emerging resistance to praziquantel in schistosomiasis therapy.

Human schistosomiasis, also known as bilharzia, is a disease caused by trematodes of three main species: Schistosoma mansoni, S. haematobium, and S. japonicum. Some 230 million individuals are infected, with 800 million at risk, and there are more than 200,000 deaths annually (1, 2). The only current treatment for schistosomiasis is praziquantel. Laboratory strains and clinical isolates of the parasite with resistance to praziquantel have been identified (3). No other drugs are currently available for the treatment of the disease, and no new drug has entered clinical trials for schistosomiasis treatment in more than 30 years (4). Moreover, no vaccines have been developed, and progress is very slow (5). There is therefore a need to develop alternative drugs for schistosomiasis treatment.

One potential target for schistosomiasis drug development is isoprenoid biosynthesis, which is involved in the synthesis of dolichols, quinones, and the isoprenoid diphosphates used in posttranslational prenylation of proteins (with FPP and GGPP), as well as in nonsterol isoprenoid synthesis (16, 17). For S. mansoni, work from different groups has shown that schistosome Ras is farnesylated (18), and Rab and Rho orthologs are geranylgeranylated (19, 20).

The mevalonate pathway thus serves as the main precursor for synthesis of sterols as well as nonsterol isoprenoids (7–11), including quinones and dolichols.

It has been determined that S. mansoni does not make cholesterol (12); the gene for squalene synthase, the enzyme that catalyzes the synthesis of squalene from FPP, is not present in the S. mansoni genome (13). Consequently, it has been proposed that the parasite obtains its cholesterol from its human host (14). However, the mevalonate pathway that produces IPP and DMAPP is present in schistosomes (15), and isoprenoids derived from mevalonate have been identified in S. mansoni and are thought to be involved in posttranslational prenylation of proteins (with FPP and GGPP), as well as in nonsterol isoprenoid synthesis (16, 17). For S. mansoni, work from different groups has shown that schistosome Ras is farnesylated (18), and Rab and Rho orthologs are geranylgeranylated (19, 20).

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media. Mevinolin also caused worm death in infected mice, but it required administration for 14 days to achieve high efficacy (6), so HMGR may not be a good target for schistosomiasis therapy.

Although the importance of the mevalonate pathway for schistosomes and its potential as a drug target have been demonstrated, there is no information available on the other enzymes in this pathway. We thus undertook a study to characterize FPPS and GGPPS in S. mansoni to determine if they might be drug targets. Bisphosphonates (Fig. 2B), which have been used to treat osteoporosis and similar diseases in millions of people (25), have been shown to be efficient FPPS (26) and, in some cases, GGPPS (27) inhibitors, and one approach for the development of drugs for neglected diseases is the repositioning of drugs currently in use (28). Bisphosphonates have also been found to be active against a variety of protozoan parasites, for example, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani (26, 29–31), Cryptosporidium parvum (32), Entamoeba histolytica (33), Toxoplasma gondii (34), and Plasmodium species (26, 33, 35–37). However, no previous investigations on FPPS or GGPPS from a parasitic helminth have been reported. Here we show that FPPS and GGPPS from S. mansoni share similarities with orthologs from other species; both schistosome enzymes are inhibited by bisphosphonates, although to differing degrees. Investigations on the substrate specificities of FPPS and GGPPS indicate that their activities are redundant, indicating that inhibition of both enzymes will be necessary for effective worm killing. Pyridinium group-containing lipophilic bisphosphonates tested against cultured ex vivo adult worms were able to kill worms, providing evidence that SmFPPS and SmGGPPS are essential for worm survival and that they are druggable targets.

**MATERIALS AND METHODS**

**Materials.** Radioactive ^3^H-IPP was obtained from American Radiolabeled Chemicals (St. Louis, MO). Escherichia coli BL21 Star(DE3) cells were obtained from Invitrogen (Carlsbad, CA). Isopropyl-β-D-1-thiogalactopyranoside (IPTG) was obtained from Gold Biotechnology (St. Louis, MO). RPMI 1640 medium was obtained from Sigma-Aldrich (St. Louis, MO).

**Clones and plasmids.** Total RNA was extracted from S. mansoni eggs isolated from mouse livers (38) by using TriReagent (Sigma-Aldrich, St. Louis, MO) following the manufacturer’s instructions. First-strand cDNA synthesis was performed on 1 µg total RNA, using SuperScript II (Stratagene, La Jolla, CA). PCR amplification of FPPS and GGPPS was accomplished using Pfu or Vent DNA polymerase and the following gene-specific primers (IDT, Coralville, IA) designed using sequences identified in the S. mansoni genome and expressed sequence tag (EST) databases after queries with known FPPS and GGPPS sequences: SmFPPS forward, 5'-caccATGCCAACCTCTGATATACATA; SmFPPS reverse, 5'-TTAACAGCACGTGGAAAAAGTAATTC; SmGGPPS forward, 5'-TTAACAGCACGTGGAAAAAGTAATTC; SmGGPPS reverse, 5'-TTATCTCTCATATTAGAAACAGG. The PCR products were cloned into pET100 (Invitrogen, Carlsbad, CA), transformed into E. coli BL21 Star(DE3) cells, plated on LB containing 50 µg/ml ampicillin, and cultured overnight at 37°C to select for ampicillin-resistant clones. The clones were confirmed by DNA sequencing.

**Recombinant protein expression and purification.** A single colony was used to inoculate 1 ml liquid broth containing 50 µg/ml ampicillin at 37°C overnight. The overnight culture was transferred to a 500-ml culture of LB containing 50 µg/ml ampicillin at 37°C and shaken at 200 rpm to an optical density at 600 nm (OD_{600}) of 0.5. Recombinant protein expression was induced in the culture with 1 mM IPTG, and the culture was incubated for a further 3 h. The cells were harvested by centrifugation (Sorvall Evolution RC) at 9,000 rpm for 15 min. Protein was obtained by lysing cells by freeze-thaw cycles followed by sonication (Branson digital sonifier) for 6 min. Sonicated cells were centri-
fuged again at 23,000 rpm for 30 min. The recombinant proteins were purified from the supernatant by nickel-affinity chromatography (GE Healthcare) using 10 mM phosphate buffer, pH 7.4, containing 10 mM imidazole, and were eluted with increasing concentrations of imidazole (100 mM, 300 mM, and 500 mM) in 10 mM phosphate buffer, pH 7.4. The purified protein was run in a 16% SDS-polyacrylamide gel to ascertain its size and purity, after which glycerol stocks of BL21 Star(DE3) containing the correct gene insert of each enzyme were made and stored at −80°C for future use.

**Radiometric assays for SmFPPS and SmGGPPS.** The FPPS enzyme assays were performed essentially as described previously (31), using 14C-labeled IPP with DMAPP, GPP, and FPP. One hundred microliters of assay buffer containing 10 mM HEPES, pH 7.4, 5 mM MgCl2, 2 mM dithiothreitol, 47 μM [4-14C]IPP (55 mCi/mmol), and 55 μM allylic diphosphate was prewarmed to 37°C. The assay was initiated by addition of 20 ng of recombinant SmFPPS and allowed to proceed for 30 min. The reaction was terminated by addition of 10 μl of 6 M HCl, and the mixture was made alkaline by addition of 15 μl of 6 M NaOH diluted with 0.7 ml of water and vortexed with 1 ml of hexane to separate the labeled alcohol products. The hexane solution was washed with water and transferred to a scintillation vial for counting. One unit of enzyme activity is defined as the activity required to incorporate 1 nmol of [4-14C]IPP into the 14C-labeled product in 1 min.

The GGPPS assays were performed as described previously (39, 40), using 14C-labeled IPP with DMAPP, GPP, and FPP. The 20-μl assay setup consisted of 25 mM HEPES, 2 mM MgCl2, 0.5% n-octyl-β-glycopyranoside, 10 μM [4-14C]IPP (55 mCi/mmol), and 10 μM allylic diphosphate. The reactions were initiated by addition of 200 ng of recombinant SmGGPPS and were allowed to proceed for 15 min at 37°C. The reactions were stopped by the addition of 2 ml of n-butanol saturated with water, followed by 1 ml of 6 M KCl, and mixtures were then vortexed for 1 min to separate the less polar products (FPP and GGPP) from the more polar, 14C-labeled substrate. The butanol phase was transferred to a scintillation vial for counting. One unit of enzyme activity was defined as the amount of protein needed to incorporate 1 nmol of [4-14C]IPP into the 14C-labeled product in 1 min.

**FIG 2** (A) Structures of substrates and products of SmFPPS and SmGGPPS catalysis. DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate. (B) Structures of bisphosphonate compounds used in this study.
TABLE 1 Comparison of SmFPPS and SmGGPPS with orthologs from other species

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* Schistosoma mansoni FPPS, accession no. CCD78373; and S. mansoni GGPPS, accession no. XP_002573680.

Kinetic analysis of $K_m$ and $V_{max}$ The Michaelis-Menten constant ($K_m$) and the maximum velocity ($V_{max}$) of SmFPPS were determined at an IPP concentration of 47 μM, with concentrations of DMAPP, GPP, and FPP varying from 0 μM to 100 μM, or with IPP from 0 μM to 100 μM while keeping the concentrations of DMAPP, GPP, and FPP constant at 55 μM. For SmGGPPS, IPP or DMAPP/GPP/FPP was maintained at 10 μM, while the second substrate was varied from 0 μM to 50 μM. The concentration ranges used to determine kinetic constants were determined in three independent preliminary experiments using a wide range of concentrations of IPP and the corresponding allylic pyrophosphate (1 μM to 100 μM). $K_m$ and $V_{max}$ for all substrate and enzyme combinations were determined by plotting velocity versus substrate concentration, using Sigmaplot (Systat Software Inc., Chicago, IL).

Inhibition of SmFPPS and SmGGPPS by bisphosphonates. Depending on solubility, bisphosphonates were typically prepared as 500 μM stock solutions in dimethyl sulfoxide (DMSO) or an aqueous solution of sodium bicarbonate (pH of ~8 to 10). Gradients of bisphosphonate concentrations (100 μM to 5 mM) were prepared by serial dilution. Inhibitors and 200 ng of enzyme were incubated at room temperature for 10 min in the assay buffer (10 mM HEPES, 150 mM NaCl, 1 mM MgCl$_2$, pH 7.5) before adding GPP and $^3$H-IPP to a 50 μM final concentration. The reactions were allowed to proceed for 20 min in a water bath at 37°C and then terminated by addition of 3 M HCl in methanol. The reaction mixtures were neutralized by NaOH-hexane, and the organic phases were collected for radioactive counting. If significant inhibition (>80%) was seen with 50 μM inhibitor, the inhibitor concentration was titrated to determine the 50% inhibitory concentration (IC$_{50}$), using Prism 5 (GraphPad Software, Inc., La Jolla, CA).

Activity of bisphosphonates against adult *S. mansoni* worms. Experiments were performed as described previously (41). Infection of mice (NIH Swiss; National Cancer Institute, Rockville, MD) with *S. mansoni* cercariae (NMRI strain) obtained from infected * Biomphalaria glabrata* snails and perfusion of adult worms (6 to 7 weeks) from mice were carried out as described previously (38). Adult worms were obtained by perfusing the portal veins of mice at 45 to 50 days postinfection, using Dulbecco’s modified Eagle’s medium (DMEM). Live worms were washed thoroughly with DMEM and incubated in 5 ml RPMI medium (Sigma-Aldrich) supplemented with 100 units/ml penicillin, 50 μg/ml streptomycin sulfate, 2 mM l-glutamine, and 10% fetal calf serum (Invitrogen). Bisphosphonates (50 μM) were added to each well, and the cultures were replenished every day with fresh medium and inhibitors. Worms were checked and counted every day for worm pairing, motility, and the presence of live and dead worms. Dead worms were identified as those worms that showed zero motility for several minutes. This study was approved by the Institutional Animal Care and Use Committee at Rush University Medical Center (IACUC number 08-058; DHHS animal welfare assurance number A3120-01).

RESULTS
Cloning and analysis of *S. mansoni* FPPS and GGPPS. Nucleotide sequences with high similarities to FPPS and GGPPS genes from other organisms were amplified from adult worm cDNA, cloned, and sequenced. The sequences obtained were identical to accessions currently in GenBank, accession number CCD78373 for FPPS and accession number XP_002573680 for GGPPS. Multiple-sequence alignments of FPPS and GGPPS were generated using CLUSTAL W 2.0.12 in order to compare the schistosome proteins to orthologs present in other species. The results indicate that the schistosome proteins share the active site motifs found in orthologous proteins (Table 1; see Fig. S1 in the supplemental material). The first aspartate-rich motif (FARM) and second aspartate-rich motif (SARM), which have been demonstrated to be the active sites in avian FPPS (42), are present in SmFPPS and SmGGPPS. In addition to these conserved motifs, SmFPPS and SmGGPPS share significant overall similarities and identities with other FPPS and GGPPS proteins (Fig. 3). For example, SmFPPS has 35% identity to human FPPS and 34% identity to *Drosophila melanogaster* FPPS, while SmGGPPS has 53% identity to human GGPPS and 46% identity to *D. melanogaster* GGPPS. Note that the amino acids proximal to the FARM are involved in determining and limiting the length of the allylic diphosphates able to bind in the active site (Fig. 3). In type I and type III FPPS, there are two highly conserved DDDXXD (FARM and SARM) repeats, whereas in type II FPPS, the FARM is DDDXXDD (Fig. 3) (43). Type I and type II FPPS primarily produce FPP, but the type III FPPS, having an aromatic amino acid (Y or F) at the fourth position and a small residue (G, A, or C) at the fifth position upstream from the FARM, is bifunctional, producing both FPP and GGPP (44). This motif in SmFPPS is similar to that seen with the type III FPPS proteins, with Gly-Phe present in the chain-length-determining region. Like other GGPPS enzymes, SmGGPPS has neither the aromatic amino acids at the fourth and/or fifth position before the FARM found in FPPS enzymes nor the two amino acid insertions that occur in FPPS and GGPPS from plants and bacteria. Instead, product chain length is determined by a bulky residue two amino acids.

*FIG 3* The most conserved motifs, i.e., the first aspartate-rich motif (FARM) and the second aspartate-rich motif (SARM), in the three different types of FPPS and GGPPS enzymes from various species. Residues that form a “lid,” effectively limiting binding to shorter allylic diphosphates, are shown with arrows. *Sae*, *Saccharomyces cerevisiae*; *Hs*, human; *Es*, *Escherichia coli*; *Bs*, *Bacillus stea-nothermophilus*; *Tg*, *Toxoplasma gondii*; *Pv*, *Plasmodium vivax*; *Sm*, *Schistosoma mansoni*; *Su*, *Sulfobolus acidocaldarius*; *Mt*, *Methanobacterium thermoautotrophicum*; *Pa*, *Pantoea ananatis*; *At*, *Arabidopsis thaliana*. 

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acids before a conserved Gly-Gln (GQ) motif (45); this motif is conserved in SmGGPPS (see Fig. S1B).

**Recombinant protein expression and purification.** SmFPFS and SmGGPPS were expressed in *E. coli* BL21 Star(DE3) cells, generating 6-His-tagged fusion proteins. The supernatants obtained from cell lysates were purified through a nickel column, and elution fractions were analyzed in a 16% SDS gel. SmFPFS eluted in the 300 mM imidazole fractions and had a molecular mass of ~44.2 kDa (see Fig. S2 in the supplemental material), while recombinant SmGGPPS eluted in the 300 mM and 500 mM imidazole fractions and had a molecular mass of ~38.3 kDa (see Fig. S2).

**Kinetic analysis of recombinant *S. mansoni* FPFS and GGPPS.** Recombinant SmFPFS and SmGGPPS proteins were analyzed for activity without removal of the 6-His tag. Both proteins were tested in reactions with 3H-IPP and allylic diphosphates (DMAPP, GPP, or FPP). Both proteins were active with all three allylic diphosphates. The *Vₘₜₐₓ* values for SmFPFS eluted from 1,077 units/mg protein with DMAPP plus IPP to 22 units/mg protein with FPP plus IPP (Table 2). The *Kₘ* values for all substrates was ~17 μM (Table 2). The *Vₘₜₐₓ* values for SmGGPPS ranged from 67 to 371 units/mg for the different substrates, while the *Kₘ* values ranged from 2.5 to 7 μM (Table 2).

**Inhibition of *S. mansoni* FPFS and GGPPS by bisphosphonates.** Ten bisphosphonates (Fig. 2B) were tested against recombinant SmFPFS and SmGGPPS. The IC₅₀ values of each inhibitor were determined by plotting curves of inhibitor concentration versus percent enzyme inhibition. IC₅₀ values with SmFPFS ranged from 0.04 μM (compounds 703 and 811) to 10 μM (compound 749), while those for SmGGPPS ranged from 2.1 μM (compound 749) to 35.5 μM (compound 811) (Table 3; Fig. 4A and B).

**Effects of inhibitors on *S. mansoni* worms cultured in vitro.** To determine the effects of bisphosphonates on *S. mansoni* worms, *ex vivo* adult worms, in 10 pairs per treatment, were cultured in the presence of a bisphosphonate at 50 μM and monitored visually for 7 days. The worm culture was replenished daily with fresh medium and 50 μM inhibitor. Of the 10 bisphosphonates tested (Table 3), only compound 715 was active, with worm death beginning after 4 days of exposure and with all worms dead on day 7 (Fig. 4C). Ten other lipophilic bisphosphonates with pyridinium ring structures, which were analogs of compound 715, were then tested on adult worms, with compounds 746, 766, 777, and 820 (Fig. 4D) resulting in worm death beginning after 4 days. Compounds affected male and female worms equally.

**DISCUSSION**

Schistosomiasis is a disease that affects approximately 200 million people worldwide, with more than 200,000 deaths annually (1). There is only one drug, praziquantel, available for treatment. In order to identify new schistosome targets for drug development, we characterized two enzymes of the mevalonate pathway. These studies were justified by previous studies with statins, inhibitors of HMGR, which result in both reductions in parasite egg production and increased worm death (6). FPFS and GGPPS are downstream of HMGR in the isoprenoid biosynthesis pathways. Their activities yield important molecules, such as dolichols, quinones, and long-chain isoprenoids involved in protein prenylation (23). Inhibition of FPFS/GGPPS enzymes is expected to reduce the amounts of these essential isoprenoid molecules and to have deleterious effects on worm metabolism. Bisphosphonates, including risedronate and zolendronate, are widely used to prevent and treat osteoporosis and have been shown to inhibit FPFS in many organisms, including humans (25) and some parasites (26). Digenerally bisphosphonates are known to inhibit human FPFS (46). No studies have been done on schistosome FPFS or GGPPS or on the activity of bisphosphonates against schistosomes. In this study, we expressed and characterized recombinant SmFPFS and SmGGPPS to determine if it might be possible to use “new-generation” lipophilic bisphosphonates for schistosomiasis treatment. We found that recombinant SmFPFS and SmGGPPS are inhibited by several bisphosphonates and that some bisphosphonates have activity against cultured, *ex vivo* adult worms, suggesting that schistosome FPFS and GGPPS may be appropriate targets for drug development for schistosomiasis.

Comparisons of SmFPFS and SmGGPPS to orthologous FPFS and GGPPS proteins indicate that they have similar conserved active site motifs. For instance, the active sites of both proteins typically contain two “aspartate-rich” motifs (40, 46). These are both present in the amino acid sequences of SmFPFS and SmGGPPS. Interestingly, from a three-dimensional structural perspective, SmFPFS is predicted to have the closest structural homology to human FPFS (based on Phyre2 [47] structure prediction), with which it has the highest (38%) sequence identity.
among the prenyltransferases whose structures have been re-
ported. Likewise, SmGGPPS is also predicted to have the closest
structural homology to human GGPPS (54% sequence identity).
The predicted structural models for SmFPPS (based on Homo
sapiens FPPS [HsFPPS]; Protein Data Bank [PDB] ID code 2F94)
and SmGGPPS (based on HsGGPPS; PDB ID code 2Q80) are
shown in Fig. S3 in the supplemental material. However, SmFPPS
and SmGGPPS do have significant sequence differences from the
human orthologs, which could be exploited for selective enzyme
inhibition and drug development.

We went on to investigate if the schistosome proteins have simi-
larity in function by investigating the substrate specificities of the
enzymes. We found that SmFPPS was active with DMAPP, GPP, and
FPP as substrates, but with substrate preferences of DMAPP >
GPP > FPP under similar reaction conditions. In previous studies
involving FPPS, the allylic substrates studied were DMAPP and GPP,
with FPPS enzymes showing a preference for DMAPP over GPP.
FPPS from Trypanosoma brucei (TbFPPS) and T. cruzi FPPS
(TcFPPS) have specific activities of 840 units/mg (31) and 325
units/mg (30), respectively, with IPP and GPP as substrates, and these
values are similar to those we determined for SmFPPS. However,
there has not been any reported activity of TbFPPS or TcFPPS with
FPP. FPPS from the rubber-producing mushroom Lactarius chrysor-
rheus has been shown to be unable to form products with FPP as the
substrate (48), and data from the Comprehensive Enzyme Informa-
tion System (http://www.brenda-enzymes.org/) indicate that only
DMAPP and GPP are substrates for FPPS enzymes from over 20
different species. However, FPPS enzymes from a number of parasitic
protozoa, including Toxoplasma gondii (44), Plasmodium vivax (49),
and Cryptosporidium parvum (50), were recently shown to be “bi-
functional” prenyl synthases that are capable of accepting DMAPP,
GPP, and FPP as substrates to produce FPP and GGPP, as well as, in
some cases, products with even longer chains. It has been proposed
that substitution of a small amino acid residue for a bulky hydropho-
bic residue in the chain-length-determining region of type III FPPS
proteins allows them to accept GPP and longer prenyl compounds as
substrates. The chain-length-determining region of SmFPPS is simi-
lar to that seen in type III FPPS, and indeed, the substrate specificity of
SmFPPS is similar as well. In addition to changes in the chain-length-
determining region, several amino acid insertions are found in type
III FPPS proteins, which may also enlarge the substrate binding
pocket to accommodate larger prenyl precursors. Similar insertions
are also found in SmFPPS (Fig. S1A in the supplemental material),
which, again, may be responsible for broadening the substrate range
of the protein and could be targeted in the development of SmFPPS-
specific inhibitors.

The inhibitory effects of bisphosphonates on recombinant
SmFPPS and SmGGPPS proteins were determined for both en-
zymes, using the commercial drugs risedronate (compound 2)
and zoledronate (compound 91), as well as a series of other species
(compound 460 and the more lipophilic bisphosphonates 703,
715, 749, 754, 782, 811, and 1186). Compounds 2 and 91 are

FIG 4 (A) IC_{50} curves for inhibitor concentration versus percent Schistosoma mansoni FPPS inhibition. (B) IC_{50} curves for inhibitor concentration versus percent S. mansoni GGPPS inhibition. (C) Survival of ex vivo adult S. mansoni worms cultured in the presence of 50 μM bisphosphonates 2, 91, and 715 as a function of time. (D) Survival of ex vivo adult S. mansoni worms cultured with 50 μM 2, 91, and 715 and with various concentrations of compounds 746, 766, 777, and 820. Worms were cultured in RPMI medium with inhibitors, with the medium and inhibitors replaced daily. Worms were observed for survival (as assessed by motility) over 6 days. The LD_{50} of the compounds for adult worms are ~25 μM.
known to inhibit FPPS. In studies with trypanosomes, compounds 2 and 91 were found to inhibit TcFPPS (30) and TcFPPS (26), with IC$_{50}$ of 37 nM and 42 nM, respectively, and human FPPS, with IC$_{50}$ in the 100 to 240 nM range (51). The IC$_{50}$ of compound 2 against SmFPPS was 0.19 µM, while compound 91 had an IC$_{50}$ of 0.14 µM against SmFPPS, which is in the same range as that against human FPPS. The lipophilic bisphosphonates were also effective SmFPPS inhibitors, with IC$_{50}$ ranging from 0.04 µM (compound 703) to 10 µM (compound 749). In general, most compounds were less active against SmGGPPS than against SmFPPS (Fig. 4A and B). An exception was compound 749, which contains two geranyl moieties. Compounds 715, 754, and 782, which are known inhibitors of GGPPS (52), had IC$_{50}$ in the micromolar range (4.1 to 9.1 µM) against SmGGPPS. Compounds 460, 811, and 1186 were the least inhibitory against SmGGPPS (23 to 35 µM).

Since these compounds had some inhibitory activity against the SmFPPS and SmGGPPS proteins, we next investigated if they had activity against cultured ex vivo adult S. mansoni worms. We initially tested all compounds listed in Table 3 for activity against adult worms. However, only compound 715, a lipophilic pyridinium bisphosphonate, had any activity (100% death at 50 µM) (Fig. 4C). The bone resorption bisphosphonate drugs risedronate (compound 2) and zolendronate (compound 91) had no activity at 50 µM (Fig. 4C). We then tested a series of compound 715 analogs (compounds 746, 766, 777, and 820) for worm-killing activity. The results are shown in Fig. 4D. Clearly, the long alkylic chain bisphosphonates have good activity, with IC$_{50}$ for worm killing of ~20 µM, while risedronate (compound 2) and zolendronate (compound 91) have no effect (Fig. 4C and D), even though they are good SmFPPS and quite good SmGGPPS inhibitors—pointing again to the need for lipophilicity in order to have worm-killing activity. It is also clear from the results described above that targeting GGPPS alone is unlikely to be a good therapeutic strategy, since FPPS can make GGPP.

Also note that compound 715 was previously reported to have activity in a mouse xenograft system, without apparent toxicity to the host (52). What is not clear at present is why some of the lipophilic bisphosphonates were inactive against worms. They have both FPPS and GGPPS activities, although given the difficulty of correlating enzyme and cell activity (36), extrapolations to animals are expected to be even more challenging. Overall, then, the results provide proof of principle that SmFPPS and SmGGPS are druggable parasite enzymes and that lipophilic bisphosphonates may serve as starting points for the development of new drugs for the treatment of schistosomiasis.

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