Alveolar echinococcosis (AE) is a disease predominantly affecting the liver, with metacestodes (larvae) of the tapeworm _Echinococcus multilocularis_ proliferating and exhibiting tumor-like infiltrative growth. For many years, chemotherapeutical treatment against alveolar echinococcosis has relied on the benzimidazoles albenzazole and mebendazole, which require long treatment durations and exhibit parasitostatic rather than parasiticidal efficacy. Although benzimidazoles have been and still are beneficial for the patients, there is clearly a demand for alternative and more efficient treatment options. Aromatic dications, more precisely a small panel of di-N-aryl-diguanidino compounds, were screened for efficacy against _E. multilocularis_ metacestodes _in vitro_. Only those with a thiophene core group were active against metacestodes, while furans were not. The most active compound, DB1127, was further investigated in terms of _in vivo_ efficacy in mice experimentally infected with _E. multilocularis_ metacestodes. This diguanidino compound was effective against AE when administered intraperitoneally but not when applied orally. Thus, thiopehe-diguanidino derivatives with improved bioavailability when administered orally could lead to treatment options against AE.

The disease alveolar echinococcosis (AE) is caused by infection with the metacestode (larval) stage of the parasitic tapeworm _Echinococcus multilocularis_. The adult tapeworm lives within the intestine of its definitive hosts, mainly foxes (_Vulpes vulpes_), as well as cats and dogs. Several genera of rodents are intermediate hosts, where asexual proliferation of the larval stage takes place (1).

AE is a zoonotic disease, and it occurs mainly in central Europe (France, Germany, Switzerland, and Western Austria), North America, and east Asia, with high fatality in humans if untreated. However, in the past 10 to 20 years, the occurrence of _E. multilocularis_ and AE has increased in northern and eastern Europe (2, 3), South Korea (4), the United States (5), Iran (6), mainland Japan (7), China (8), Kyrgyzstan (9), and Switzerland (10). It is very likely that the urbanization of foxes in many central European cities is responsible for the emergence of AE in regions where the disease was not noticed before (11).

The current strategy for treating human AE consists of surgical measures complemented by chemotherapy using benzimidazole compounds (mebendazole or albendazole). These compounds inhibit parasite proliferation, making them parasitostatic, but they do not cure the disease, meaning patients have to undergo chemotherapy for extended periods of time, often lifelong. Although benzimidazoles clearly have beneficial effects for patients in terms of life quality and survival, the current treatment results in high costs and elevated risk of adverse effects (12–17). A number of compounds have been investigated in the past as treatment alternatives for AE, using _in vitro_-cultured parasites and/or _in vivo_ rodent models. These include other benzimidazole derivatives, broad-spectrum anti-infective agents, and anticancer drugs (reviewed in references 18–20). However, none of the compounds investigated has been translated into clinical application, either due to low efficacy, high toxicity, or simply because the pharmaceutical industry has not been prepared to invest in a disease without a meaningful market return (21).

Although introduced in 1942, pentamidine is still an important broad-spectrum antimicrobial compound, with activities against trypanosomiasis, leishmaniasis, and malaria, as well as pneumocystis pneumonia caused by _Pneumocystis jiroveci_ and _Candida albicans_ infections (22). Novel diamidines and arylimidamide analogues have been proven effective against visceral leishmaniasis as well as _Trypanosoma cruzi_ and _Neospora caninum_ infections in animal models (23–25). The exact mode of action of pentamidine derivatives, such as diamidines and arylimidamides, has not been elucidated so far. These dicatonic molecules bind to the minor groove of mitochondrial and nuclear DNA at AT-rich sites and then inhibit one or more of the DNA-dependent enzymes (such as topoisomerases or nucleases) or directly impede the transcription process.

The more recent achievements in the _in vitro_ culture of _E. multilocularis_ metacestodes led to the development and validation of a drug screening assay which is based on the detection of the enzyme phosphoglucose isomerase (PGI) of _E. multilocularis_. PGI is abundant in metacestode vesicle fluid and released into the metacestode culture supernatant by dying parasites, while intact, viable metacestodes do not release this enzyme (26). Recently, we demonstrated that DB1127, a dicatonic thiophene derivative (di-N-aryl-diguanidino compound), triggered the release of high levels of PGI activity from metacestodes in a dose-dependent manner, was substantially active at concentrations as low as 1 μM, and induced structural damage upon _E. multilocularis_ metacestodes (21).
In this study, we investigated the anti-echinococcal activities of a small panel of diguanidino compounds, which are structurally related to DB1127. These included derivatives with a furan core structure and others with a sulfur substitution in the furan ring (thiophene). DB1127 was also administered to experimentally infected mice, and the administration route of the drug was proven to be crucial for its effectiveness.

**MATERIALS AND METHODS**

*In vitro culture of E. multilocularis metacestodes.* If not stated otherwise, all culture media and reagents were purchased from Gibco-BRL (Zürich, Switzerland), and biochemical reagents were from Sigma (St. Louis, MO). The culture of *E. multilocularis* (isolate H95) was carried out as previously described (27, 28). In short, metacestodes dissected from experimentally infected BALB/c mice were pressed through an autoclaved tea sieve. The metacestodes were incubated in antibiotic solutions: 20 μg/ml levofloxacin (Aventis, Meyrin, Switzerland) and 20 μg/ml ciprofloxacin (Bayer, Zürich, Switzerland) in phosphate-buffered saline (PBS) overnight. The sedimented material was washed several times with 1× PBS, and 1 ml was added to a cell culture flask containing 5 × 10^4 rat hepatoma (CRL-1600; ATCC) cells/50 ml cultivation medium (Dulbecco’s modified Eagle medium [DMEM], 10% fetal calf serum [FCS], 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate). These cocultures were incubated at 37°C and 5% CO2, with medium changes once a week. Splitting of cultures was carried out when exceeding 15 ml total metacestode volume. Metacestodes were used for experimental procedures when they reached diameters of ~4 mm.

**Compounds.** The diguanidino compounds investigated in this study were synthesized by David W. Boykin and Chad E. Stephens. Nitazoxanide was synthesized and kindly provided by Christian Leumann (Department of Chemistry, University of Berne), and albendazole was purchased from Sigma (St. Louis, MO).

*In vitro drug treatment of Echinococcus multilocularis metacestodes.* *E. multilocularis* metacestodes were collected after 1 to 2 months of culture. The screening for effective compounds against the parasite was performed as described by Stadelmann et al. (26). In short, after extensive washing with PBS and selection of intact vesicles, they were distributed in 24-well plates. Treatments were carried out in medium without phenol red (DMEM, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, 2 mM L-glutamine). The drugs were prepared as stocks of 10 mM in dithiothreitol (DMSO) and added to the wells at a final concentration of 20 μM (0.2% DMSO) for initial screening or less for studies on dose-dependent effects. After 5 days of incubation at 37°C and 5% CO2, the medium within the wells was collected and stored at −20°C. In parallel, specimens were viewed by light microscopy to assess potential drug-induced morphological damage.

**Assessment of toxicity of compounds for E. multilocularis metacestodes.** The damage induced upon vesicles incubated with selected drugs under axenic conditions was assessed indirectly by measuring the release of PGI into the medium supernatant after 5 days of *in vitro* treatment (26, 29). The assay was performed in 96-well microtitre plates (Sarstedt) under axenic conditions, i.e., in the absence of hepatoma cells. Per well, 95 μl of assay buffer (100 mM Tris–HCl [pH 7.6], 0.5 mM nicotinamide adenine dinucleotide [NAD; Fluka], 2 mM EDTA [Merck], and 1 U glucose-6-phosphate dehydrogenase) was mixed with 20 μl of each supernatant aliquot (described above). Measurements were performed in triplicates. The reaction was started by addition of fructose-6-phosphate (Fluka) to 1 mM. NAD reduction to reduced nicotinamide adenine dinucleotide (NADH) was measured by reading the A_{340} every minute from 0 to 30 min with a 96-well plate reader (2300 EnSpire multilabel reader; Perkin-Elmer, Turku, Finland). Enzyme blanks (no substrate) and substrate blanks (no enzyme) were also included. Absorbance values of the enzyme blanks were subtracted from the enzyme reaction values afterwards. The PGI activity of the untreated group was subtracted from the activity of the treated groups, as it represents the activity baseline. PGI activity was calculated from the corresponding linear regression parameters (ΔA_{340}/Δt) and is presented as a percentage relative to the values obtained by treatment of vesicles with 1% Triton X-100. Linear regression analysis was performed using Excel (2007).

**Assessment of toxicity of compounds for HFF, Vero cells, and RH cells.** Human foreskin fibroblasts (HFF) and rat hepatoma (RH) cells were seeded in a 96-well microtiter plate (Greiner Bio-One) at a cell density of 10,000 cells/well. They were cultured at 37°C and 5% CO2 in DMEM without phenol red and supplemented with 1% L-glutamine, 10% FCS, 50 U/ml penicillin G, and 50 μg/ml streptomycin for 48 h, at which time the monolayer became confluent. Monkey kidney epithelial (Vero) cells were cultured similarly, but the initial density of cells per well was 5,000. The compounds DB869, DB871, and DB1127, dissolved in DMSO, were added to provide final concentrations of 0.5, 1, 5, 10, and 20 μM. As a negative control, the cells were treated with medium, and the same amount of DMSO (final concentration, 0.2%) was present in the treated groups. As a positive control, the cells were treated with 1% Triton X-100. After incubation at 37°C and 5% CO2 for 3 days, the cell vitality was assessed by alamarBlue assay. In short, 1 μl of a 200× solution (2 g/liter) of resazurin was added to each well to a final concentration of 1×. Fluorescence at 595 nm was measured in a multilabel plate reader (2300 EnSpire multilabel reader; Perkin-Elmer, Turku, Finland) at 0 and 4 h after the addition of resazurin to the cells. The values obtained at 0 h were subtracted from the ones obtained at 4 h. The percent survival was calculated by standardizing the values to those of the untreated group (100%). A positive control was not included in the calculations. For each incubation condition, sextuplets were measured. Linear regression analysis was performed using Microsoft Excel (2007).

**In vivo studies on the efficacy of DB1127 in experimentally infected BALB/c mice.** The *in vivo* effects of DB1127 were investigated and compared to those of standard oral albendazole treatments. Two experiments were performed, one assessing the intraperitoneal (21) and one the oral (30) delivery route. DB1127 was prepared as a stock solution in DMSO and diluted 1,000 times with 1% carboxymethyl cellulose (CMC) in saline solution. The intraperitoneal dose was of 10 mg/kg of body weight and 1% DMSO (final concentration). For oral drug delivery, the drug was directly emulsified in a 1:1 mixture of honey–1% CMC (M-Budget honey; Migros, Zürich, Switzerland) to 50 mg/kg. Albendazole was also emulsified in a 1:1 mixture of honey–1% CMC to 200 mg/kg. The animals received the drugs in a final volume of 100 μl.

For experiment 1 (assessment of intraperitoneal injection of DB1127), 24 female BALB/c mice (age, 9 weeks; average body weight, 25 g) were housed in a temperature-controlled light cycle room with food and water ad libitum. Experiments were carried out according to the Swiss Animal Welfare regulations. All mice were infected intraperitoneally with 100 μl of metacestode vesicle suspension (29) and were randomly allocated into 3 groups of 8 animals each. At 6 weeks postinfection, mice were given the following daily treatments: group 1 (untreated control group) received 100 μl of the honey–CMC mixture orally; group 2 (albendazole control group) received albendazole (200 mg/kg) in 100 μl diluted honey orally; and group 3 received DB1127 (2 mg/kg in 100 μl PBS-CMC) intraperitoneally. The treatments were performed daily for groups 1 and 2 and three times weekly for group 3, for an overall period of 4 weeks.

For experiment 2 (oral administration of DB1127), 32 female BALB/c mice (age, 9 weeks; average body weight, 25 g) were infected with *E. multilocularis* metacestodes as described above and were divided into 4 groups of 8 animals each. Group 1 (untreated control) received 100 μl CMC-honey only; group 2 (albendazole control group) received albendazole (200 mg/kg) in 100 μl CMC-honey; group 3 received DB1127 (50 mg/kg) in 100 μl CMC-honey; group 4 received DB1127 (50 mg/kg) in combination with albendazole (200 mg/kg) in 100 μl CMC-honey. Albendazole was administered daily, while DB1127 was provided three times a week for a period of 6 weeks.

At the end of the studies, the animals were euthanized. After necropsy,
the total parasite tissue was collected and the parasite weight determined and presented as box plots. The data were submitted to a two-tailed distributed Student's t test, with two-sample equal variance determinations performed using the untreated group and each of the treatment groups belonging to each experiment.

RESULTS AND DISCUSSION
Effects of diguanidino compounds against *E. multilocularis* metacestodes in vitro. The structures of the limited panel of diguanidino derivatives investigated in this study are depicted in Table 1. Initial PGI assays were carried out by exposing metacestodes to 20 μM each compound over a period of 5 days (Fig. 1). Significantly elevated PGI activity was recorded in medium supernatants of metacestodes cultured in the presence of DB1127, DB869, and DB871 (Fig. 1). The term “% metacestode death” in Fig. 1 relates to the enzymatic activity in drug-treated parasites relative to PGI activity obtained from vesicles killed and permeabilized by the addition of Triton X-100 (designated 100%). The morphological alterations due to drug treatment, such as loss of turgor and vesicle collapse, were also visualized by light microscopy (Fig. 1B). Only those compounds with a thiophene backbone structure were active against metacestodes, while compounds with a furan ring were not; thus, the presence of a thiophene ring is important for antimetacestode activity. This becomes particularly evident when comparing the structures of DB869 and

<table>
<thead>
<tr>
<th>Code</th>
<th>IC50 (μM)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB856</td>
<td>ND</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>DB857</td>
<td>ND</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>DB859</td>
<td>ND</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>DB869</td>
<td>12.3</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>DB871</td>
<td>11.3</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>DB872</td>
<td>ND</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>DB944</td>
<td>ND</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>DB1127</td>
<td>6.1</td>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>

*ND, not determined.*
DB859, which are identical, with the exception that the thiophene ring in the former is replaced by a furan ring in the latter (Fig. 1A).

The dose dependency of DB1127-, DB869-, and DB871-mediated effects was assessed at different concentrations. The broad-spectrum drug nitazoxanide, previously shown to exhibit profound antimetacestode activity in vitro (26, 31), was used as a positive control. PGI enzyme activity values of 50% (Fig. 2, 50% metacestode death) were achieved at a nitazoxanide concentration of 1.4 μM. For DB1127, 6.1 μM was required in order to achieve similar PGI activity release. For DB869 and DB871, 12.3 and 11.2 μM, respectively, were needed to induce 50% metacestode death (Table 1). Thus, methyl groups on the terminal benzene rings of these aromatic dications appear to be irrelevant in terms of anti- Echinococcus activity, since methyl groups are present in both DB1127 and DB871 but not in DB869 (Table 1). In contrast, DB1127 is the only compound with a thiophene group flanked by two methylated benzene rings, and it is possible that these methyl groups are important for the increased activity of DB1127 compared to those of the DB869 and DB871. While the three compounds exhibit a highly similar molecular structure, the addition of the methyl group on the internal benzene rings could provide DB1127 with an increased membrane permeability, which, in turn, could increase the chances of the drug passing through several layers of membrane, including the plasma membrane and the membrane of a potential target organelle. This hypothesis is consistent with their CLogP values, which are the following: DB869, 5.72; DB871, 6.72; and DB1127, 7.12. Which targets are actually affected by DB1127 is not clear. Current evidence suggests that aromatic dicationic compounds bind to AT-rich sites in the DNA minor groove; thus, they inhibit transcription or the interaction with DNA-binding enzymes, such as topoisomerases or nucleases (32). This indicates that these compounds influence gene expression; thus, many diverse cellular functions could be affected. Clearly, more analogues need to be investigated to draw definitive conclusions, and other important structural requirements may be relevant for antimetacestode activity.

**Cytotoxicity in HFF, Vero cells, and RH.** Dicationic compounds, such as pentamidine and its derivatives, often exhibit a certain degree of toxicity. Cardiotoxicity, nephrotoxicity, and pancreatic complications have been reported (33). The cytotoxicity of DB1127, DB869, and DB871 was comparatively assessed in mammalian cells of human, monkey, and rat origin by alamarBlue assay. In this assay, viable cells reduce resazurin to resorufin, a fluorescent substance, which can be quantified at 595 nm, and this is used as a measure of viability. The term “survival in %” in Fig. 3 relates to the fluorescence measured at 595 nm in relation to the fluorescence measured from untreated control cultures (designated 100%). Table 2 shows the observed 50% inhibitory concen-

![FIG 2 Dose responses upon treatments of E. multilocularis metacestodes with thiophene diguanidino compounds.](image1)

![FIG 3 Cytotoxicity assays.](image2)

<table>
<thead>
<tr>
<th>Code</th>
<th>HFF</th>
<th>RH</th>
<th>Vero</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB869</td>
<td>7.9</td>
<td>5.5</td>
<td>&gt;20</td>
</tr>
<tr>
<td>DB871</td>
<td>3.3</td>
<td>5.5</td>
<td>&gt;20</td>
</tr>
<tr>
<td>DB1127</td>
<td>3.2</td>
<td>3.6</td>
<td>16.5</td>
</tr>
</tbody>
</table>

**TABLE 2 IC50s (μM) of compounds DB679, DB681, and DB1127 in HFF, RH, and Vero cells**
nounced so far. In conclusion, DB1127, exhibiting the most pro-
induce cell proliferation, but no evidence for this has been gener-
native possibility is that in low concentrations these compounds
microscopic inspection of cultures during drug treatments, which
increased energy consumption. This idea is supported by light
seek to compensate for the impairment mediated by the drugs by
increased energy consumption. This idea is supported by light
microscopic inspection of cultures during drug treatments, which
did not reveal any obvious alterations (data not shown). An alter-
native possibility is that in low concentrations these compounds
induce cell proliferation, but no evidence for this has been gener-
ated so far. In conclusion, DB1127, exhibiting the most pro-
ounced in vitro effects against E. multilocularis metacestodes, ap-
peared to be more cytotoxic than DB869 and DB871 in HFF and
Vero cells, while all three compounds were similarly toxic for RH.

In vivo studies employing DB1127 treatment in E. multilocu-
laris-infected mice. The in vivo efficacy of DB1127 was investi-
gated in mice by employing two modes of drug administration. In
experiment 1, DB1127 was administered by intraperitoneal injec-
tion at a dose of 2 mg/kg three times a week, and the effect of this
treatment was compared to that of the standard albendazole treat-
ment (200 mg/kg applied orally on a daily basis) (37, 38) over a
treatment duration of 4 weeks. In a pilot experiment, two unin-
fected mice were treated with DB1127 at 2 mg/kg for 2 weeks
without exhibiting any overt adverse side effects. The morphology
of the intraperitoneal cavity remained unaffected despite the fre-
quent injections of the drug (data not shown). Thus, 2 mg/kg was
chosen as the treatment dose. As indicated in Fig. 4A, the standard
oral albendazole treatment resulted in a significant reduction in
the parasite burden by 84%, and DB1127 treatment reduced the
parasite weight significantly by 70% compared to levels for the
untreated control. No distress or other behavioral changes were
observed in the animals during the treatment.

In experiment 2, DB1127 was applied by the oral route, as was
done for albendazole. In a pilot experiment, two uninfected mice
per group were treated with DB1127 at 10, 20, and 50 mg/kg,
respectively, for 2 weeks without exhibiting any adverse side ef-
fects. Thus, a treatment dosage of 50 mg/kg was chosen. After a
treatment period of 6 weeks, albendazole chemotherapy resulted in
a significant reduction in parasite burden (Fig. 4B). However,
no reduction in parasite weight was recorded in DB1127-treated
mice, and no improvement of treatment efficacy was found in
mice receiving albendazole-DB1127 combination treatment. In
fact, the combined application of DB1127 with albendazole com-
promised the treatment efficacy of the benzimidazole. This indi-
cates that drug interactions occur, the nature of which are un-
known at present and need to be investigated further. For
instance, the inclusion of DB1127 could affect the absorption of
albendazole. Dicationic compounds, when applied orally, are
known to be poorly absorbed. The poor bioavailability is attribut-
able, in large part, to the high pKₐ of the amide functional group;
thus, at physiological pH, these drugs are dicationic. If DB1127
interacts with albendazole when combined and applied orally, this
could have an impact on adsorption of the latter. Reuter et al. (39)
demonstrated that in vitro treatment of E. multilocularis metaces-
todes with either albendazole or oxamflutarin B had a profound
destructive impact on the parasites, and sequential treatments
with the two compounds killed metacestodes. However, simulta-
neous application of the two compounds had an inhibitory effect
on vesicle destruction. Antagonistic effects have been commonly
observed in experimental treatments of other helminth infections.
For instance, a recent study on the activity of albendazole in com-
bination with oxantel pamoate against Trichuris muris in mice
showed that while oxantel pamoate treatment alone was highly
active, the combination with albendazole revealed antagonistic
effects. On the other hand, highly synergistic effects were observed

![Figure 4](http://aac.asm.org/Downloaded from http://aac.asm.org on October 31, 2017 by guest)
when oxantel pamoate was administered in combination with another benzimidazole, mebendazole (40).

Interestingly, the treatment efficiency of albendazole was variable between the two experiments, as seen by the differences in the reduction in parasite weights compared to those of the controls (84% in experiment 1 and 36% in experiment 2). However, the parasite weights in the controls recovered at the end of the experiment differed substantially in the two experiments, such as a median weight of 1.2 g in the experiment 1 control group and slightly below 0.6 g in the control group of experiment 2. First, this reflects the inherent variation in the experimental system for AE, such as batch-to-batch variations of parasite material used for infection experiments, and points out the need for more standardization of cultured material in order to obtain parasites with highly similar infectivity. Second, these results indicate that chemotherapy has a lower impact in hosts infected with a lower parasite load for reasons that are currently unknown. Third, the duration of treatment (4 weeks in experiment 1 and 6 weeks in experiment 2) might have an influence. It remains to be shown whether Echinococcus metacestodes have acquired the ability to adapt to drug pressure by, e.g., upregulation of intrinsic detoxification factors, such as glutathione transferase or P-glycoprotein homologues. Thus, a constant exposure to a certain drug level might become inefficient with time, leading to regrowth of parasites after an initial reduction in parasite weight. Clearly, more work needs to be done to clarify these aspects of the experimental system for murine AE.

In summary, three di-N-aryl-diguanidino compounds with a thiophene core (DB869, DB871, and DB1127) displayed potential toxicity against E. multilocularis metacestodes in vitro, while compounds with a furan core lacking the sulfur substitution did not have any effect. The superior in vitro activity of DB1127 at lower concentrations could be attributed to the methyl groups of the benzene rings flanking the thiophene group. DB869, DB871, and DB1127 showed similar and high cytotoxicity for RH cells, while toxicity for HFF and Vero cells was lower. Although it is more cytotoxic than the others, DB1127 did not exhibit adverse effects to the mitochondria or P-glycoprotein homologues. Thus, a combination of DB1127 and a thione transferase or P-glycoprotein homologue might be considered. Thus, thiophene-diguanidino derivatives, perhaps prodrugs that are currently unknown. Third, the duration of treatment (4 weeks in experiment 1 and 6 weeks in experiment 2) might have an influence. Second, these results indicate that chemotherapy has a lower impact in hosts infected with a lower parasite load for reasons that are currently unknown. Third, the duration of treatment (4 weeks in experiment 1 and 6 weeks in experiment 2) might have an influence. It remains to be shown whether Echinococcus metacestodes have acquired the ability to adapt to drug pressure by, e.g., upregulation of intrinsic detoxification factors, such as glutathione transferase or P-glycoprotein homologues. Thus, a constant exposure to a certain drug level might become inefficient with time, leading to regrowth of parasites after an initial reduction in parasite weight. Clearly, more work needs to be done to clarify these aspects of the experimental system for murine AE.

ACKNOWLEDGMENTS

We are grateful to Bruno Gottstein and Markus Spiliotis (Institute of Parasitology, Bern) for continuous support and helpful discussions and Christian Leumann (Department of Chemistry, University of Bern) for the synthesis of nitazoxanide. The study was financed through the Swiss National Science Foundation (grant no. 31003A-125990), the Swiss Life Foundation, and the Bangerter-Rhyner Foundation.

REFERENCES


