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2,6-Dihydroxybenzoic Acid Anilides as Fasciolicides

D. Düwel and H. Metzger*

Pharma Research Laboratories, Farbwerke Hoechst AG, D 623 Frankfurt am Main-80, Germany. *Received August 17, 1972*

The inhibitory constants of various substituted 2,6-dihydroxybenzanilides (DHB-anilides) toward the succinate dehydrogenase complex of the rat and *Fasciola hepatica* have been determined. The values of these constants are compared with the biological findings of the compounds as fasciolicides in sheep. The strongest inhibitors are those dihalogen-DHB-anilides and DHB-3',5'-$(CF_3)_2$-anilides which can be retained in the body for a prolonged period of time.

Chemotherapeutic research directed toward a more selective synthesis of active compounds has experienced increasing importance during the past few years. The metabolic pathways of the organisms to be treated have been examined carefully and compared with those of their hosts,[1,2] since species differences in the sensitivity of enzymes toward chemical agents can serve for the development of highly active compounds.[3-6]

Within the framework of this trend has been the approach to fascioliasis, an important problem in veterinary medicine.[7,8] The metabolism of the liver fluke (*Fasciola hepatica*) has been examined at various places for possible sites of action.[9-18] One such site, *F. hepatica* succinate dehydrogenase (SDH), has recently been shown to be inhibited by 2,6-dihydroxybenzanilides (DHB-anilides).[2]

In this paper results of *in vitro* inhibition of the *F. hepatica* and rat SDH enzymes are compared with *in vivo* fasciolicide activity in an attempt to obtain a structure-activity relationship among the DHB-anilides.

Methods. The compounds used in the present study were prepared by conventional means.[19a] Specific details including physical constants and spectral data will be reported elsewhere.[19b]

Liver fluke succinate dehydrogenase (SDH) inhibitory activities were determined on extracts of uniform *Fasciola* material from Wistar rats (strain Hoechst), while rat SDH inhibitory activities were determined on extracts of rat myocardial tissue.[2]

The *in vivo* activities of the DHB anilides were determined in sheep which had been infected orally with 250–300 metacercariae of *F. hepatica*. With the beginning of the excretion of eggs (at the end of the prepatent period), the infected sheep were treated orally with the test compounds. The reduction of egg excretion (*vs.* controls) over 28 days was determined and expressed as per cent reduction (method of fecal examination, ref 8). The maximum tolerated dose (MTD) of each compound was determined in NMRI mice (strain Hoechst) using at least four mice per dose (observation period, 14 days).

Results and Discussion

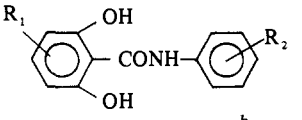
The findings of the various *in vitro* and *in vivo* tests are shown in Tables I–IV. The compounds have been grouped in such a manner as to illustrate the effects of systematic structural variation. Thus, for example, Table I illustrates the effect of variations in the anilide portion with the DHB fragment held constant, while Table II shows the effect when the anilide portion is held constant and the DHB moiety is varied.

Examination of the data reveals that within each group there is considerable variation in the succinate dehydrogenase inhibitory constants against both the live fluke ($K_{I\text{-}SDH\text{-}F}$) and the rat ($K_{I\text{-}SDH\text{-}R}$) preparations. There is also some variation between groups. What is perhaps most interesting, however, is the variation in Q or $K_{I\text{-}SDH\text{-}R}/K_{I\text{-}SDH\text{-}F}$. Q represents an idealized chemotherapeutic index, wherein a high value ($Q \gg 1$) indicates that the liver fluke enzyme is more sensitive to an agent than the rat enzyme. Conversely, a low value ($Q \ll 1$) means higher sensitivity of the mammalian enzyme as compared with the parasite enzyme. It can be seen from the data in Tables I and II that it is indeed possible to achieve a high degree of selectivity in many cases (Q often >20).

It is evident by comparing groups that the effect of anilide substituents on Q is largely similar to, but independent from, the effects of substituents in the DHB moiety. Thus, if one ranks the substituents in order of increasing Q values, it would appear that the order is approximately the same within each group. It would further appear that as the substituents become more hydrophilic, the Q value of the compound becomes greater, *i.e.*, greater selectivity for the fluke enzyme, making the compounds of interest as fasciolicides. This apparent dependence of Q on lipophilicity has been confirmed by a Hansch regression analysis.[20]

The maximum tolerated dose (MTD) also appears to depend in an inverse manner on lipophilicity. The MTD values that do not fit this general scheme are of compounds that are metabolized (and thus strongly protein bound) or not well absorbed by the host organism.

Table I. Comparison of the Inhibition Constants of 2,6-Dihydroxybenzoic Acid Anilides (DHB-anilides) on the Succinate Dehydrogenases of Rat Heart ($K_{I\text{-}SDH\text{-}R}$) and of Liver Flukes (*Fasciola hepatica*) ($K_{I\text{-}SDH\text{-}F}$) with Biological Findings[a]



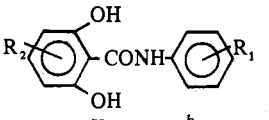
R_1	R_2	$K_{I\text{-}SDH\text{-}R}$, mol/l.	$K_{I\text{-}SDH\text{-}F}$, mol/l.	$\frac{K_{I\text{-}SDH\text{-}R}}{K_{I\text{-}SDH\text{-}R,\ GSS}}$[b]	MTD[c] ($\times 10^{-5}$ mol/kg)	Q[d]	Effectiveness in sheep[e] ($\times 10^{-5}$ mol/kg)	
3H	4'-Phenothiazinyl	2.35×10^{-8}	2.35×10^{-7}	0.003		0.1		
	3',5'-di-CF_3	2.74×10^{-7}	5.48×10^{-7}	0.036	438	0.50	27.4	+++
	4'-I	2.82×10^{-6}	3.1×10^{-6}	0.37	704	0.91		
	4'-Br	6.49×10^{-6}	5.2×10^{-6}	0.86	>1600	1.2	65	+
	4'-Cl	7.59×10^{-6}	9.48×10^{-6}	1.00	>900	0.8		
	3'-Cl	7.59×10^{-6}	1.29×10^{-5}	1.00	>1900	0.59		
	4'-$COOCH_3$	1.39×10^{-5}	3.5×10^{-5}	1.83	>1000	0.4		
	2'-Cl	1.52×10^{-5}	3.8×10^{-6}	2.0	>800	4		
	4'-F	2.35×10^{-5}	3.24×10^{-5}	3.1	>1200	0.72		
	4'-$CONH_2$	$>3.6 \times 10^{-5}$	2.94×10^{-5}	>4.7	>1800	>1.2		
	4'-OH	$>4 \times 10^{-5}$	$>4 \times 10^{-5}$	>5.3				
3-C_3H_7CO	3',5'-di-CF_3	9.20×10^{-7}	6.21×10^{-6}	0.077	367	0.15	2.3	+++
	4'-Br	4.23×10^{-6}	13.2×10^{-6}	0.35	>850	0.32	26.5	–
	4'-Cl	1.2×10^{-5}	8.1×10^{-6}	1.00	958	1.48	30	–
	4'-H	$>3.3 \times 10^{-5}$	3.3×10^{-5}	>2.8	>1000		33	+
3-CN	3',5'-di-CF_3	4.10×10^{-7}	2.05×10^{-7}	0.09	25.6	2	3.85	+
	4'-C_6H_4	4.85×10^{-7}	7.88×10^{-8}	0.107	121	6	6.1	–
	3'-CF_3	3.11×10^{-6}	1.71×10^{-7}	0.68	124	18		
	4'-Cl	4.54×10^{-6}	1.73×10^{-7}	1.00	69	26	10.4	–
	4'-CH_3	9.33×10^{-6}	4.48×10^{-7}	2.06	149	21		
	4'-F	2.02×10^{-5}	8.46×10^{-7}	4.45	74	24	3.7	+
	4'-OCH_3	3.03×10^{-5}	7.04×10^{-7}	6.67	282	43	7.04	–
	4'-H	3.19×10^{-5}	8.27×10^{-7}	7.03	79	38	7.9	–
	4'-OH	$>3.7 \times 10^{-5}$	7.78×10^{-7}	>8.1	296	>45	11.1	–
3-NO_2	4'-Phenothiazinyl	5.31×10^{-8}	2.76×10^{-8}	0.009		1.9		
	3',5'-di-CF_3	4.87×10^{-7}	2.93×10^{-7}	0.084	3.4	1.7	0.6	+++
	3'-CF_3	3.8×10^{-6}	6.14×10^{-7}	0.65	36.5	6	14.6	+++
	4'-Br	3.68×10^{-6}	4.53×10^{-7}	0.63	35.4	8	4.25	+++
	4'-NO_2	5.0×10^{-6}	1.25×10^{-6}	0.86	>31.5	4	4.7	+++
	4'-Cl	5.83×10^{-6}	9.73×10^{-7}	1.00	19.4	6	3.24	+++
	4'-$COOCH_3$	1.02×10^{-5}	1.2×10^{-6}	1.76	>90	8.5		
	4'-H	2.92×10^{-5}	1.82×10^{-6}	5.00	14.6	16	5.5	--
	4'-OCH_3	$>3.3 \times 10^{-5}$	7.57×10^{-6}		41		16.4	++
3-Cl	4'-C_4H_9	1.69×10^{-7}	4.08×10^{-9}	0.16		41.5		
	4'-Cl	1.07×10^{-6}	4.03×10^{-8}	1.0		27		
	4'-NO_2	1.3×10^{-6}	2.05×10^{-7}	1.21		6.3		
	4'-$COOCH_3$	1.43×10^{-6}	7.8×10^{-8}	1.34		18.4		
3,5-Cl_2	4'-C_4H_9	4.41×10^{-8}	1.98×10^{-9}	0.24		22	14.1	–
	4'-Cl	1.8×10^{-7}	6.0×10^{-9}	1.00	6	30	0.6	+++
	4'-$COOCH_3$	3.51×10^{-7}	3.09×10^{-8}	1.87		11		
	4'-OCH_3	5.73×10^{-7}	5.79×10^{-8}	3.05		9.9		
	4'-NO_2	5.83×10^{-7}	3.5×10^{-8}	3.24		17		
	4'-$COCH_3$	1.23×10^{-6}	8.22×10^{-8}	6.83	58.8	15	8.8	++
	4'-CN	1.24×10^{-6}	8.67×10^{-8}	6.89		14		
	4'-$CONH_2$	1.07×10^{-5}	8.79×10^{-7}	59		12		
	4'-$NHCOCH_3$	1.49×10^{-5}	2.25×10^{-7}	83		66	14	–
	4'-COOH	$>2.9 \times 10^{-5}$	9.9×10^{-6}	>160				
Oxyclozanide[f]		5.26×10^{-6}	2.76×10^{-7}			19	3.76	+++
3,4',5-Tribromosalicylanilide and 4',5-dibromosalicylanilide (1:1)[f]		4.15×10^{-7}	6.59×10^{-8}			6.3	7.3	+++

[a] The DHB parts are kept constant. [b] The $K_{I\text{-}SDH\text{-}R}$ of each group is divided by the $K_{I\text{-}SDH\text{-}R}$ of a group specific standard substance (GSS; 4'-chloro-DHB-anilide). [c] MTD is the maximal tolerated dose of the drug as determined in mice. [d] Q is the quotient of $K_{I\text{-}SDH\text{-}R}/K_{I\text{-}SDH\text{-}F}$. It represents an idealized index for chemotherapy. [e] The effectiveness in sheep indicates the rate of reduction (%) of liver fluke eggs at a given dose of the drug: 0–20%, ineffective (–); up to 50%, slightly effective (+); 50–75%, effective (++); >75%, highly effective (+++). [f] Established drugs.

The true criterion for interest in a compound as a fasciolicide is its *in vivo* effectiveness (Tables I and II). From the data it would appear that all the effective compounds have low $K_{I\text{-}SDH\text{-}F}$ values; the converse, however, is not true. Further, there appears to be no real correlation between high Q values and good *in vivo* potency. Thus, for high *in vivo* effectiveness, factors other than $K_{I\text{-}SDH\text{-}F}$ and Q values are also important. A compound must be absorbed in a minimal dose (D) and remain unchanged in the host for a certain minimum period (T). The essential element, then, for an effective fasciolicide is the product of the dose and the time which the compound has to act on the parasite, *i.e.*, $D \times T$.

The dose has to exceed the minimal curative dose (MCD) but should not reach the maximum tolerated dose (MTD). Simple dihalogen-DHB-anilides (Table I) are quite active and selective (low $K_{I\text{-}SDH\text{-}F}$ and high Q values) in the enzyme tests but are less effective *in vivo*. They are relatively well

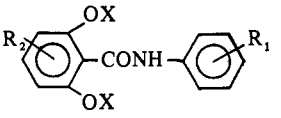
Table II. Comparison of $K_{I\text{-}SDH\text{-}R}$ and $K_{I\text{-}SDH\text{-}F}$ with Biological Findings[a]



R_1	R_2	$K_{I\text{-}SDH\text{-}R}$, mol/l.	$K_{I\text{-}SDH\text{-}F}$, mol/l.	$\frac{K_{I\text{-}SDH\text{-}R}}{K_{I\text{-}SDH\text{-}R,\ GSS}}$[b]	MTD[c] ($\times 10^{-5}$ mol/kg)	Q[d]	Effectiveness in sheep[e] ($\times 10^{-5}$ mol/kg)	
4'-Cl	3,5-di-Cl	1.80×10^{-7}	6.00×10^{-9}	0.03	6	30	0.6	+++
	3-NO_2, 5-I	5.75×10^{-7}	2.76×10^{-7}	0.10	6	2	11.5	+++
	3-NO_2, 5-Br	6.46×10^{-7}	1.29×10^{-7}	0.11	3	5		
	3-Cl	1.07×10^{-6}	4.03×10^{-8}	0.18		27		
	3-NO_2, 5-Cl	1.57×10^{-6}	4.36×10^{-7}	0.27	4	4	1.2	+++
	3-CN	4.54×10^{-6}	1.73×10^{-7}	0.78	69	26	10.4	–
	3-NO_2	5.83×10^{-6}	9.73×10^{-7}	1.0	19.4	6	3.24	+++
	3-H	7.59×10^{-6}	9.48×10^{-6}	1.1	947	0.8		
	3-C_3H_7CO	1.2×10^{-5}	8.1×10^{-6}	2.05	958	2	30	–
	3-*i*-C_3H_7CO	4.8×10^{-5}	$>3 \times 10^{-5}$	8.2	>898		3	–
4'-Br	3,5-di-Br	1.07×10^{-7}	5.36×10^{-9}	0.03	3.2	19		
	3,5-di-Cl	2.12×10^{-7}	1.33×10^{-8}	0.06	6.6	16	5.3	–
	3-I	3.92×10^{-7}	2.30×10^{-8}	0.11	737	17	4.6	–
	3-CH_3CO, 5-I	8.4×10^{-7}	1.05×10^{-6}	0.23	336	0.8	21	+++
	3-CH_3CO, 5-Br	1.4×10^{-6}	1.40×10^{-6}	0.40	47	1	7	++
	3-CH_3CO, 5-Cl	1.64×10^{-6}	1.2×10^{-6}	0.45	52	1.4	7.8	+++
	3-NO_2	3.68×10^{-6}	4.53×10^{-7}	1.00	35.4	8	4.25	+++
	3-H	6.49×10^{-6}	5.2×10^{-6}	1.76	>1600	1.2	65	+
	3-CH_3CO	$>2.86 \times 10^{-5}$	$>2.86 \times 10^{-5}$	>7.7	>910		2.86	+
	3-*i*-C_3H_7CO	$>2.64 \times 10^{-5}$	$>2.6 \times 10^{-5}$	>7.2	>850		13.2	+++
	3-$C_5H_{11}CO$	$\geq 2.4 \times 10^{-5}$	$\geq 2.4 \times 10^{-5}$	>6.5	394		24.6	+
3',5'-$(CF_3)_2$	3-NO_2, 5-I	1.68×10^{-7}	1.49×10^{-7}	0.33	1.12	1.1		
	3-C_3H_7CO, 5-I	2.9×10^{-7}	7.66×10^{-7}	0.59	17.8	0.38	5.4	++
	3-*i*-C_3H_7CO, 5-I	3.57×10^{-7}	1.07×10^{-6}	0.73	53.5	0.33	2.7	+++
	3-*i*-C_3H_7CO, 5-Br	3.89×10^{-7}	1.07×10^{-6}	0.80	19.5	0.36	5.8	+++
	3-CN	4.10×10^{-7}	2.05×10^{-7}	0.84	25.6	2	3.85	+
	3-NO_2	4.87×10^{-7}	2.93×10^{-7}	1.00	3.41	1.7	0.6	+++
	3-*i*-C_3H_7CO, 5-Cl	5.33×10^{-7}	5.33×10^{-7}	1.09	5.33	1	1.07	+++
	3-NH_2	8.95×10^{-7}	5.26×10^{-7}	1.83	211	1.7	7.9	–
	3-C_3H_7CO	9.20×10^{-7}	6.21×10^{-6}	1.88	367	0.15	2.3	+++
	3-CH_3CO	9.83×10^{-7}	2.09×10^{-6}	2.0	393	0.47	2.5	+++
	3-*i*-C_3H_7CO, 5-NO_2	11.3×10^{-7}	9.58×10^{-7}	2.3	6.25	1.2	1.04	+++
	3-*i*-C_3H_7CO	$>14 \times 10^{-7}$	$>2.3 \times 10^{-5}$	>2.87	23		2.3	+++
	3-$C_5H_{11}CO$	19.4×10^{-7}	$>2.16 \times 10^{-5}$	3.98	86		21.6	–

[a] The anilide parts of the compounds to be tested are kept constant. GSS = 3-NO_2-DHB. [b–e] See footnotes of Table I.

Table III. Influence of Acylation of the 2,6-Dihydroxy Group of the DHB-Anilides on the $K_{I\text{-}SDH\text{-}R}$ and $K_{I\text{-}SDH\text{-}F}$ and on Biological Parameters



R_1	R_2	X	$K_{I\text{-}SDH\text{-}R}$, mol/l.	$K_{I\text{-}SDH\text{-}F}$, mol/l.	MTD[c] ($\times 10^{-5}$ mol/kg)	Effectiveness in sheep[e] ($\times 10^{-5}$ mol/kg)	
4'-Cl	3,5-di-Cl	H	1.80×10^{-7}	6.00×10^{-9}	6	0.6	+++
	3,5-di-Cl	CH_3CO–	$>2.4 \times 10^{-5}$	$>2.4 \times 10^{-5}$	6	1.8	+++
4'-Cl	3-NO_2	H	5.83×10^{-6}	9.73×10^{-7}	19.4	3.24	+++
	3-NO_2	CH_3CO–	$>2.5 \times 10^{-6}$	$>2.5 \times 10^{-5}$	10		
4'-Br	3,5-di-Cl	H	2.12×10^{-7}	1.33×10^{-8}	6.6		
	3,5-di-Cl	CH_3CO–	2.17×10^{-5}	1.73×10^{-5}	10.8	3.24	+++
3',5'-$(CF_3)_2$	3-NO_2	H	4.87×10^{-7}	2.93×10^{-7}	3.41	0.6	+++
	3-NO_2	CH_3CO–	6.88×10^{-6}	18.2×10^{-6}	40.5	0.5	+++
3',5'-$(CF_3)_2$	3-H	H	2.74×10^{-7}	5.48×10^{-7}	438	27.4	+++
	3-H	CH_3CO–	1.78×10^{-5}	$>2.2 \times 10^{-5}$	356	22	+++

[c,e] See footnotes of Table I.

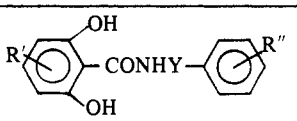
absorbed but rapidly eliminated. Thus, they barely reach the necessary product of dose and time (Figure 1, type A).

This difficulty, however, can be overcome by modification of the DHB-anilides. Acylation of the free hydroxyl groups (Table III) produces compounds with poor *in vitro* potency which are slowly metabolized *in vivo* (deacylated) to give the potent hydroxy compounds and thus a larger $D \times T$ product (Figure 1, type B).

Also more active *in vivo* than predicted by the Q values are 5-halogen-3-nitro- (or 3-acyl-) DHB-anilides (Table II). With these compounds presumably, the nonhalogen component of the DHB moiety might be altered by host metabolism, whereby the compounds become more potent and/or remain in the circulation for a prolonged time (Figure 1, type B).

Phenoxy enlargements (Table IV) lower the inhibitory

Table IV. Influence of an Enlargement of the DHB-anilides by Phenoxy Substituents on $K_{I\text{-}SDH\text{-}R}$ and $K_{I\text{-}SDH\text{-}F}$ and on Biological Data



R'	Y	R''	$K_{I\text{-}SDH\text{-}R}$, mol/l.	$K_{I\text{-}SDH\text{-}F}$, mol/l.	MTD[c] ($\times 10^{-5}$ mol/kg)	Q[d]	Effectiveness in sheep[e] ($\times 10^{-5}$ mol/kg)	
3,5-di-Cl		4'-Cl	18.00×10^{-8}	6.00×10^{-9}	6	30	0.6	+++
3,5-di-Cl	2-Cl-C_6H_4O	4'-Cl	0.87×10^{-8}	1.7×10^{-9}	5.4	5	0.65	+++
3-NO_2		4'-Cl	58.30×10^{-7}	9.73×10^{-7}	19.4	6	3.24	+++
3-NO_2	C_6H_5O	4'-Cl	0.82×10^{-7}	0.45×10^{-7}	24.9	1.8	2.5	++
3-NO_2	2-Cl-C_6H_4O	4'-Cl	0.46×10^{-7}	0.46×10^{-7}	22.9	1.0	2.3	+
3-NO_2		3',5'-di-CF_3	48.7×10^{-8}	29.3×10^{-8}	3.41	1.7	0.6	+++
3-NO_2	C_6H_5O	3',5'-di-CF_3	6×10^{-8}	6×10^{-8}	20	1	2	+++
3-NO_2	2-Cl-C_6H_4O	3',5'-di-CF_3	3×10^{-7}	1.1×10^{-7}	15	1	3.7	+++
Rafoxanide[f]			7.68×10^{-9}	5.6×10^{-9}		1.4	1.35	+++

[c-f] See footnotes of Table I.

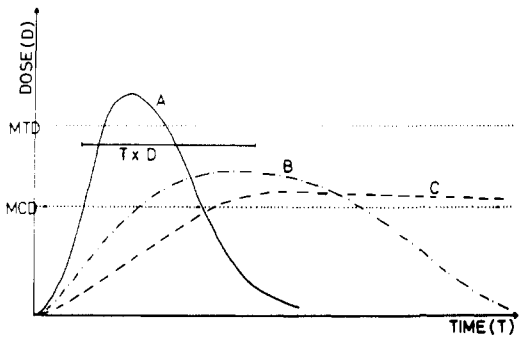


Figure 1. Dose-response curve of differently substituted DHB-anilides as fasciolicides: MTD = maximum tolerated dose; MCD = minimal curative dose; A, B, C = concentrations in the blood of compounds with different pharmacokinetic properties (see text).

constants ($K_{I\text{-}SDH\text{-}F}$) and Q values of the compounds, presumably by increasing the lipophilicity. The *in vivo* effectiveness is increased but to a lesser degree than might be expected from the decrease in $K_{I\text{-}SDH\text{-}F}$ values. These findings might indicate that the compounds are less easily absorbed or that their unspecific binding to body lipids is considerably stronger (Figure 1, type C). The use of these compounds as fasciolicides might involve residue problems.

In summary, then, although for good *in vivo* fasciolicide activity with DHB anilides one must have potent (low $K_{I\text{-}SDH\text{-}F}$) and preferably selective (high Q values) compounds, it would appear that in many cases the factors determining usefulness are biopharmaceutic or pharmacokinetic in nature.

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2,6-Dihydroxybenzoic Acid Anilides Active against Liver Flukes. A Hansch Analysis

E. Druckrey* and H. Metzger

Farbwerke Hoechst AG, D 623 Frankfurt am Main-80, Germany. *Received August 17, 1972*

With the aid of Hansch analysis quantitative structure-activity relations are established between 2,6-dihydroxybenzanilides substituted in both phenyl rings and their inhibitory activity on the enzyme succinate dehydrogenase, isolated from rats and liver flukes.

The liver fluke (*Fasciola hepatica*) infests sheep and cattle and represents a major problem in veterinary medicine.[1,2] The most probable point of attack of several active substances against *F. hepatica* is that enzyme system which controls the transformation of fumarate into succinate and vice versa.[3] Since liver flukes are very much dependent on this enzyme,[4-6] its inhibition must greatly reduce the viability of the parasites.