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THE CHEMOTHERAPY OF SCHISTOSOMIASIS

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INTRODUCTION

Several major developments have occurred in the chemotherapy of schistosomiasis since Archer & Yarinsky reviewed the subject in 1972 (1). Perhaps the outstanding discovery has been the broad-spectrum, well-tolerated drug praziquantel, which is rapidly becoming the drug of choice in the treatment of schistosomiasis. Two other new broad-spectrum, clinically effective drugs that have made their appearance in recent years are oltipraz and amoscanate. We have also made some progress in understanding the mode of action of some of the older antischistosomal drugs such as hycanthone, oxamniquine, niridazole, and metrifonate, but little is known about the mechanism of action of the newer drugs. In order to focus attention on this problem, the Steering Committee of the Scientific Working Group on Schistosomiasis sponsored a symposium in Geneva January 30–February 1, 1984 (2) that was devoted exclusively to the biochemistry and chemotherapy of this disease. Wherever possible, this chapter devotes particular attention to the mode of action of clinically active agents and some of their relevant analogues.

HYCANTHONE

Hycanthone 2 has been shown to be a bioactive metabolite of lucanthone, 1. It is a potent antischistosomal drug when given orally, but it is more potent when administered parenterally. It is more active in hamsters than in mice against experimental *Schistosoma mansoni* infections (3, 4). It is clinically effective against *S. mansoni* and *S. haemotobium* infections when given in single intramuscular doses of about 3 mg/kg (1). Hycanthone is an antitumor agent in

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mice (5) and is a frameshift mutagen (6). It has been reported to be teratogenic (7) and possibly carcinogenic (8, 9).

The effects of lucanthone and hycanthone on nucleic acid and protein biosynthesis have been studied in bacterial and mammalian systems (10–12). The primary effect of both drugs is a pronounced but reversible inhibition of RNA synthesis; DNA and protein synthesis are affected to a lesser extent. Such effects are to be expected of intercalating drugs. It has been demonstrated that lucanthone and hycanthone intercalate into DNA (4, 13). Both drugs interfere with DNA-dependent RNA polymerase.

Hycanthone and lucanthone show delayed effects on schistosomes. Hepatic shifts with both drugs do not commence until about 72 hours after administration (14–16), yet almost all of the drugs disappear from the blood of the host 24 hours post-administration (17, 18). Despite indications that hycanthone and lucanthone have a similar mode of action, it is not clear why lucanthone has to be oxidatively metabolized before it exerts its schistosomicidal effects. The evidence that supports such a hypothesis has been summarized (1).

Recently, Cioli and his co-workers found that hycanthone inhibits the uptake of ^{3}H-uridine by adult hycanthone-sensitive *S. mansoni* worms (19), whereas under comparable experimental conditions lucanthone is ineffective in inhibiting uptake of this pyrimidine base by the schistosomes. These observations confirm the previously stated hypothesis concerning the bioconversion of 1 to 2. Cioli & Knopf (20) have studied the action of hycanthone both in vivo and in vitro using a new technique based on the transfer of schistosomes into the mesenteric veins of hamsters. They concluded that the action of 2 against *S. mansoni* is not due to a host-derived metabolite of hycanthone.

Cioli, Pica-Mattoccia, Rosenberg, & Archer (19) have proposed that the manifold effects of hycanthone can be accounted for on the basis of the mechanism of action shown in Figure 1.

Lucanthone, 1, is bio-oxidized to hycanthone, 2, as proposed earlier (5). The latter is converted enzymically to the ester, 3, presumably by either a kinase that results in the formation of a phosphate or by a sulfotransferase that affords a sulfate ester. The formation of the acctatc ester from acetylCoA cannot be ruled out. Miller & Hulbert (21) suggest that under certain conditions hycanthone and hycanthone acetate may act as alkylating agents. In either case, 3 possesses a good leaving group that allows a non-enzymic dissociation to the ion 4-4a to occur. This ion intercalates into DNA and then monoalkylates this macromolecule to give the covalently bound drug-DNA complex, 5. In the case of hycanthone, these reactions occur in the mammalian host as well as in the schistosome. A similar mechanism has been proposed without supporting evidence to account for the mutagenic action of hycanthone (22). The mechanism shown in Figure 1 is compatible with the findings of Cioli & Knopf (20),

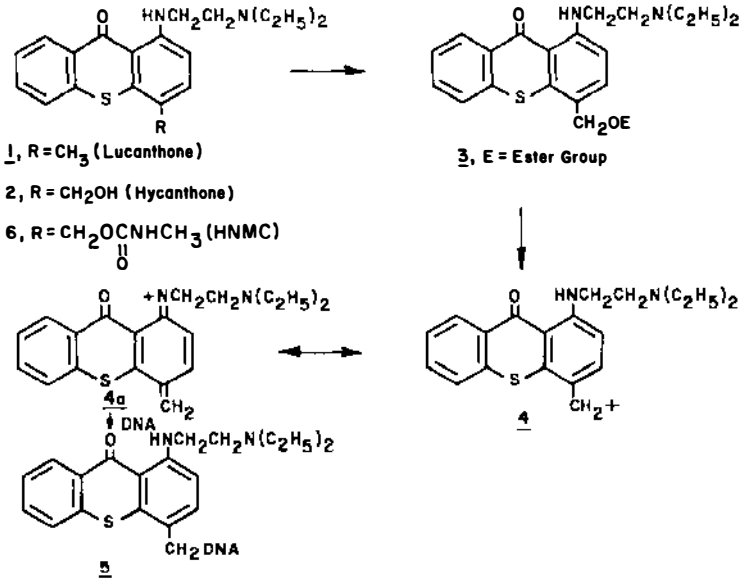


Figure 1 The mode of action of hycanthone

who concluded that the schistosomicidal activity of hycanthone does not depend on host bioactivation of the compound.

It has been known for some time that hycanthone and some of its congeners are antitumor agents (23, 24), but it is difficult to account for this property on the basis of intercalation alone. However, the mode of action shown in Figure 1 can also serve as the molecular basis for the antitumor activity of hycanthone.

In order to test this hypothesis, a surrogate, 6, of the ester, 3, was prepared because it was believed that sulfate or phosphate esters of 2 would be difficult to synthesize and use because of their high instability and reactivity. The N-methylcarbamate, 6 (HNMC), was readily prepared by treating hycanthone with methyl isocyanate (19).

The apparent association constant (Kapp) of the HNMC-calf thymus DNA complex is approximately fifteen times greater than that of the hycanthone-DNA complex, yet the ΔTm values of these complexes are nearly identical. Such observations can be rationalized on the basis of single-stranded DNA monoalkylation, as shown in Figure 1. Comparative dialysis experiments of these complexes showed that, in the time required for half of the hycanthone to dialyze away, only 2% of the HNMC dialyzes. The antitumor activities of 2 and HNMC in leukemic mice are almost identical, but HNMC exerts its action at one-tenth the dose of 2 and is also far more toxic. Such an increase in potency and toxicity could result from alkylation of DNA.

Cioli and coworkers have shown (25) that in adult hycanthone-sensitive *S.*

mansoni, hycanthone-induced inhibition of ^{3}H-uridine uptake is not reversed after removal of the drug from adult schistosomes but, in the case of hycanthone-resistant worms, ^{3}H-uridine uptake is restored after removal of the drug. According to the scheme outlined in Figure 1, hycanthone resistance is due to the absence of the enzyme that converts 2 to 3. Accordingly, it predicts that a surrogate of 3 that can undergo non-enzymic conversion to 4-4a in the schistosome should be active in hycanthone-resistant worms. When the above experiment is repeated with equimolar concentrations of HNMC, ^{3}H-uridine uptake is blocked in both hycanthone-sensitive and hycanthone-resistant worms. The in vitro experiments utilizing ^{3}H-uridine incorporation are duplicatible in vivo (26). *S. mansoni*–infected mice were treated with hycanthone; schistosomes were obtained by perfusion at various times after drug administration and the worms were tested for their ability to incorporate precursors of DNA, RNA, and protein. In hycanthone-sensitive adult worms precursor incorporation was inhibited, whereas in the case of immature or resistant worms, no such inhibition was noted. There was a close correlation between inhibition of macromolecule biosynthesis and parasite death. These results are also compatible with the mechanism shown in Figure 1, where the postulated lethal event is monoalkylation of schistosomal DNA.

When HNMC was administered in low doses (owing to the high toxicity of the drug) over a period of several days to mice infected with hycanthone-resistant *S. mansoni* worms, the mice were cured of their infection. Thus, HNMC is effective in vivo as well as in vitro.

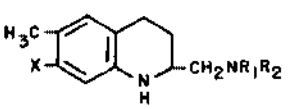
It is well known that hycanthone has no effect on either immature *S. mansoni* or on adult *S. japonicum*. Recent experiments (27) on interbreeding between hycanthone-sensitive and hycanthone-resistant schistosomes have led to the conclusion that hycanthone resistance behaves like an autosomal recessive trait, which in turn suggests that hycanthone-resistant schistosomes are deficient in some factor(s). According to Figure 1, the missing factor is the enzyme that converts 2 to 3. A similar deficiency can account for the ineffectiveness of 2 in the immature *S. mansoni* and *S. japonicum*. Cioli and co-workers found that HNMC, 6, blocks ^{3}H-uridine uptake in both the immature forms of *S. mansoni* and the adult forms of *S. japonicum* (19). These results are compatible with the mechanism shown in Figure 1.

Thus far, the molecular mechanism of the mode of action of hycanthone as shown in Figure 1 can account for the manifold activities of the drug. Furthermore, the same mechanism can account for the cross-resistance with oxamniquine and IA-IV (16). Hillman and colleagues have suggested (28, 29) that hycanthone acts by binding irreversibly to the acetylcholine receptors of *S. mansoni*. This in turn leads to the paralysis of the digestive tract of the worm and eventual death by starvation. Although the evidence discussed above does not directly rule out such a hypothesis, it does not easily account for the many

actions of hycanthone in mammalian and bacterial systems as well as in schistosomes by acetylcholine receptor blockade.

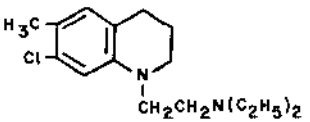
OXAMNIQUINE

A series of tetrahydroquinolines containing an aromatic methyl group adjacent to an electronegative substituent of the general formula 7 has been prepared in the laboratories of Pfizer Ltd. (30, 31). This group of compounds is closely related to the Mirasan analogue, Bayer 1593A, 8, (32).



7, X= NO_2, CN or Cl

R_1, R_2 = H or lower alkyl



8, Bayer 1593A

1.

The Pfizer compounds were active in *S. mansoni* infected mice; the most active member of the series was UK 3883 (7, X = NO_2, R_1 = H, R_2 = isopropyl). Armed with the knowledge that hycanthone is a metabolite of lucanthone and can be prepared by microbiological oxidation of 1 with *Aspergillus sclerotiorum* (1), the Pfizer group carried out a similar experiment on UK 3883 (33). They found that a similar conversion occurs that results in the formation of the hydroxymethyl analogue, oxamniquine, 9, which has proved to be a potent schistosomicide against experimental *S. mansoni* infections (34, 35). The drug is ineffective against *S. haemotobium* and *S. japonicum* infections (36, 37) but is a drug of choice in the treatment of *S. mansoni* infections in man (38). In South America, the recommended single oral dose is 12.5 mg/kg for patients weighing more than 40 kg and about 15 mg/kg for lighter individuals (39). In Zimbabwe, the dose necessary to achieve acceptable cure rates is 60 mg/kg given in four equal portions over a period of two days (40). This discrepancy in therapeutic regimens is probably a reflection of the differences in strain sensitivity to the drug. A large number of patients have been treated in Brazil with uniformly good cure rates at an acceptable level of mild side effects (41).

The genetic and mutagenic effects of oxamniquine are considerably weaker than those of hycanthone (42–44), but they are not entirely absent. Despite the fact that hycanthone and oxamniquine differ widely in their mutagenic potential, they are cross-resistant. In every case where hycanthone-resistant *S. mansoni* worms have been isolated from either man or animals, they have been found to be resistant to oxamniquine also (45–48). These observations suggest that the two drugs have a common mode of action with regard to their effect on

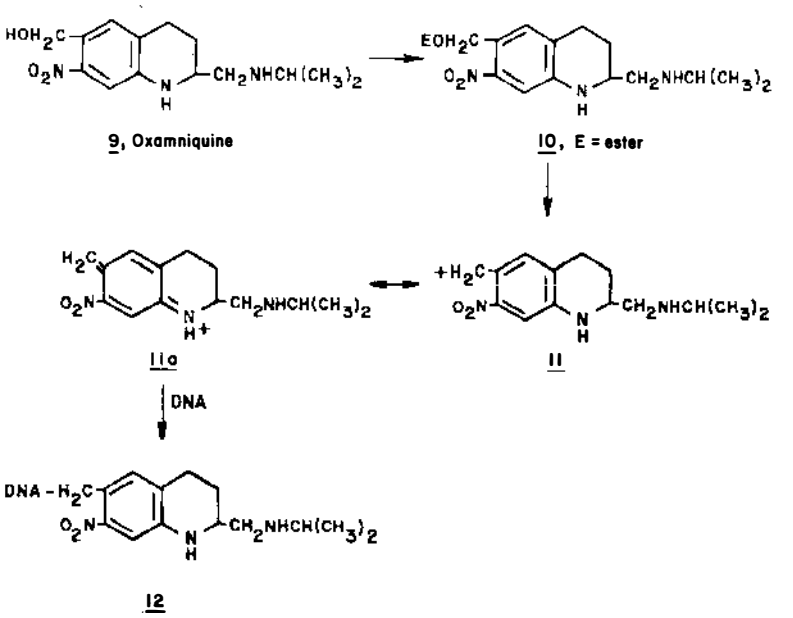


Figure 2 Proposed mode of action of oxamniquine

schistosomes. Accordingly, the scheme shown in Figure 2 has been proposed to account for the antischistosomal activity of oxamniquine.

As in the case of hycanthone, oxamniquine is enzymically converted, presumably by the same enzyme, to the ester, 10, where E is either a phosphate or a sulfate function. This species now possesses a good leaving group and may dissociate non-enzymically to give the ion 11-11a, which can alkylate DNA. The major difference between the mechanisms shown in Figures 1 and 4 is that in the latter case it is postulated that the ion 11-11a does not intercalate because oxamniquine does not possess a multiplanar ring system, which is a structural requirement for intercalation (49). Cioli (unpublished observations) has found that oxamniquine does not affect the Tm of calf thymus DNA, an observation that strongly supports the view that the drug is not an intercalating agent. Gale et al have remarked, "The most potent frameshift mutagens do seem to be intercalating agents, but whether intercalation is necessary for drug-induced mutagenesis (it is clearly not sufficient) or merely incidental remains to be established" (50). The lower mutagenic action of oxamniquine may be attributable to the fact that it is not an intercalating agent.

Recently, Cioli (51) has studied the effect of in vitro exposure of schistosomes to oxamniquine, hycanthone, and some related drugs on the in vivo survival of these worms. Adult hycanthone-sensitive *S. mansoni* worms were exposed for one hour at 37°C to concentrations of the drugs listed in Table 1. At

Table 1 Survival of *S. mansoni* transplanted into Nile rats after in vitro exposure to drugs

Drug	Concentration	Worm recovery relative to untreated controls (%)		Egg counts relative to untreated controls (%)	
		Total	Males only	Liver	Intestine
Oxamniquine	10 μg/ml	70	67	66	98
	20 μg/ml	37	26	52	36
	50 μg/ml	30	3	23	4
UK-3883	50 μg/ml	121	120	98	133
Hycanthone	0.5 μg/ml	23	0	11	0.1
Lucanthone	10 μg/ml	113	116	97	129

the end of 21–30 days, live worms were recovered and the schistosome eggs were counted.

At concentrations of 50 μg/ml oxamniquine causes a marked reduction in both worm recovery and egg counts but is far less potent than hycanthone. The corresponding methyl precursors, UK-3883, 7($X = NO_2$, $R_1 = H$, $R_2 =$ isopropyl), and lucanthone 1 are inactive. However, when UK-3883 and lucanthone are administered orally to *S. mansoni*–infected mice and the synthesis of macromolecules measured in vitro in worms obtained 1–3 days post-treatment, both drugs inhibit uptake of ^{3}H-thymidine. These results support the previous conclusion that both UK-3883 and lucanthone must be bio-oxidized to furnish the active metabolites, oxamniquine and hycanthone. The uptake of ^{3}H-uridine by hycanthone-resistant schistosomes is inhibited in the presence of oxamniquine, but the inhibition disappears when the worms are thoroughly washed prior to measurement of the uptake of the pyrimidine base. When *S. japonicum* worms are exposed to oxamniquine, similar results are obtained. The lack of activity of oxamniquine is due to a deficiency in these particular species of the enzyme necessary to convert oxamniquine to the ester 10 (Figure 2).

1A-IV

A series of benzothiopyrano (4.3.2-cd) indazoles has been prepared by Elslager and his associates according to the scheme shown in Figure 3 (52–55). Treatment of the dichlorothioxanthenone, 13, with the dialkylaminoalkyl hydrazines 14 furnishes the benothiopyranoindazoles 15. Microbiological conversion with *A. sclerotiorum* gives the desired analogues of hycanthone, 16. This procedure is identical to the one used in the original preparation of

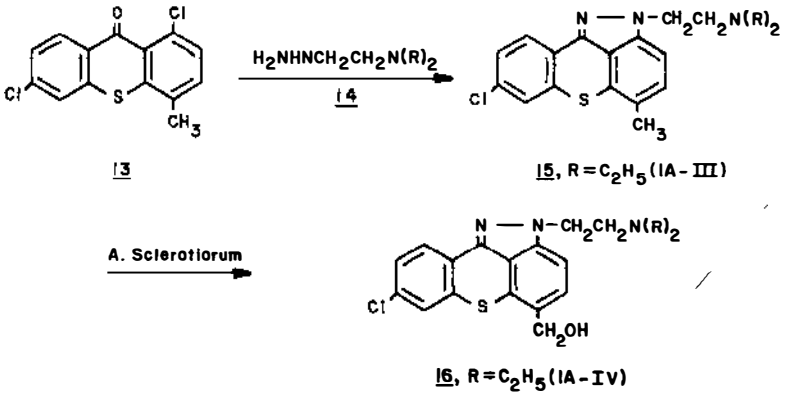


Figure 3 Synthesis of 1A-IV and analogues

hycanthone (1) except that, in the present instance, the hydrazines, 14, have been substituted for diethylaminoethylamine.

According to Elslager (55), 1A-IV and hycanthone are approximately equiactive when given intramuscularly, in the diet, or by gavage to mice infected with a Puerto Rican strain of *S. mansoni*. Single intramuscular injections of lucanthone or its analogue 1A-III (15, R = C_2H_5) have been ineffective in reducing the worm burden in hamsters, but hycanthone and 1A-IV have been curative in doses of 25–200 mg/kg. What is surprising is the observation that 1A-III, 1A-IV, and hycanthone are equally effective in mice when given in single intramuscular doses. Nevertheless, it has been concluded that 1A-IV is a bioactive metabolite of 1A-III.

Waring (56) has shown that 1A-III and 1A-IV reverse the supercoiling of closed circular duplex DNA and has concluded that these drugs are intercalating agents similar to lucanthone and hycanthone. The resemblance between hycanthone and 1A-IV is further strengthened by the finding that this pair of drugs is cross-resistant (57). Although the data on which to base a mechanism of action of 1A-IV are limited, it is tempting to postulate that 1A-IV and hycanthone act in a similar fashion, as shown in Figure 1.

The major difference between these drugs is that 1A-IV is claimed to be far less mutagenic than hycanthone (58, 59). Their mutagenic activity has been studied using the *S. typhimurium* strains TA-98 and TA-100 under in vitro and in vivo conditions. Waring (56) has commented that there does not appear to be a simple correlation between the ability of a drug to interact with DNA in the test tube and to induce mutagenesis in vivo. These observations can be rationalized on a molecular basis if we assume that 1A-IV is a poor substrate for the mammalian esterifying enzyme but a good one for the analogous enzyme in schistosomes. Investigations are in progress in the reviewer's laboratory to test this hypothesis.

PRAZIQUANTEL

A joint program sponsored by E. Merck, Darmstadt, and Bayer, A. G., on schistosomiasis chemotherapy has resulted in the discovery of the broad-spectrum schistosomicide, praziquantel, 18 (60). A vast literature of over 400 papers has appeared since the first publication on this drug, including a comprehensive review published in 1983 (61). Praziquantel is effective in mice, Syrian hamsters, and the multimammate rat, *mastomys natalenisis,* experimentally infected with *S. mansoni, S. haemotobium, S. japonicum, S. intercalatum,* and *S. matthei*. The drug is effective against young and adult *S. mansoni*. Praziquantel is active in monkeys and baboons infected with *S. haemotobium, S. mansoni,* and *S. japonicum* (62).

Extensive structure-activity studies have been carried out with a series of congeners of 18. The two most critical structural features of praziquantel are position −4 and position −2. An oxo group must be present at C-4; compounds with different substituents at this position are inactive in vivo and in vitro. Maximum activity has been observed when a cyclohexylcarbonyl group is present at position −2, but the p-aminobenzoyl and benzoyl analogues are also quite active. It should be noted that praziquantel possesses a chiral center at position C−11b. A precursor of 18 has been resolved and the optical isomers converted to (+) and (−) -praziquantel. Only the latter is biologically active (60, 61).

Praziquantel itself has a rapid onset of action both in vivo and in vitro. The drug appears to be less potent in mice than in larger animals, but this difference has been attributed to the relatively rapid drug elimination in mice (63).

A multi-center clinical trial of praziquantel was carried out using patients infected with *S. haemotobium, S. japonicum,* and *S. mansoni* in such countries as Brazil, Japan, the Philippines, and Zambia (64, 65). The initial double blind study used doses of 1 × 20 mg/kg, 2 × 20 mg/kg, and 3 × 20 mg/kg. This was followed by a single blind trial in Zambia using two different regimens; one employed a dose schedule of 3 × 20 mg/kg given at four-hour intervals and the other was a single oral dose of 50 mg/kg. Regardless of the dose regimen, there was only one failure in the 73 patients, who were followed for six months. Tolerance to the drug was very good. Since the initial clinical trials a number of other studies have been performed. The cure rates were uniformly excellent in all, regardless of the nature of the schistosome infection. The most common side effects are abdominal pain, headache, dizziness, and skin involvement, such as urticaria, which may be an allergic response to dying parasites. The currently recommended dosing schedules are: a single oral dose of 40 mg/kg for *S. haemotobium* and *S. mansoni* infections and 2 × 30 mg/kg given in divided doses in one day for *S. japonicum* (63). Praziquantel is well absorbed when given orally to man or animals. When ^{14}C-labelled drug is administered orally

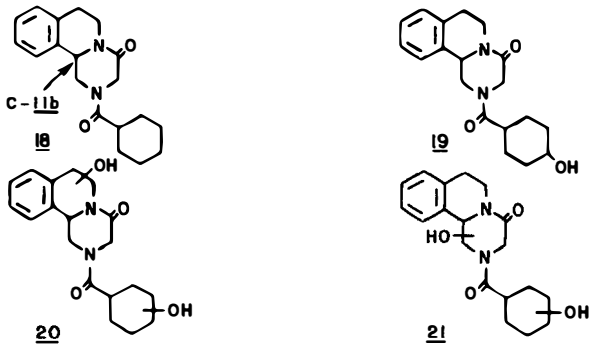


Figure 4 Metabolites of praziquantel

to volunteers in doses of either 20 mg/kg or 50 mg/kg, the maximum serum concentrations of unchanged praziquantel vary between 0.2 μg/ml and 1.0 μg/ml reached one to two hours post-administration. It was demonstrated earlier that at concentrations of 1 μg/ml the drug is lethal to adult *S. mansoni* worms (63). Praziquantel appears to be extensively metabolized, since only 5 to 7% of the total radioactivity present in the serum is unchanged drug. The half life of unchanged drug is about 1.5 hours. Between 80–85% of the ^{14}C-label is excreted via the kidney within four days. Thus, the drug and its metabolites are rapidly absorbed, rapidly metabolized, and rapidly eliminated. The major metabolite of 18 in man is the monohydroxylated compound 19, whose structure is secure. Two minor metabolites, probably derived by further oxidation of 19, are dihydroxylated derivatives, which on the basis of mass spectroscopic studies have been provisionally assigned structures 20 and 21 (see Figure 4) (63). In all likelihood, the position of the hydroxyl group in the cyclohexane ring of 20 and 21 is the same as it is in 19. The other hydroxyl in 21 is probably located at C−11b, because this is a benzylic carbon adjacent to a nitrogen atom.

Praziquantel is effective in patients infected with hycanthone- and oxamniquine-resistant *S. mansoni* (66). It is not mutagenic in a number of different species, including bacteria, yeasts, insects, and mammalian cells (67). The drug has no effect on DNA or protein synthesis and does not affect the uptake of nucleic acid precursors (63). On the basis of these observations, the mode of action of praziquantel clearly differs from those of hycanthone and oxamniquine.

Direct exposure of adult schistosomes to praziquantel results in immediate tetanic contraction of the musculature followed by a rapid vacuolization of the syncytial tegument. These effects occur at drug concentrations in the same range as therapeutic serum levels. In vitro, ^{14}C-praziquantel is rapidly taken up by schistosomes, but after transfer to a drug-free medium 93% of the radioactivity disappears from the worms. Although praziquantel is not an ionophor,

the rapid contraction of the worms has been attributed to a change in calcium flux. In male schistosomes, praziquantel causes a rapid uptake of calcium ion with a concomitant loss of potassium. The muscle contractions can be abolished by lowering the ambient calcium ion levels or by increasing the concentration of magnesium ion (68). Vacuolization of the tegument is reversible and thus is not a lethal event (69). Death of the schistosome occurs when tegumental damage becomes severe and irreversible. Despite the body of evidence that points to the tegument as the locus of the schistosomicidal action of praziquantel, the exact mechanism whereby lethality is produced is not yet understood.

The general consensus at present is that praziquantel is effective orally in treating all human forms of schistosomiasis, is relatively well-tolerated, and thus far has produced no known cases of drug resistance. It is rapidly becoming the drug of choice for treating schistosomiasis.

OLTIPRAZ

Barreau and his colleagues synthesized oltipraz [4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione] 22 in 1976 (70). The drug is an effective schistosomicidal agent against experimental *S. mansoni* infections in mice and monkeys. In animals, oltipraz has little or no effect on the cardiovascular, respiratory, or central nervous systems. It responds negatively in the usual laboratory tests for mutagenic and immunosuppressant activity (71). In human *S. mansoni* infections, 3.0–4.5 g of the drug given in three divided doses in one day proved to be curative (72). Similar results have been reported from the Sudan. Two groups of young males were given 25 mg/kg and 35 mg/kg in two divided doses in one day. Cure rates of greater than 90% were observed. The most frequent side effects were vomiting and mild abdominal pain (73). Oltipraz is also effective in treating *S. haemotobium* and *S. intercalatum* infections. The most frequent side effects, in addition to those observed in other studies (73), are headache and paresthesias of the extremities, which appear to increase after exposure to sunlight. The cure rate in the *S. haemotobium* patients was 90% in those receiving a total dose of 25 mg/kg given in one or two days, and 87% in the *S. intercalatum*-infected patients, who received total doses ranging from 1.25 g –4.50 g over a three-day period. These clinical results suggest that oltipraz is a broad-spectrum, orally effective antischistosomal drug (74).

In contrast to praziquantel, oltripraz is a very slow-acting drug; approximately two months are required before the full schistosomicidal effects become manifest. One of the first signs of schistosomicidal activity of a drug in laboratory *S. mansoni* infections is the shift of the worms from their usual habitat in the mesenteric veins to the liver. This effect was first reported by Bang & Hairston in 1946 (75). In the case of oltipraz, the hepatic shift does not

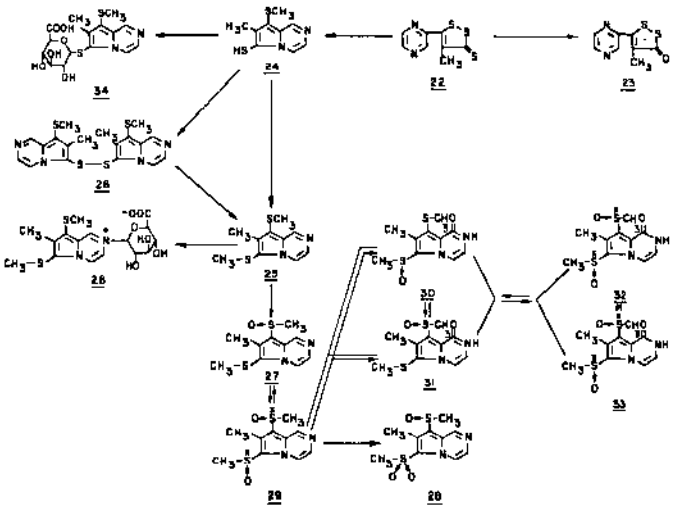


Figure 5 Metabolites of oltipraz

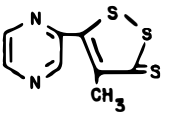
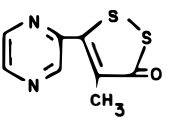
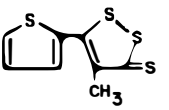
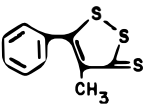
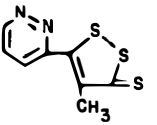
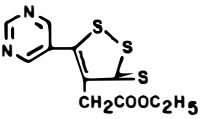
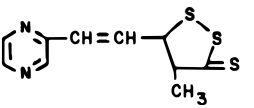
occur until nine days after drug administration (76). The first major effect of oltipraz observed is a reduction of schistosomal glutathione (GSH) levels. For example, two days after administration of 150 mg/kg of the drug to *S. mansoni*-infected mice, the GSH levels in the worm isolated from the mesenteric veins fall from 2.36 μM/g in untreated mice to 1.42 μM/g in those receiving the drug. This is a transient effect, since over the course of the next 15 days GSH levels return to control levels and then fall again. At higher doses, the GSH levels are depressed for several days before returning to normal, and at 250 mg/kg there does not appear to be any significant early reduction in GSH levels. However, at the higher doses, the initial control values are lower than normal. Despite these variable results, there does seem to be a distinct trend toward initial lowering of GSH levels in the schistosomes. In a series of oltipraz congeners, there seems to be a correlation between antischistosomal activity and lowering of GSH levels (Table 2).

Bieder and his associates have performed a thorough study of the metabolism of ^{14}C-oltipraz in rodents, monkeys, and man (77). Thirteen metabolites were isolated, purified, and identified. Their structures and interrelationships are shown in Figure 5.

With the exception of 23, which is the direct hydrolysis product of 27, all of the other metabolites result from a deep-seated rearrangement of oltripraz that this reviewer postulates to proceed via the unstable intermediate 24. The rest of the metabolites can be derived from 24, as shown in Figure 5. The percent distribution of the metabolites in rodents and monkeys is given in Table 3.

One possible mechanism that accounts for the formation of metabolites 25

Table 2 Antischistosomal activity and the effect on GSH of oltipraz congeners

Number	Structure	Antischistosomal activities	Single oral dose (mg/kg)	GSH levels[a]
35972 RP Oltipraz		+	250	1.42
36,642 RP		−	250	2.39
36,731 RP		−	250	2.42
37,528 RP		−	250	2.39
36,733 RP		+	50	1.70
38,650 RP		+	50	1.70
40,863 RP		−	200	2.37

[a]GSH content of worms two days after drug administration (μM/g). Control values were in the range 2.19–2.63 μM/g.

and 34 is shown in Figure 6. This differs from the scheme suggested by Bieder et al (77) in that GSH rather than CH_3S^- initiates the rearrangement. The methylation of the thiols can take place via well-known methyl transfer reactions. This scheme requires the intervention of mammalian glutathione, even though Bueding has been unable to show any reduced GSH levels in the host (76).

Very little is known about the mode of action of oltipraz. As far as this

Table 3 Percent distribution of the principal metabolites of ^{14}C-oltipraz in non-hydrolyzed urine[a]

Metabolites	Species		
	Mouse	Rat	Monkey
22, 23, 25, 26	9.4	14.4	7.9
27, 28	15.6	12.1	3.4
29	35.8	16.9	2.8
30	<1.0	2.0	8.1
31	8.2	26.1	15.2
32, 33	5.5	5.3	25.0
34, 35	20.5	23.2	47.6

[a]A possible mechanism to account for the formation of metabolites 25 and 34 is shown in Figure 6. This differs from the scheme suggested by Bieder et al (77) in that GSH rather than $\overline{CH_3S}^-$ initiates the rearrangement.

reviewer is aware, no studies have been reported on either the interaction of oltipraz with or the effect on the biosynthesis of macromolecules. Any proposed mechanism must account for the very slow onset of the drug and its effect on schistosomal GSH levels. It is tempting to suggest that a scheme slightly modified from that shown in Figure 6 can be used to account for the mode of action of oltipraz. As shown in this scheme, ring closure of 36 occurs by a nucleophilic attack of a pyrazine nitrogen atom on the carbon in the dithio ester, 36. In the schistosome this electrophilic carbon atom reacts with a macromolecule to give a covalently bound drug complex. If the macromolecule is an essential enzyme, then such a reaction could result in the slow death of the parasite. An investigation into the question of whether oltipraz does bind covalently in the schistosome would be a welcome test of the plausibility of this suggestion.

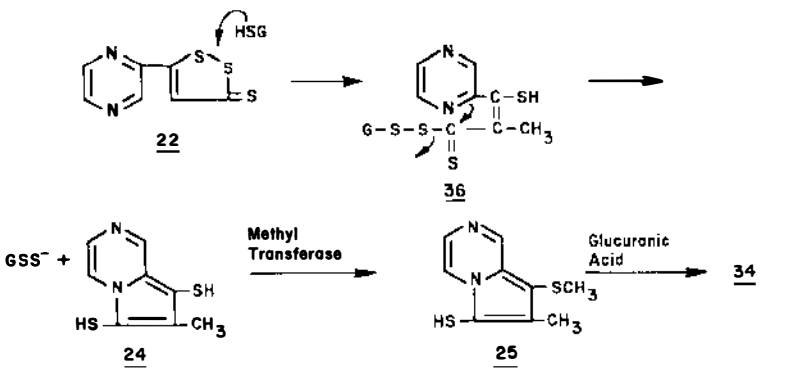


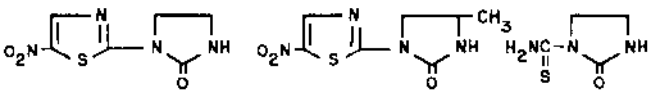
Figure 6 Proposed mode of action of oltipraz

NIRIDAZOLE

Niridazole, 39, a nitrothiazole, was first reported to have schistosomicidal activity over 20 years ago (78). The early studies were reviewed in 1972 (1); thus, this review deals with more recent developments.

The drug is an effective schistosomicide against *S. mansoni, S. haemotobium,* and *S. japonicum* infections in man (78, 79). The usual dose is 25–30 mg/kg given over a period of five to ten days. Factors other than the need for multiple dosing and the occurrence of occasional severe CNS side effects, which militate against the clinical acceptability of niridazole, are immunosuppression (80), mutagenicity, and carcinogenicity (81–81b). Recent studies support the hypothesis that some of the side effects of niridazole, in addition to its effectiveness as an antischistosomal compound, are related to the metabolism of the drug (82).

Faigle & Kebule showed that schistosomes absorb ^{14}C-niridazole and convert it to unidentified metabolites (83). Tracy, Catto, & Webster found that the radio label is bound covalently to schistosomal macromolecules (84). For example, when adult *S. mansoni* worm pairs are incubated with 70 μM of ^{14}C-niridazole for sixteen hours, about 30% of the total radioactivity that the parasites incorporate is precipitable with trichloroacetic acid (TCA). Vigorous attempts to dissociate the radiolabel from the TCA-precipitated material have been unsuccessful. About 85–90% of the radioactivity is associated with the protein fractions, 3–5% with RNA, and 4–7% with DNA. When this experiment is carried out in *S. mansoni*-infected mice, about 43% of the total parasite radio label is covalently bound to the macromolecular fraction, showing that covalent drug binding occurs under therapeutic conditions as well as in vitro.



39 40 41 2.

Covalent drug binding has been demonstrated using cell-free schistosome preparations. Under these conditions, it has been found that the initial step in the metabolism of niridazole involves an NADPH-dependent enzymatic reduction of the nitro group. Such a reduction may lead to an as yet unidentified drug metabolite that can bind covalently to schistosomal macromolecules. 4'-Methylniridazole, 40, is inactive as a schistosomicide (84). The compound is taken up by adult worms but, in contrast to niridazole, 40, is recovered unchanged. Thus, 40 is not a substrate for schistosomal nitro-reductase. Blumer et al (85) found that the antibiotic and mutagenic activities of niridazole toward auxotrophs of *S. typhimurium* are linked to the levels of bacterial nitro

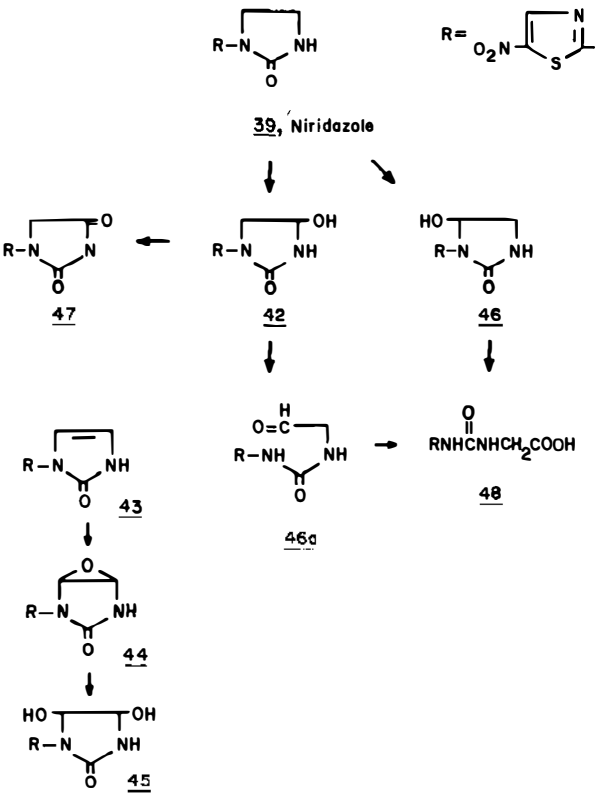


Figure 7 Metabolism of niridazole

reductase. Resistant strains are less sensitive to the mutagenic effects of the drug and have lower levels of nitro reductase.

An immunosuppressive metabolite was isolated from the urine of rats and of a patient treated with niridazole (86). The compound was subsequently shown to be 41 (87, 88). The compound is not formed in the liver but is produced by intestinal flora (89). Most, if not all, of the immunosuppressant action of 39 is due to this metabolite (90).

In contrast to the reductive metabolism of niridazole that is the predominant pathway in the schistosome, mammalian metabolism of the drug is oxidative in nature (82, 87). Hepatic microsomes obtained from DBA/2J mice were incubated with either niridazole or its metabolites in the presence of NADPH. Ethyl acetate extracts of the mixtures were analyzed by high-performance liquid chromatography (hplc). The oxidative metabolic pathway is shown in Figure 7.

Niridazole is hydroxylated to give 4- and 5-hydroxyniridazole (42 and 46 respectively). The former is dehydrated to afford 4,5-dehydroniridazole, 43,

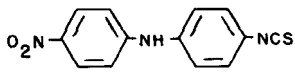
which is oxidized to the epoxide, 44. Finally, 44 is hydrated to give the diol, 45. 4-Ketoniridazole, 47, is formed from 4-hydroxyniridazole and not from either the dehydro derivative, 43, or the epoxide 44. 4-Hydroxyniridazole, 46, exists in tautomeric equilibrium with the open-chain form 46a, which is oxidized to the hydantoic acid, 48, by means of a NAD^+-dependent aldehyde dehydrogenase.

The delayed CNS toxicity that is an occasional toxic manifestation of niridazole therapy may be due to a metabolite rather than to the drug itself (87). 4-Ketoniridazole, 47, is more potent than niridazole in producing CNS toxicity, but the overt signs are different (91). On the other hand, the delayed onset of CNS toxicity has been correlated with oxidative metabolism of the drug.

The microsomal oxidative metabolism of ^{14}C-niridazole leads to covalent drug binding, and the requirements for such binding are the same as those necessary for oxidative metabolism. The 4,5-diol, 45, is the only stable end product of the microsomal oxidation of 4,5-dehydroniridazole, 43. Blumer et al have suggested that the reactive epoxide, 44, may be responsible for niridazole-induced tumor formation (85). The recent investigations of Webster and his colleagues (82) have established in part the molecular basis both for the antischistosomal action of niridazole and for the toxic effects of the drug. Although the proximate active metabolites remain to be identified, it is clear that the pathways leading to the antischistosomal action are different from those leading to the toxic effects of the drug.

AMOSCANATE

In 1976, Striebel (92) reported that 4'-isothiocyanato-4'-nitrodiphenylamine, amoscanate, 49, was an anthelmintic, active against intestinal nematodes, filariae, and schistosomes.



49, Amosconate 3.

The yellow crystalline compound is insoluble in water and common organic solvents. This lack of solubility has created problems from the beginning with respect to proper dosage forms of the drug. The first experimental studies were carried out with material having a particle size of 30–50 μm. The curative single oral dose against *S. mansoni* in mice was 300 mg/kg. When micronized material was used, this dose dropped to 120 mg/kg. Ball mill treatment for 14 days in a suspension of 1% Cremophor EL (BASF) and 25% glycerol reduced the particle size to 0.5 μm. With particles of this size, the curative dose was 5–7.5 mg/kg (94). Amoscanate is active against hycanthone-resistant strains of

S. mansoni. Bueding also has reported that amoscanate is not mutagenic when tested against Salmonella strains TA-98 and TA-100 in either the presence or absence of liver microsomes (93). However, six species of mammals receiving 49 excreted an unidentified mutagenic metabolite probably produced by intestinal flora rather than by the metabolic action of the hosts (94).

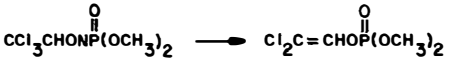
Amoscanate has proved effective in experimental *S. haemotobium* and *S. japonicum* infections in mice (92) and is active in monkeys against the three common human forms of schistosomiasis. It does not produce any major organ toxicity (95). The drug is also active in humans (96).

Little is known about either the metabolic fate or the mode of action of amoscanate. Striebel (96a) has reported that the compound binds irreversibly to the amino groups of amino acids and proteins in vitro and in vivo by reaction with the isothiocyanate to form thiourea derivatives. About 80% of the drug in plasma is precipitated by perchloric acid and is not extractable with acetone. Presumably, the drug is covalently linked to plasma proteins.

Voge & Bueding have studied the tegumental alterations in *S. mansoni* induced by subcurative doses of amoscanate by means of scanning electron microscopy (97). The changes noted include swelling, constriction, and erosion of large surface areas. Most of the lesions were completely repaired 62 days after exposure to the drug. The auth
surface changes are the result of drug-induced functional damage regardless of the type of drug used.

METRIFONATE

Metrifonate (trichlorfon, bilarcil) is an organophosphorous compound that by virtue of its inhibitory affect on cholinesterases has been used as an insecticide under the names Dipterex and Dylox. The compound is an effective schistosomicide in animals (98–100).



50, Metrifonate 51, Dichlorvos 4.

Whatever side effects are produced result from the expected depression of plasma cholinesterase levels. The drug shows moderately high host-mediated mutagenic activity in *S. typhimurium* strain TA-100 (11) but is neither teratogenic nor carcinogenic in healthy rats and dogs (101).

It is generally believed that the schistosomicidal activity of metrifonate is not mediated via the drug itself but by its metabolite, dichlorvos, 51, which is a far

more potent inhibitor of cholinesterase (102–104). The potency of metrifonate increases with pH and time, factors that do not affect the activity of 51. After metrifonate administration to patients, the plasma levels of 50 and 51 were 31 μM and 0.3 μM respectively. This level of dichlorvos is sufficient to cause a substantial inhibition of cholinesterase within fifteen minutes.

The most surprising aspect of the therapeutic profile of metrifonate is that the drug does not appear to be effective against human *S. mansoni* infections but it is quite active against *S. haemotobium* in man despite the fact that both species of schistosomes are susceptible to the drug in vitro (102, 105). The reasons for this discrepancy are not clear.

CONCLUDING COMMENTS

During the past fifteen years, major advances have occurred in the chemotherapy of schistosomiasis. Three new broad-spectrum compounds, amoscanate, oltipraz, and praziquantel, have appeared on the therapeutic horizon. Praziquantel has had extensive clinical use and has established itself as a drug of choice for the treatment of all human forms of the disease. These drugs emerged from routine screening of compounds: the serendipitous method of drug discovery has again produced active compounds. Unfortunately, the mechanisms by which these drugs exert their effects are not known. Considerable progress has been made with regard to the molecular basis for the action of two drugs that have fallen out of clinical favor in recent years, namely, hycanthone and niridazole. Insights into the modes of action of these drugs can be exploited to design new molecules free of the undesirable effects of the parent drugs. This has been accomplished inadvertantly in the case of oxamniquine. Its mechanism of action in schistosomes is thought to be almost identical with that of hycanthone but, because of highly significant structural differences, its mammalian effects are far more benign.

Investigators have begun to apply rational design to the chemotherapy of schistosomiasis. elKouni and his colleagues (106) have shown that the pathways in de novo pyrimidine biosynthesis in *S. mansoni* differ from the mammalian pathway. This difference, as well as some others, can be exploited by offering the chemist targets for new synthetic enzyme inhibitors.

Finally, from the clinical standpoint, the future looks brighter than ever for the more than 200 million individuals who suffer from schistosomiasis. The newer drugs can be given in one or two doses and are reasonably well-tolerated. As a result, widespread clinical therapy is now possible for the first time.

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