General Procedure for N-5-Alkylation of 3-Alkylthiotriazinoindoles. NaH (4.8 g, 50% dispersion in oil; 0.104 mole) was added to a stirred suspension of the appropriate 3-alkylthio compd (0.1 mole) in anhyd $HCONMe_2$ (200 ml) and the appropriate alkyl halide (0.106 mole) in anhyd C_6H_6 was then added and the mixt refluxed for 24 hr, cooled, and filtered. After the removal of solvent, the residue was extd with hot cyclohexane or EtOH. On cooling the product crystd. For analysis, a sample was recrystd from MeOH or EtOH.

General Procedure for 3-Aminotriazinoindoles. A soln of the appropriate thione or 3-alkylthio compd and the amine (3 ml/g of S compd) was heated at 160–180° until the evolution of H_2S or alkanethiol was complete (5–6 hr). For low boiling amines, an EtOH soln in a pressure vessel was used. On cooling, the mixt was stirred with an excess of H_2O, and the product was filtered off, washed with H_2O, and dried. For analysis, a sample was recrystallized from EtOH.

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Anthelmintic Activity in Sheep of Some Compounds Related to Pyrantel and Morantel

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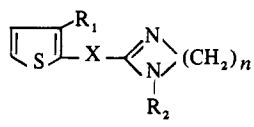
The anthelmintic activity in sheep of pyrantel, morantel, and other cyclic amidines, of a series of thiazoline and dihydrothiazine analogs of pyrantel, and of a related series of 1-(2-arylvinyl)pyridinium compounds correlated with those previously reported for the *Nematospiroides dubius* rodent screen. The rodent screen was shown to be a good early indicator for activity against the gastrointestinal nematodes of sheep for these classes of compounds, although it became clear subsequently that for some species, *e.g.*, *Trichostrongylus colubriformis*, the screen was not always valid.

The discovery of a new series of highly active anthelmintic compounds exhibiting broad spectrum activity in domestic animals has been reported.[1] Details of the structure-activity relationship of a large number of compounds in this and closely related series using the mouse nematode *Nematospiroides dubius* have been published.[2-5] Two compounds, *trans*-1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)vinyl]-pyrimidine (pyrantel)† and its 3-methyl-2-thienyl analog (morantel),‡ have been extensively evaluated in sheep,[6-9] cattle,[10] pigs,[11,12] horses,[13-14] dogs,[15-18] and man.[19,20]

Evaluation of these series in sheep has involved primarily the testing of compounds showing sufficient promise in the primary mouse screen. Some compounds showing little activity in mice have, however, also been tested in sheep because of the need to establish a correlation between the

Table I. Pyrantel, Morantel and Other Thiophene-Substituted Cyclic Amidines

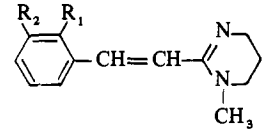
Compound number[a]	X	*n*	R_1	R_2
				
1	CH_2S	2	H	H
8	CH_2CH_2	2	H	H
10	CH_2CH_2	3	H	H
21/22	CH_2CH_2	2	H	CH_3
37/38	CH_2CH_2	3	H	CH_3
66	CH=CH	2	H	CH_3
70	CH=CH	3	H	H
71 (pyrantel)	CH=CH	3	H	CH_3
74 (morantel)	CH=CH	3	CH_3	CH_3
78	CH=CH	3	C_2H_5	CH_3
79	CH=CH	3	Br	CH_3

[a]Number corresponding with identification used in McFarland, *et al.*[2]

†Pyrantel tartrate, Banminth, Strongid.

‡Morantel tartrate, Banminth II.

Table II. Substituted Styryl Tetrahydropyrimidines

Compound number[a]	R_1	R_2
		
82	H	H
84	CH_3	H
93	Cl	H
97	Br	H
102	H	OCH_3
103	OC_2H_5	H
106	NO_2	H

[a]Corresponding to number used for identification in McFarland, *et al.*[2]

Table III. Anthelmintic Activity in Sheep of Compounds Shown in Table I. Worm Counts.

Compound	Salt	Dosage, mg/kg	No. of sheep	Infection	Day of infection when treated	% efficacy in controlled trials[a]										
						Abomasum				Small intestine				Large intestine		
						H	*O*	*T*	*Imm*	*Tr*	*N*	*C*	*Imm*	*Oes*	*Trich*	*Chab*
1	Pamoate	40	4	Natural	Adult	48	52	78		55	92					
8	HCl	40	4	Natural	Adult	45	47	92		41		90				55
10	HCl	40	4	Natural	Adult	88	86	91		75	100	100				95
		60	9	Expt *N. battus*	7, 14, and 21						100					
21	Tosylate	25	4	Natural	Adult	92	93	93	98	62	31	77	40	0	0	100
37	Tosylate	25	4	Natural	Adult	0	53	36	65	34	98	96	20	100	0	32
		50	16	Natural	Adult	97	98	99	98	84	100	100	20	100	93	100
		50	12	Expt *N. battus*	3, 7, 14, and 21						100					
	Citrate	50	10	Natural	Adult		100	98	99	80	100	100	64			
38	Tartrate	45	13	Natural	Adult	100	97	98	89	84	100	100	95			
71 (pyrantel)	Citrate	50	5	Natural	Adult		100	100	100	100	100	100	75			
	Tartrate	12.5	9	Natural	Adult	100	90	100	87	87	100	100	94	99	30	93
		12.5	9	Expt *T. col. N. battus*	7, 14, and 21					52–84	99–100					
		25	9	Expt *T. col. N. battus*	7, 14, and 21					81–100	100					
		50	4	Natural	Adult		97	100	94	97	100		98		80	100
	HCl	12.7	4	Expt *H. cont. T. col.*	Adult	99				68						
		17	4			99				87						
74 (morantel)	Fumarate	12.5	4	Natural	Adult	100	100	100	100	82	100	100	96		0	88
			8	Expt *T. col.*	Adult					84						
		25	4	Natural	Adult	100	100	100	100	85	100	100	97		0	100
			8	Expt *T. col.*	Adult					99						
	HCl	2.5	5	Natural	Adult	94	91	98		64	100	100				
		7.5	5	Natural	Adult	100	98	100		90	100	100				
		10	5	Natural	Adult	100	99	99		97	100	100				
74 (morantel)	HCl	5	12	Expt *H. cont. T. col.*	Adult	97				89						
		10	18		Adult	99				96						
	Tartrate	3	5	Natural	Adult	93	85	85	87	23	98	100	63	93	66	100
		6	5	Natural	Adult	100	100	100	100	85	100	100	100	85	12	50
		10	4	Natural	Adult	100	100	100	100	98	100	100	100	98	0	97
		25	5	Natural	Adult	100	100	100	100	100	100	100	100	100	61	100
		10	16	Expt *H. cont. T. col. N. battus*	3, 7, 14, and 21	87–100				90–99	99–100					
78	Tartrate	3	4	Expt *T. col.*	Adult					20						
		6	4		Adult					74						
		12.5	4		Adult					96						
		22.4	4	Expt *H. cont. T. col.*	Adult	99				99						

[a] *T. col. = Trichostrongylus colubriformis; H. cont. = Haemonchus contortus; H = Haemonchus; O = Ostertagia; Tr = Trichostrongylus; N = Nematodirus; C = Cooperia; Imm = unidentified immature 4th stage; T = Trichostrongylus axei; Oes = Oesophagostomum; Trich = Trichuris; Chab = Chabertia.*

mice and sheep results and so validate the primary screening procedure. A total of 34 compounds has been evaluated, some in more than one salt form, in controlled trials yielding worm burden data or in egg count reduction trials.

Experimental Section

Worm Count Reduction Trials. Natural Infections. Sheep naturally infected with a range of species were purchased from farms with a history of parasitic gastroenteritis. Animals were allocated to treatment and control groups on the basis of fecal egg count. Worms were recovered separately from the various parts of the alimentary tract 5–7 days after treatment, counted, and identified according to standard methods.

Experimental Infections. Pure strains of *Haemonchus contortus*, *Trichostrongylus colubriformis*, and *Nematodirus battus* were maintained in culture animals and groups of worm-free lambs infected with 1 or more of these species. Animals were randomly assigned to equal treated and control groups. Activity against adult worms was assessed by treatment when the infections had become patent, and the animals were killed for worm counts 5 days later. Activity against immature stages was assessed by treatment at various times during the prepatent stage. All animals were killed together when the worms in controls and those remaining in treated groups had reached the adult stage.

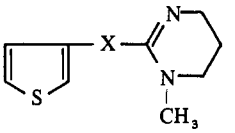
Egg Count Reduction Trials. Natural Infections. Sheep harboring natural infections and showing moderate to high McMaster egg counts[21] were grouped equally into treated and control groups according to egg counts based on 2 or 3 samples. The reduction in egg count compared with the pretreatment level was assessed 5–7 days after treatment provided the control animals did not show a marked alteration during the treatment period. Pretreatment feces samples were subjected to culture for identification of third stage larvae of the species present.[22]

Experimental Infections. Worm-free lambs infected with cultures of *T. colubriformis*, *H. contortus*, or both were equally grouped into treatment and control groups. The mean of 2 or 3 samples before treatment was compared with the mean of similar samples 3–7 days posttreatment, and the degree of egg count reduction was calculated.

Results and Discussion

For convenience the compounds tested are considered in the same chemical groups as those referred to in papers on the structure-activity relationships in rodents and are identified by the same numbering, *viz.* pyrantel and other cyclic amidines;[2] 1-(2-arylvinyl)pyridinium salts[3] and thiazoline and dihydrothiazine analogs of pyrantel.[5]

Pyrantel and Other Cyclic Amidines. The chemical structures of compounds within this group which have been tested in sheep are shown in Tables I and II, and in the structure (A, B) below and the parasitological details of



A, X = CH=CH
B, X = CH_2CH_2
3-thienyl analogs

worm count trials in Tables III and IV and egg count trials in Table V. These compounds possess broad spectrum activity against the range of gastrointestinal helminth infections of sheep. Activity of an especially high order was demonstrated throughout the series against the important abomasal species *H. contortus*, *Ostertagia circumcincta*, *O. trifurcata*, and *T. axei* and against the intestinal genera *Nematodirus* and *Cooperia*. Marginally less activity was apparent against *T. colubriformis* and *T. vitrinus*, while an appreciable level of activity against *Trichuris ovis* is demonstrated at high doses. Information on *Oesophagostomum columbianum* and *Oes. venulosum* and *Chabertia ovina* is limited but sufficient to show a high level of activity with most members of the series.

Table IV. Anthelmintic Activity of Compounds Shown in Table II, and of Dihydrothiazine Analogs and of Thiophene-Substituted Noncyclic Amidines. Worm Count Trials.

							% efficacy in controlled trials							
							Abomasum			Small intestine			Large intestine	
Class	Compound No.	Salt	Dosage, mg/kg	No. of sheep	Infection	Day of infection when treated	*H*	*O*	*T*	*Tr*	*N*	*C*	*Imm*	*Trich*
II[d]	82	Tartrate	25	3	Expt *T. col.*	Adult				0				
II	84	Fumarate	25	4	Expt *T. col.*	Adult				29				
		Tartrate	22	5	Natural	Adult	100	100	100	70	100			
			33	5	Natural	Adult	100	100	100	88	99			
			50	5	Natural	Adult	100	100	100	94	100			
II	93	Tartrate	25	3	Expt *T. col.*	Adult				39				
			49	5	Expt *T. col.* and *H. cont.*	Adult	100			81				
b	63	Br^-	12.5	3	Expt *T. col.*	Adult				51				
			25	4	Expt *T. col.*	Adult				93				
b	66	Br^-	12.5	3	Expt *T. col.*	Adult				0				
			25	3	Expt *T. col.*	Adult				30				
			30	4	Expt *T. col.* and *H. cont.*	Adult	100			88				
b	66	Cl^-	7.5	6	Expt *T. col.*	Adult	37			10				
			15	6	and *H. cont.*	Adult	97			62				
			30	11	Expt *T. col.* and *H. cont.*	Adult	99			80				
			34	4	Expt *T. col.* and *H. cont.*	Adult	98			92				
			50	15	Expt mixed	7, 17, and 21	99–100	100	100	98–99	86–95			
b	75	Br^-	30	5	Expt *T. col.*	Adult	89			45				
			40	10	and *H. cont.*	Adult	98			67				
			53	5		Adult	100			76				
c	9	HCl	25	4	Expt *T. col.*	Adult				99				

[a] *T. col.* = *Trichostrongylus colubriformis*; *H. cont.* = *Haemonchus contortus*; *H* = *Haemonchus*; *O* = *Ostertagia*; *T* = *Trichostrongylus axei*; *Tr* = *Trichostrongylus*; *N* = *Nematodirus*; *C* = *Cooperia*; *Trich* = *Trichuris*; *Imm* = *unidentified immature 4th stage*. [b] Dihydrothiazine analogs. [c] Thiophene-substituted noncyclic amidines. [d] Roman numerals indicate Table in which structure is to be found.

Table V. Anthelmintic Activity of 3-Thienyl Analogs, Thiophene-Substituted Noncyclic Amidines, Dihydrothiazine Analogs, and Compounds Shown in Tables I, II, and VI. Egg Count Reduction Trials.

Class	Compound No.	Salt	Dosage, mg/kg	No. of sheep	Infection	Larval differentiation[a] Pretreatment, % *H*	*O*	*T*	*C*	*Chab*	*Oes*	Egg count reduction, % *Trichostrongyles*	*Nematodirus*
I[b]	66	Tosylate	11.3	4	Expt *T. col.* and *H. cont.*				Nd			0	
I	70	Maleate	9.7	4	Expt *T. col.* and *H. cont.*				Nd			0	
I	78	Fumarate	25	3	Natural	48	17	15	4	16		93	100
II	93	Tartrate	9.5	4	Expt *T. col.* and *H. cont.*				Nd			48 86	
II	97	Tartrate	15	4	Expt *T. col.* and *H. cont.*				Nd			0	
II	97	Tartrate	25	4	Natural	15	7	70	3	2	3	87	100
II	102	Bicarbonate	25	4	Expt *T. col.* and *H. cont.*				Nd			0	
II	103	Tartrate	25	4	Expt *T. col.* and *H. cont.*				Nd			0	
VI	2	Br^-	25	3	Natural	35	8	51	1	5		48	100
VI	62	Br^-	25	4	Expt *T. col.* and *H. cont.*				Nd			0	
VI	66	Br^-	25	3	Natural	48	17	15	4	16		97	100
			6	4	Expt *T. col.*				Nd			69	100
			15	4	and *H. cont.*				Nd			73	100
VI	115	Cl^-	25	4	Expt *T. col.* and *H. cont.*				Nd			0	
VI	116	Cl^-	15	4	Expt *T. col.* and *H. cont.*				Nd			0	
c	6	Fumarate	25	5	Expt *T. col.* and *H. cont.*				Nd			80	
c	21	Pamoate	45	4	Natural		36	48	8	8		57	100
c	9	HCl	6	4	Natural				Nd			77	88
			25	4	Expt *T. col.* and *H. cont.*				Nd			77	
c	9	Pamoate	10	2	Expt *T. col.*				Nd			0	
			50	4	and *H. cont.*				Nd			96	
c	41	HCl	25	4	Expt *T. col.* and *H. cont.*				Nd			74	
c	45	HCl	25	4	Expt *T. col.* and *H. cont.*				Nd			55	
d	10	HCl	25	5	Expt *T. col.* and *H. cont.*				Nd			50	
d	36	Tosylate	25	5	Expt *T. col.* and *H. cont.*				Nd			50	
e	A	Fumarate	25	3	Natural	35	8	51	1	5		63	100
e	B	Fumarate	25	5	Expt *T. col.* and *H. cont.*				Nd			0	

[a] *T. col.* = *T. colubriformis*; *H. cont.* = *H. contortus*; *H* = *Haemonchus*; *O* = *Ostertagia*; *T* = *Trichostrongylus axei*; *C* = *Cooperia*; *Chab* = *Chabertia*; *Oes* = *Oesophagostomum*; Nd = not done. [b] Roman numerals indicate table where structure is to be found. [c] Dihydrothiazine analogs. [d] Thiophene-substituted noncyclic amidines. [e] 3-Thienyl analogs.

Generally speaking, a higher order of activity is shown by the compounds with the larger basic ring, *i.e.*, where $n = 3$. Thus, **10** is more active than **8**, **37** more active than **21**, and **71** more active than **66** although the middle comparison does not hold for abomasal species. Similarly, methylation of the basic ring at R_2 gives rise to a marked increase in activity over the corresponding unsubstituted analogs. Thus, **21** is more active than **8**, **37** more active than **10**, and **71** more active than **70**. A further increase in activity is clearly shown when the saturated linkage ($X = CH_2CH_2$) of **10** and **37** is replaced by the transvinyl linkage ($X = CH{=}CH$) of **70** and **71**. In compounds A and B where the 2 rings are linked through the 3 position of the thiophene ring instead of the 2 position as in pyrantel, activity is reduced except against species of the genus *Nematodirus*.

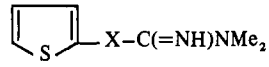
A further demonstration of these structure-activity relationships is shown in Table II. Generally, the logical replacement of the thiophene ring by the Ph ring leads to a reduction in activity. However, within that framework, ortho substitution with Me (**84**), Cl (**93**), and Br (**97**) gives compounds which are more active in sheep than the unsubstituted compound, **82**. This difference is more marked in the Ph compounds than in the thiophene series. Other substituted Ph compounds such as the OH, OR, and NO_2 compounds, while being much more readily accessible than their thiophene analogs, showed at best only modest activity in primary screens and virtually nil at the dose levels used in sheep.

Further information on the structure-activity relationships has been derived from examination of variants in the basic ring. For example the open chain amidine analogs of pyrantel were less active in sheep as indeed they were in the primary screens. Of other basic heterocyclic analogs screened the most interesting were the dihydrothiazines. These compounds were highly active, and in contradistinction to the tetrahydropyrimidines, the saturated chain compounds were more active than their vinylene analogs although strict comparison of the worm count and egg reduction

figures should be treated with some reserve. However, their relative toxicity to sheep precluded their further development, their therapeutic ratio being much lower than those of pyrantel or morantel salts.

One further series which was given considerable attention because of the high level of activity in primary screening was the series of pyridinium salts outlined in Table VI. Most of the structure-activity relationships established in other series held for this one, *e.g.*, **63** was more active than **62**. An exception to the general pattern is observed since **63** was marginally more active than **66**. However, this series was in general less potent than the pyrantel series.

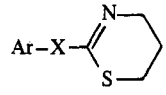
Thus, with certain minor exceptions, the structure-activity relationships in these series in sheep were found to parallel those demonstrated within the primary screen.



10,[a] X = CH_2CH_2
36, X = CH=CH

thiophene-substituted noncyclic amidines

[a]Number corresponding with identification in McFarland and Howes.[4]



dihydrothiazine analogs

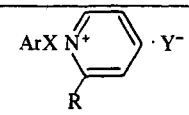
6,[a] Ar = 2-thienyl; X = CH_2CH_2
9,[a] Ar = 3-Me-2-thienyl; X = CH_2CH_2
21,[a] Ar = 2-furyl; X = CH_2CH_2
41,[a] Ar = 3-Me-2-thienyl; X = CH=CH
45,[a] Ar = *o*-tolyl; X = CH=CH

[a]Number corresponding with identification in McFarland, *et al.*[5]

Compounds **71** (pyrantel) and **74** (morantel) are the most active against the major nematode infections of sheep and the recommended therapeutic dosages are 25 mg/kg and 10 mg/kg, respectively. Against species of the genera *Nematodirus* and *Cooperia* the optimum dose is lower still. Details of the laboratory evaluation of these 2 compounds, which have been developed for commercial use, have been reported elsewhere.[6-9]

Acknowledgments. The authors wish to acknowledge the help of Jean Berry and Marcia A. Blore in these investigations.

Table VI. Pyridinium Salts

Compound number[a]	Ar	X	R	Y
				
2	3-Me-2-thienyl	$COCH_2$	H	Br
62	2-Thienyl	CH=CH	H	Br
63	3-Me-2-thienyl	CH=CH	H	Br
66	*o*-Tolyl	CH=CH	H	Cl
75	*o*-ClC_6H_4	CH=CH	H	Br
115	2-Thienyl	CH_2CH_2	H	Cl
116	2-Thienyl	CH_2CH_2	CH_3	Cl

[a]Number corresponding to identification used in McFarland and Howes.[3]

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