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Cestocidal Activity of 4-Alkoxy-1-naphthamidines against Dog and Cat Tapeworms

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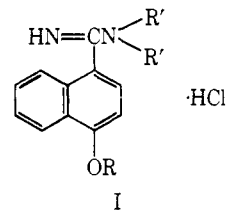
Structure–activity relationships in a series of 39 naphthamidines tested against 4 species of dog and cat tapeworms have been evaluated. The compounds fell into 4 groups that varied in activity and toxicity. The group containing those compounds in which R′ was either *n*-Pr or *i*-Pr and OR ranged from pentyloxy to decyloxy was the most active and next to the least toxic. Since most members of this group are equal to or better than bunamidine hydrochloride, further comparative studies are in order.

A number of years after a preliminary paper on the 4-alkoxy-1-naphthamidines[1] was published some members of the series were found active against the mouse pinworm, *Syphacia obvelata*,[2a] and against tapeworms in mice, dogs, cats, and sheep.[2b] The activity proved to be better against tapeworms than against pinworms and the most active compound at that time was selected for extensive veterinary investigation. This compound (Table I, **18**) was given the name bunamidine hydrochloride and has been used extensively throughout the world since then.

Over 20 additional naphthamidines have been made and tested against 2 tapeworms of mice (*Hymenolepis nana, Oochoristica symmetrica*) and against 4 species in dogs and cats (*Dipylidium caninum, Hydatigera taeniaeformis, Spirometra mansonoides, Taenia pisiformis*). The results of the mouse work have been published[3] and the present paper will report the results of treating dogs and cats in America and dogs in England.

The 39 naphthamidines selected for the dog and cat studies have the basic structure I[1,2b] in which R ranges from CH_3 to $C_{13}H_{27}$ and R′ from C_2H_5 to C_8H_{17}.

The objective was to test each of the naphthamidines active against mouse tapeworms against 4 dog and cat



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cestodes, to determine what structure–activity relationships existed, to evaluate the whole series or smaller groups, and to find out if any compounds appeared to be superior to bunamidine hydrochloride.

Materials and Methods

A (Tuckahoe). Selection of Animals.—All stray dogs and cats purchased by the Laboratory were examined for eggs with the $ZnSO_4$ flotation technique and for proglottids by examining the pan beneath the wire bottom of the cages. An animal positive for any species of tapeworm was selected for trial and was kept in an individual cage during the test. Dogs were given canned dog food and H_2O and cats, canned cat food and milk.

Infections.—All infections were natural, except for *Spirometra mansonoides*. Although on occasion we have found this species in cats and have carried out the entire life cycle in the laboratory, most of the infections used in this series were obtained from spargana given to us.[4]

Dose.—Throughout these trials a uniform dose of 50 mg/kg was used, except for a few cases in which the shortage of drug made it necessary to reduce the level to 25 mg/kg. Gaps in Table I indicate that the supply of drug was exhausted before all species had been tested.

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(1) E. Lorz and R. Baltzly, *J. Amer. Chem. Soc.*, **73**, 93 (1951).

(2) (a) M. Harfenist, *J. Med. Chem.*, **6**, 361 (1963); (b) R. Baltzly, R. B. Burrows, M. Harfenist, K. A. Fuller, J. E. D. Keeling, O. D. Standen, C. J. Hatton, V. J. Nunns, D. A. Rawes, B. D. Blood, V. Moya, and J. L. Lelijveld, *Nature (London)*, **206**, 408 (1965).

(3) M. Harfenist, R. B. Burrows, R. Baltzly, E. Pedersen, G. R. Hunt, S. Gurbaxani, J. E. D. Keeling, and O. D. Standen, *J. Med. Chem.*, **13**, 97 (1970).

(4) Spargana were donated by Dr. J. F. Mueller, State University of New York, Upstate Medical Center, Syracuse, N. Y.

Procedure.—After an animal was dosed, it was observed frequently for evidence of side effects, which were recorded. Each fecal specimen was collected and washed through a sieve, the sieve contents were poured a little at a time into a black-bottomed pan, and searched for worms, or fragments, using a magnifier-illuminator. All findings of any species of parasite were recorded. After 48 hr the animal was necropsied, all remaining worms were collected and counted, and with the aid of the magnifier-illuminator the entire intestinal wall was examined for attached scolices. Where possible, the percentage of worms killed was computed. Since tapeworms disintegrate rapidly after death, particularly when remaining in the intestine for some hours, no signs of dead tapeworms were found in many animals.

In an earlier study of one of the naphthamidines,[5] the average tapeworm burden of a large number of untreated animals was used as a basis for estimating percentage of elimination of worms in treated animals. This procedure was followed throughout most of the trials done with the large group of naphthamidines. In the last fourth of the dogs and cats we used the PAF sedimentation technique[6] on fecal specimens of all treated animals, since it indicated whether or not the drug had activity.

All specimens passed during the first 24 hr after dosing were examined with the PAF technique as well as by checking the sieve contents in a black-bottomed pan. If a large number of eggs and calcareous bodies were seen in the sediment, that was an indication of activity and at necropsy it could be determined whether scolices or short portions remained attached. If few or no eggs or calcareous bodies were seen in the sediment, the lack of activity could be confirmed when the necropsy was done. An occasional drug caused quick disintegration of worms and destruction of eggs, so that only fragmented eggs and calcareous bodies are found. Unlike the other species of tapeworms, eggs of *Dipylidium caninum* were rarely found in the sediment of the PAF procedure. After the saline-Et_2O combination was spun down, the supernatant was poured into a small beaker, which was set aside for about 1 hr for most of the Et_2O to evaporate. The contents were then swirled and poured into a centrifuge tube, more saline was added to the 12-ml mark, the tube was centrifuged, the supernatant was poured off, and a few drops of saline were added to the sediment. The sediment was mixed, and a drop was placed on a slide and examined for *D. caninum* eggs. Calcareous bodies from *D. caninum* are more likely to be found in the PAF sediment, whereas the eggs themselves are recovered more readily from the sediment of the supernatant.

B (Frant).—The procedures followed are essentially those used in earlier work with bunamidine.[7]

Infections.—The infections of *Taenia pisiformis*, the only species used, were experimental, Gravid proglottids were obtained from dogs, ground lightly in a mortar, a little formalin was added to inhibit bacterial growth, and the suspension was set aside at room temp for at least 2 days. Each rabbit was given orally an aq suspension containing approximately 40,000 eggs. After about 14 weeks the rabbits were necropsied, and the cysts were removed and pooled. Puppies (6–8 weeks old) were given a drink of protein hydrolysate[8] to stimulate gastric secretion, and then were given 10–20 cysts in a gelatin capsule. Mature tapeworms recovered after this procedure ranged from 40 to 100%. The puppies were placed in individual cages, with wire mesh floors, about 6 weeks after infection, and a check was made for eggs or proglottids in feces. When found positive, they were used in experiments.

Dose.—Where sufficient compound was available, each drug was tested at several different levels, generally 100, 50, 25, 12.5, and 6.25 mg of base/kg, unless prior studies on mice had indicated that the higher levels might be too toxic for dogs. In a few cases the highest level tested was 25 mg of base/kg. The activity incorporated in the chart was that obtained with the 50 mg of base/kg level, unless indicated otherwise.

Procedure.—For 72 hr after dosing all feces were collected and examined for proglottids and eggs. Since the majority of worms quickly disintegrated after death, clearance could rarely be expressed in percentage. After 10 days the dogs were necropsied, the entire intestine was slit open, and the contents were washed into a 44-mesh sieve. All material retained by the sieve was searched for worms or fragments.

Results

The data from these trials are presented in Table I. *Dipylidium* was found to be the most sensitive to these compounds, with 29 of 35 trials (83%) resulting in complete elimination. *Taenia* was the most resistant and the 2 strains were found to be quite different, only 5 of 36 trials (14%) with the American strain giving perfect results compared with 12 of 28 (43%) with the English strain. *Hydatigera*, 24 of 37 (65%), and *Spirometra*, 24 of 39 (62%), were intermediate and fairly close to each other in response.

A large chart of squares was made to arrange the compounds in systematic order and to place the test results in their appropriate positions. The systematic arrangement of the various compounds is shown in Figure 1. When the test results were studied on the chart, it was found that the compounds fell into 4 groups, with slight similarity of activity on both sides of the group interfaces. These 4 groups (A–D) are outlined on Figure 1 and each encloses the compounds of the group.

A summary of the results obtained in each group is presented in Table II. Group A had less than one-fifth of the trials resulting in complete elimination and the average percentage of clearance was 32. Group D, at the other end of the series, was somewhat better, with less than half the trials being perfect and an average percentage of 40. Group B, which contains bunamidine (**18**) was better than either of the others, with 70% of the trials being perfect and the average clearance being 76%. However, in group C every trial run against *Dipylidium*, *Hydatigera*, and *Spirometra* resulted in complete worm removal, the majority of *Taenia* infections were eliminated, and the overall clearance for the group was 91%. Complete removal of the English strain of *T. pisiformis* occurred only in Groups B and C, but the American strain responded completely only to 5 compounds in group C.

Five compounds of group C (**11**, **12**, **30**, **32**, **34**) removed all tapeworms of each animal tested (Table I). Two (**11**, **12**) were not tested against the English strain of *T. pisiformis* and 1 (**32**) was not used against *Hydatigera* or the American *Taenia* because of insufficient compound.

Acute oral toxicities in mice have been done on most of these compounds,[9] and the information was extracted from the files for the present use. The range and (average) number of mg/kg po for the 4 groups are: group A, 400–5000 (1730); group B, 200–750 (425); group C, 180–1200 (620); and group D, 250-1200 (835). Group A was the least toxic and the least active. Group D was about twice as toxic as A, but somewhat more active. Of the 2 active groups, C was more effective and less toxic than B. The 5 best compounds, all in group C, had these LD_{50}'s po: **11**, 250; **12**, 500; **30**, 400; **32**, 750; and **34**, >1000. This information suggests that **34** is half as toxic as **18** (LD_{50} 540)[3] and more active.

Of the 186 animals used for these trials, 33 vomited, 3 cats died, and several dogs had some diarrhea. (Since

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(6) R. B. Burrows, *Amer. J. Clin. Pathol.*, **48**, 342 (1967).
(7) (a) C. J. Hatton, *Vet. Rec.*, **77**, 408 (1965); (b) *ibid.*, **81**, 104 (1967).
(8) PROTOGEST.

(9) Our thanks to R. V. Fanelli and B. R. Sezesny, Pharmacology Department, The Wellcome Research Laboratories, Tuckahoe, N. Y., who determined the acute toxicities.

TABLE I
ACTIVITY OF THE VARIOUS NAPHTHAMIDINES, AT 50 MG/KG, AGAINST FOUR CESTODES OF DOGS AND CATS

Compd	R of OR	R′ of NR′	% tapeworms eliminated				
			Dc[a]	*Ht*	*Sm*	*Tp*-A	*Tp*-E
1	CH_3	C_7H_{15}	0	100	0	0	73
2	CH_3	C_8H_{17}	100	100	50	14	0
3	C_2H_5	C_7H_{15}	100	0	0	0	50[b]
4	C_2H_5	C_8H_{17}	100	100	100	0	0
5	C_3H_7	C_7H_{15}	100	50	0	33	0
6	C_3H_7	C_8H_{17}	50	75	0	0	0
6a	C_4H_9	C_4H_9	100	100	100	0[b]	100
7	C_4H_9	C_5H_{11}	100	100	100	50	100
8	C_4H_9	C_6H_{13}	100	33	50	0	0
9	C_4H_9	C_7H_{15}	90	0	0	0	50[c]
11	C_5H_{11}	C_3H_7	100	100	100	100	
12	C_5H_{11}	$CH(CH_3)_2$	100	100	100	100	
13	C_5H_{11}	C_4H_9	100	100	100		50[b]
14	C_5H_{11}	C_5H_{11}	100	0	100	4	
15	C_5H_{11}	C_6H_{13}	100	0	0	50	0
16	C_5H_{11}	C_7H_{15}		0	50	0	0
17	C_6H_{13}	C_3H_7	100	100	100	0	
18	C_6H_{13}	C_4H_9	100	100	100	50	100
19	C_6H_{13}	C_5H_{11}	95	100	100	1	100
20	C_6H_{13}	C_6H_{13}	8	0	25	0	0[b]
23	C_7H_{15}	C_3H_7	100	100	100	100	0[b]
24	C_7H_{15}	$CH(CH_3)_2$	100	100	100	81	100
25	C_7H_{15}	C_4H_9	100	100	100	0	100[b]
27	C_7H_{15}	C_6H_{13}	100	0	0	0	
29	C_8H_{17}	C_3H_7	100	100	100	0	100[b]
30	C_8H_{17}	$CH(CH_3)_2$	100	100	100	100	100
31	C_8H_{17}	C_4H_9	100	100	100	0	0
32	C_9H_{19}	C_3H_7	100		100		100
33	C_9H_{19}	C_4H_9	100	100	33	0	
34	$C_{10}H_{21}$	C_3H_7	100	100	100	100	100
35	$C_{10}H_{21}$	$CH(CH_3)_2$	100	100	100	14	100
36	$C_{10}H_{21}$	C_4H_9	100	100	100	0	100
37	$C_{11}H_{23}$	C_2H_5	100	0	0	0	
38	$C_{11}H_{23}$	C_3H_7	100	100	100	0	0[b]
39	$C_{11}H_{23}$	$CH(CH_3)_2$	0	100	100	0	50[b]
40	$C_{12}H_{25}$	C_2H_5		50	100	0	
41	$C_{12}H_{25}$	C_3H_7		100	0	0	
42	$C_{12}H_{25}$	$CH(CH_3)_2$	100	0	100		
43	$C_{13}H_{27}$	C_3H_7			0	0	
Summary							
Cleared/treated			29/35	24/37	24/39	5/36	12/28
Av % worms eliminated			90	70	67	22	53

[a] Abbreviations for tapeworms: *Dc, Dipylidium caninum; Ht, Hydatigera taeniaeformis; Sm, Spirometra mansonoides; Tp, Taenia pisiformis;* A, American, E, English. [b] Dosed at 25 mg/kg rather than at 50. [c] Dosed at 100 mg/kg rather than at 50.

TABLE II
EFFECTIVENESS OF THE VARIOUS GROUPS OF NAPHTHAMIDINES AGAINST THE DIFFERENT SPECIES OF CESTODES

Species[a]	Group A		Group B		Group C		Group D	
	C/T[b]	%	*C/T*	%	*C/T*	%	*C/T*	%
Dc	7/11	77	9/10	99+	10/10	100	3/4	75
Ht	3/12	38	9/10	90	9/9	100	3/6	58
Sm	1/12	23	9/10	93	10/10	100	4/7	57
Tp—A	0/12	8	0/9	12	5/9	66	0/6	0
Tp—*E*	0/11	16	6/8	81	6/7	86	0/2	25
Total	11/58	32	33/47	76	40/45	91	10/25	40

[a] See footnote *a*, Table I. [b] *C/T*, animals cleared/animals treated; %, av per cent of worms removed from group treated.

a few animals had infections with 2 species of tapeworms and since a few experiments were repeated, the number of trials (Table II) and the number of animals used are not the same). Twenty-one per cent of the 98 dogs and 14% of the 88 cats vomited. Vomition was less in groups A and B, being 13% for dogs and 9% for cats. Group C, which had the best activity, had the most emesis, 36% in dogs and 25% in cats. However, this apparently had little effect on the activity of the drugs, since 14 of 17 group C animals that vomited were freed of tapeworms and had an average clearance of 88%, which compares favorably with the 26 of 28 freed and 93% clearance in those that did not vomit. In group D, 33% of the dogs and 14% of the cats vomited.

The cats purchased for use were strays that were not used to cages, and it was not unusual for 1 or more of each dozen to be found dead in the cage at some time during the first week. Those that survived the first week generally adjusted to cage life. All 3 cats that died were dosed within a few days of arrival, in no case did other animals receiving the same drug die, and 2 of the 3 drugs were among the least toxic, with LD_{50}'s po of over 1000 mg/kg. These deaths are not blamed on the drugs.

Several dogs in America and several in England had diarrhea, but this was not attributed to the drugs. Often changes of diet and/or habitat may cause a tem-

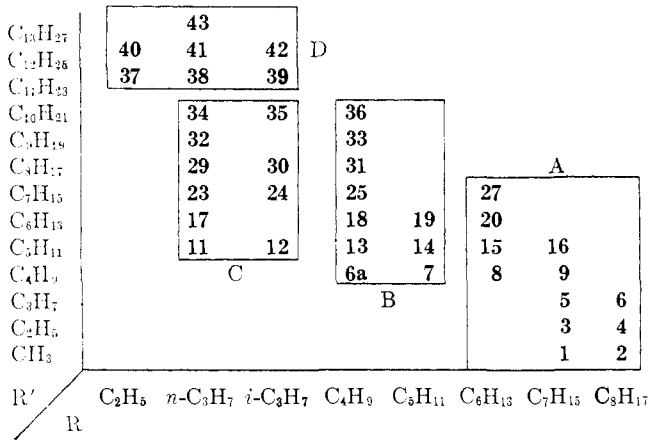

Figure 1.—Compounds arranged according to systematic position and grouped according to activity.

porary looseness, regardless of whether the dog had been treated or not. The majority of dogs in these trials had normal bowel movements or were somewhat constipated.

Discussion

After bunamidine hydrochloride was selected as the best of the naphthamidines made prior to 1965, a number of papers have appeared concerning its activity against 14 species of tapeworms of mice, dogs, cats, sheep, horses, and poultry. These papers are listed in a prior article.[3] In addition to the various papers dealing with the effect of bunamidine on different cestode species, one article[10] dealt with the effect of 8 naphthamidines against *Echinococcus granulosus* in Argentine dogs. All 8 of these compounds fell into groups A and B, 6 had moderate activity, 2 had good activity, and bunamidine hydrochloride was considered the better of the 2 good ones.

Hatton[7b] tested 3 different salts of bunamidine: hydrochloride, hydroxynaphthoate, and *p*-chlorobenzenesulfonate. He reported that the hydroxynaphthoate produced fewer side effects than either of the others, but was rather inactive when given on an empty stomach. When mixed with food it was quite active. On the other hand, Burrows and Lillis[5] found the hydrochloride more effective against *T. pisiformis* when given on an empty stomach than when given at or near mealtime.

The most effective group of naphthamidines made to date are those in group C, which contains the compounds in which OR ranges from pentyloxy to decyloxy and R′ is either *n*-Pr or *i*-Pr. Differences between compounds having $(n\text{-Pr})_2$ or $(i\text{-Pr})_2$ are minor and not consistently favorable to either.

Now that the most active group of the naphthamidines appears to be pinpointed, several other problems must be attacked. These involve additional trials at various dose levels of most of the compounds of the group, the relation of dosage time to feeding time, the testing of different particle sizes, the preparation of the hydroxynaphthoate salt of the better compounds, and the evaluation of several different types of formulations of the better drugs.

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Syntheses of Halogenated Phenanthrene Amino Alcohols as Antimalarials[1]

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A series of phenanthrene-1-amino alcohols has been prepared and evaluated against *Plasmodium berghei* in mice. The target compounds contain halogen at the 2, 6, 6,7, 6,7,9, or 10 positions, and were made through sequences beginning with chlorobenzene, *o*-dichlorobenzene, methyl 1-phenanthrylate, or methyl 3,4-dihydro-1-phenanthrylate. Some of these halogen-substituted phenanthrene-1-amino alcohols showed moderate curative activity against *P. berghei* in mice.

As part of the current U. S. Army Research Program on Malaria, we undertook the syntheses of phenanthrene aminomethylmethanols. The aim was activity against the drug-resistant strain of *Plasmodium falciparum*. In a similar program during World War II, a considerable number of phenanthrene aminomethylmethanols had been prepared, and some showed considerable activity against other types of malaria.[2] Most of the earlier phenanthrene derivatives had the dialkylamino alcohol group at the 3 or 9 position, a few were located at the 2 position, and one[3] was found[4] to be at the 1 position. We have described our earlier syntheses of basic phenanthrenemethanols with the dialkylamino alcohol side chain attached at the 1 and at the 4 position, and with H, Cl, or Br at the 9 or 10 position.[4] We now report the syntheses of phenanthrene-1-amino alcohols substituted with Cl or Br at the 2, 6, or 6 and 7 positions, and with Cl, Br, or H at the 9 or 10 positions.

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