2-Acetylpyridine Thiosemicarbazones. 13. Derivatives with Antifilarial Activity[1,2]

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Several members of a series of 2-acetylpyridine thiosemicarbazones possess in vivo and in vitro macrofilaricidal properties. The most promising of the group tested is N^4-(2-aminophenyl)-2-[1-(2-pyridinyl)ethylidene]-hydrazinecarbothioamide (**4**), which suppressed 100% of the macrofilariae of *Brugia pahangi* and 94% of those of *Acanthocheilonema viteae* in the jird at a dose of 25 mg/kg per day × 5. Compounds **4** and **14** were also shown to inactivate or kill *Onchocerca gutturosa* and *Onchocerca volvulus* adult worms as measured by the loss of their motility or the inhibition of the conversion by the worms of the dye MTT to formazan.

Filariasis is the term applied to a group of systemic nematode diseases caused by the transmission of infective larvae of filariae to man by the bite of certain mosquitos or blackflies. The macrofilariae (adult worms) inhabit the body cavities, tissues, or circulatory system of the host. Restriction of the lymphatic vessels by adult worms can cause dramatic enlargement of the lower extremities, such as that seen in elephantiasis. Among the important members of this family of filarial nematodes that infect man are *Wuchereria bancrofti*, *Brugia malayi*, *O. volvulus*, and *Loa loa*.

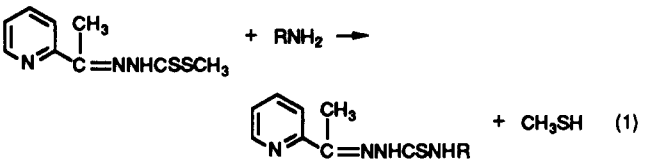
The drug ivermectin effectively kills the microfilariae (prelarval forms) of *O. volvulus*, which are responsible for onchocerchiasis (river blindness), but is not effective against macrofilariae of this species.[3] An older drug, diethylcarbamazine, removes most of the microfilarial forms of *W. bancrofti*, *B. malayi*, *L. loa*, and *O. volvulus* and has been reported to kill the adult worms of *B. malayi* and *L. loa*;[4] however, it is not effective against adult worms of other species and its use is associated with severe allergic reactions. The World Health Organization considers the development of a safe and effective macrofilaricidal drug to be an urgent requirement.[5]

The search for new macrofilaricides has led to the examination of representative 2-acetylpyridine thiosemicarbazones, a class shown to have chemotherapeutic activity against malaria parasites,[6] herpes viruses,[7] and a broad range of bacteria.[8] In addition, this group of compounds has been demonstrated to inhibit ecdysis in the large milkweed bug (*Oncopeltus fasciatus*)[9] and other insects.[10]

In this study, a series of 2-acetylpyridine thiosemicarbazones was screened against two species of filariae, *B. pahangi* and *A. viteae*, with the jird as the host. Two of the most promising 2-acetylpyridine thiosemicarbazones, i.e., **4** and **14**, were also tested in vitro against *O. gutturosa* and *O. volvulus*.

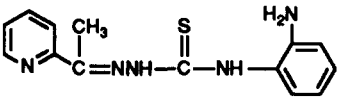
Chemistry

All new thiosemicarbazones utilized in this study were prepared by the condensation of 2-acetylpyridine with methyl hydrazinecarbodithioate to afford an intermediate *S*-methyl 3-[1-(2-pyridinyl)ethylidine]hydrazinecarbodithioate.[6a] Reaction of this dithioester with the appropriate amine proceeded with the evolution of methanethiol to yield the desired thiosemicarbazones (eq 1).[6a] Although



(1)

some of the final products (**2**, **4**–**11**, **18**) themselves possess a free amino group (a type of thiosemicarbazone not prepared by us previously), no precautions were necessary to avoid bis-thiosemicarbazone formation despite the use of diamines in the displacement reaction. In the case of compound **8**, the starting diamine was not symmetrical and, thus, X-ray crystallography was utilized to ascertain which of the two possible isomers had been isolated.



4

The dihydrochloride salt of **4**, i.e., **5**, was prepared with the aim of having a water-soluble thiosemicarbazone. Unfortunately, one of the amino functions present is a weak base and forms an unstable salt that dissociates rapidly in aqueous solution to form an insoluble monohydrochloride. Difficulties encountered with **5** have caused us to continue our subsequent testing with the free base **4**.

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(1) Paper number 12 in this series: Klayman, D. L.; Acton, N.; Scovill, J. P.; Bruce, J. *Arzneim.-Forsch./Drug Res.* **1986**, *36*, 10.
(2) These results were presented in part at the 200th Meeting of the American Chemical Society, Washington DC, August 26–31, 1990; Abstract No. 159 and The 39th Annual Meeting of the American Society of Tropical Medicine and Hygiene, New Orleans, Louisiana, November 4–8, 1990; Abstract No. 179.
(3) Taylor, H.; Green, B. M. *Am. J. Trop. Med. Hyg.* **1989**, *4*, 460.
(4) Wilson, T. *Trans R. Soc. Trop. Med. Hyg.* **1950**, *44*, 49.
(5) WHO Document OCP/GVA/85.1B (1985).
(6) (a) Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. *J. Med. Chem.* **1979**, *22*, 855. (b) Klayman, D. L.; Scovill, J. P.; Bartosevich, J. F.; Mason, C. J. *J. Med. Chem.* **1979**, *22*, 1367.
(7) Shipman, C.; Smith, S. H.; Drach, J. C.; Klayman, D. L. *Antiviral Res.*, **1986**, *6*, 197.
(8) Dobek, A. S.; Klayman, D. L.; Scovill, J. P.; Dickson, E. T.; Tramont, E. C. *Antimicrob. Agents Chemother.* **1980**, *18*, 17.
(9) (a) Kelly, T. J.; Redfern, R. E.; DeMilo, A. B.; Borkovec, A. B. *Pestic. Biochem. Physiol.* **1982**, *17*, 35. (b) DeMilo, A. B.; Redfern, R. E.; Borkovec, A. B. *J. Agric. Food Chem.* **1983**, *31*, 713.
(10) Pederson, L.-E. K.; Svendsen, A.; Klemmensen, P. D. *Pestic. Sci.* **1984**, *25*, 462.

Biology

In Vivo. The acute toxicity of the test compounds was determined in the noninfected jird, an animal commonly known as a gerbil. The maximum tolerated dose and, if supplies of chemical were adequate, several graded doses were administered to the animals in which the infection of two species of nematodes had been previously established. After a period of ca. 2 months, the animals were sacrificed and the number of worms in the subcutaneous tissue and fascial plane of muscle (*A. viteae*) and peritoneal cavity (*B. pahangi*) was ascertained.

In Vitro. The testing of two candidate drugs showing promise was performed by two newly devised techniques. The first measured the reduction of motility of male *O. gutturosa* and *O. volvulus* worms caused by the drug, and the second assayed the reduced viability of live-treated macrofilaria as reflected by their diminished ability to take up the dye MTT and convert it to a colored product, formazan. The quantity of formazan formed was measured colorimetrically.

Results and Discussion

As can be seen from Table I, most of the 2-acetylpyridine thiosemicarbazones had some suppressive activity against *B. pahangi* and/or *A. viteae*. Compound **4** is the most active and does not cause toxicity at the maximum administered dose of 100 mg/kg per day ×5. At a dose of 25 mg/kg per day × 5, the compound cleared all of the macrofilariae of *B. pahangi* and virtually all those of *A. viteae*, whereas the hydrochloride salt of **4**, compound **5**, completely eliminated the macrofilaria of both species at the same dose. Other compounds that showed significant suppression of macrofilariae of both species are **14** and **8** (closely related in structure to **4**). The congeners of **4** in which the aromatic NH_2 group is located in the 3- and 4-positions relative to the N^4-thiosemicarbazone nitrogen atom, i.e., **6** and **7**, are considerably less suppressive. It thus appears that an ortho relationship of aromatic amine functions is important for activity. Compound **18**, a variant of **4** in which the pyridine ring is replaced by phenyl, was active only against *B. pahangi*. Compound **14**, in which the N^4 is part of an azacycloheptane ring, showed great efficacy against *B. pahangi*, but was toxic at a dose of 100 mg/kg per day × 5.

Both compounds **4** and **14** immobilized *O. gutturosa* in vitro within the 7-day trial at concentrations as low as ca. 10^{-8} M, whereas, against *O. volvulus*, both compounds immobilized or virtually immobilized the worms at a concentration of 3.1×10^{-6} M by day 5 (Table II). Activity was confirmed by the MTT assay which showed a significant inhibition of formazan formation for both compounds (Table III). This level of activity compares favorably with that of other known filaricides in vitro. For example Townson et al.[13] demonstrated that the minimum concentration of the drug Mel W (the most active macrofilaricide in this system) required to immobilize the parasites within 7 days was 7.81×10^{-7} M.

In preliminary studies, compound **4** also showed 100% reduction of microfilariae of *A. viteae* after 56 days at doses ranging from 25 to 100 mg/kg per day × 5. It is concluded that the 2-acetylpyridine thiosemicarbazone class of compounds has potential as antifilarial agents. Compound **4**, in particular, is now undergoing further testing in dogs.

Experimental Section

Chemistry. All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra of solid samples were obtained with KBr disks on a Perkin-Elmer Model 283 spectrophotometer. NMR spectra were run on a JEOL FX90Q spectrometer using Me_4Si as an internal standard. All spectra were in accord with the assigned structures. Analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI, and results were within ±0.4% of the theoretical values.

N^4-(2-Aminophenyl)-2-[1-(2-pyridinyl)ethylidene]hydrazinecarbothioamide (4). *o*-Phenylenediamine (4.32 g, 0.04 mol) and 9.0 g (0.04 mol) of *S*-methyl 2-[1-(2-pyridinyl)ethylidene]hydrazinecarbodithioate[6a] were heated under reflux in 150 mL of 95% EtOH for 24 h. MeSH was evolved during the course of the reaction. The solvent was evaporated under reduced pressure to about one-half of the original volume and the mixture was cooled. The brownish crystals that formed were collected and recrystallized from EtOH-$CHCl_3$ to give 4.2 g (40%) of N^4-(2-aminophenyl)-2-[1-(2-pyridinyl)ethylidene]hydrazinecarbothioamide (**4**) as white crystals: mp 178–180 °C dec; IR 3280, 3170, 2950, 1470, 1200 cm^{-1}. Anal. ($C_{14}H_{15}N_5S$) C, H, N, S.

N^4-(2-Aminophenyl)-2-[1-(2-pyridinyl)ethylidene]hydrazinecarbothioamide Dihydrochloride (5) and Monohydrochloride. Compound **4** was dissolved in 1 N HCl, filtered, and lyophylized. The resulting yellow powder was purified by dissolving it in a small volume of MeOH and adding the solution to a large volume of anhydrous Et_2O to give **5** as a yellow powder: mp 185–186 °C dec. The compound crystallizes with 1 mol of MeOH. Anal. ($C_{14}H_{15}N_5S \cdot CH_3OH \cdot 2HCl$) C, H, N, S, Cl.

Compound **5**, when dissolved in water, dissociates to form a fine orange powder that separates from moderately concentrated solutions as **4**·HCl: mp 202–203 °C dec. Anal. ($C_{14}H_{15}N_5S \cdot HCl$) C, H, N, S, Cl.

Biology. In Vivo. Potential filaricidal compounds were evaluated for macrofilaricidal activity against a dual infection of the nematodes *B. pahangi* and *A. viteae* in which the host was the male Mongolian jird (*Meriones unguiculatus*) weighing 50–60 g.[11] *B. pahangi* was maintained by alternate passage through Beagle dogs and *Aedes aegypti* (selected Liverpool strain). *A. viteae* was cyclically maintained in jirds and the soft tick (*Ornithodoros tartakovskyi*) as described by McCall.[12]

The test compounds were suspended in 0.5% hydroxycellulose and 0.1% Tween 80 by sonication at 20 kilocycles for 10 min. The exact dosage level used depended on the sample size and toxicity of the chemical. Compounds were administered once daily at 100 mg/kg per day by subcutaneous injection. If sufficient material was available, toxicity determinations were made on a noninfected jird with a single dose of 200 mg/kg administered either orally or subcutaneously. If there was no apparent toxicity, as evidenced by excessive weight loss or death after 72 h, testing was begun at 100 mg/kg per day × 5; however, if toxicity was noted, the dosage was adjusted accordingly.

The nematodal infection was established in groups of 80 jirds, as described by McCall et al.[11] Each jird was given five male and five female adult worms of *A. viteae* by subcutaneous transplantation, and within 7–14 days later, each animal received 10 male and 10 female *B. pahangi* adult worms by transplantation into the peritoneal cavity. One to two weeks later, 68 of the dually infected jirds were allocated to 17 test groups, each containing four jirds, and a control group of at least six jirds. The compound was given once daily for the ensuing 4 days, and weekly thereafter. A test compound was considered toxic if death occurred or if there was a >15% group mean weight loss.

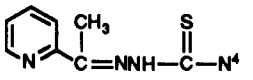
On days 50–60 after the treatment was started, all of the jirds were sacrificed and the adults of *A. viteae* and *B. pahangi* in the skin and peritoneal cavity, respectively, were counted. The number of live worms was ascertained and identified as to species and sex and when possible, dead and encapsulated worms were also counted. A compound is considered active if the number of macrofilariae is significantly lower ($p < 0.05$) than that in the controls. A 60% reduction in the adult worm burden is considered significant.

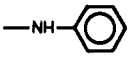
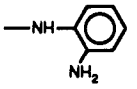
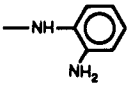
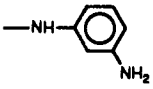
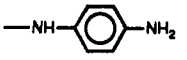
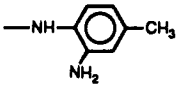
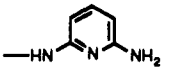
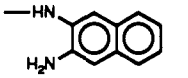
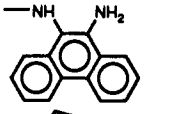
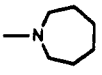
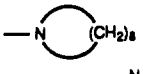
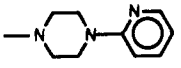
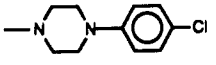
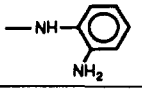
In Vitro. Male *O. gutturosa* were isolated by dissection from the nuchal ligament connective tissues of naturally infected cattle

(11) McCall, J. W.; McTier, T. L.; Rowan, S. J. In *Proceedings of a Symposium on Onchocerchiasis/Filariasis*; April 8–10, 1986; Condor, G. A., Williams, J. F., Eds.; Upjohn Co.: Kalamazoo, MI, 1986; pp 23–33.

(12) McCall, J. W. *J. Georgia Entomol. Soc.* **1981**, *16*, 283.

Table I. Activity of 2-Acetylpyridine Thiosemicarbazones against Macrofilariae in Jirds



no.	mp,[a] °C	solvent	% yield	N^4	dose, mg/kg/day (5 days)	% suppression *B. pahangi*	% suppression *A. viteae*
1	*b*	–	–	$-NH_2$	100	toxic	
					1.56	41	60
					12.5	53	42
2	128–129	EtOAc	30	$-NHCH_2CH_2NH_2$	25	26	72
					12.5	-3	-40
3	*b*	–	–		50	97	25
					25	17	44
4	*c*				100	100	100
					50	100	100
					25	100	94
					12.5	16	28
5[d]	*c*				100	100	100
					50	100	100
					25	100	100
					12.5	29	36
6	110–113	THF	65		100	17	43
7	192–194	THF	70		200	60	25
					100	39	34
					25	5	9
8[e]	178–179	EtOH	33		100	100	72
					75	100	72
					50	100	75
					25	29	44
9	199.5–200.5	CH_3CN	44		100	55	14
					50	5	14
10	192–194	CH_3CN	30		100	49	37
					50	–	4
11	279–281	CH_3CN	19		100	33	23
					50	5	23
12	*f*	–	–		12.5	86	31
					1.56	46	–
13	*f*	–	–		12.5	65	14
					6.25	18	25
14	*f*	–	–		100	toxic	
					25	84	100
					12.5	100	-16
15	*f*	–	–		50	16	74
16[d]	*f*	–	–		6.25	-5	100
					1.56	–	40
17	199–202 dec	CH_3CN	60		100	14	85
					25	6	21
18[g]	188–189	EtOH	75		200	71	19
					100	15	9
					25	6	15

[a] No chemical or physical data are given if the compound was reported previously. [b] Reference 6a. [c] See the Experimental Section. [d] Dihydrochloride salt. [e] X-ray data will be published in a separate paper by Dr. Judith Flippen-Anderson. [f] Reference 6b. [g] The pyridine ring is replaced by phenyl. Compound 18 is, therefore, N^4-(2-aminophenyl)-2-(1-phenylethylidene)hydrazinecarbothioamide, $C_{14}H_{16}N_4S$.

following slaughter at a UK abattoir.

Drug preparation consisted of dissolving the test chemical in a small volume of DMSO before adding it to the test medium.[13] The latter consisted of Eagle's minimum essential medium, 10% heat-inactivated fetal calf serum, 100 IU of penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, and sodium bicarbonate (2 g/L). An equal quantity of DMSO not exceeding 0.016% was added to control wells.

The experiments were performed by adding a male *O. gutturosa* to each well of a 24-well plate containing 1.8 mL of medium (±drug) in the presence of LLCMK2 feeder cells.[13] Cultures were maintained in an atmosphere of 5% CO_2 in air.

(13) Townson, S.; Connelly, C.; Dobinson, A.; Muller, R. *J. Helminth.* **1987**, *61*, 271.

Table II. Mean Motility Scores for Treated *O. gutturosa*[a] and *O. volvulus*[b]

compd	drug concn, M	motility[c] 0.5[d]	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	24	48	72	96	168
							(a) *O. gutturosa*								
control		10	9.6	9.8	9.5	9.3	9.2	9.3	8.6	8.8	8.3	8.3	8.8	7.5	7.8
4	5×10^{-5}	10	10	9.5	8.5	9.5	9.3	9.5	9.5	9.5	9.5	8.3	1.6	1.5	0
4	1.94×10^{-7}	9.8	9.8	8	9	ND	ND	ND	ND	ND	8	4	1.5	1.8	0
4	1.21×10^{-8}	9	8.5	4.3	3.3	ND	ND	ND	ND	ND	9	7.3	6.8	3.8	1
4	3.05×10^{-9}	8.8	8.8	8.3	7.5	7.5	7.5	7.3	5.8	7.5	7.3	6.3	7.5	6.8	4.5
14	5×10^{-5}	6	4.8	4.8	4.8	3.8	2.2	2.3	1.5	1.8	0	0	0	0	0
14	3.1×10^{-6}	9.5	9.8	9.8	9.8	8.5	9.3	9	9	9.5	4	2.8	0	0	0
14	1.95×10^{-7}	8.8	9.5	9.5	9.3	9.3	10	10	10	9.5	8	2.5	1	0	0
14	4.88×10^{-8}	8.5	9	8.5	8.8	8.3	9	8.5	8.8	9.8	4	4	3.5	1.5	0
14	3.05×10^{-9}	9.8	9	8.5	9.8	8.3	8.8	8.3	7.8	9.5	9.3	6.8	6	5.5	3.5
							(b) *O. volvulus*								
control		8.0	8.4	8.4	7.6	ND	ND	ND	ND	ND	7.6	7.6	7.6	8	ND
4	5×10^{-5}	5.0	5.0	4.4	6.0	ND	ND	ND	ND	ND	4.4	3.6	2.4	0.6	ND
4	3.1×10^{-6}	8.0	5.0	4.4	6.0	ND	ND	ND	ND	ND	1.6	2.0	1.2	0.4	ND
control		7.6	7.4	7.0	7.0	ND	ND	ND	ND	ND	7.6	7.6	7.0	7.0	ND
14	5×1^{-5}	8.0	8.4	8.0	8.0	ND	ND	ND	ND	ND	1.6	1.0	1.0	0.3	ND
14	3.1×10^{-6}	6.6	6.6	6.4	6.6	ND	ND	ND	ND	ND	3.4	0.6	1.0	0.0	ND

[a] *O. gutturosa* culture system included LLCMK2 feeder cells. [b] *O. volvulus* culture system excluded LLCMK2 feeder cells. [c] Scores: O=immotile; 10=maximum control motility. ND = not done. [d] All time in hours.

Table III. Colorimetric Quantification of Male *O. gutturosa* and *O. volvulus* Viability Using the Tetrazolium Dye MTT

compd	drug concn, M	% inhibn of formazan formation compared to controls	significance, p
		(a) *O. gutturosa*	
4	5×10^{-5}	72.9	0.001
4	1.94×10^{-7}	84.8	0.0001
4	1.21×10^{-8}	27.9	0.04
14	5×10^{-5}	90.3	0.0007
14	3.1×10^{-6}	90.2	0.0002
14	1.95×10^{-7}	70.4	0.002
14	4.88×10^{-8}	31.7	0.09
14	3.05×10^{-9}	19.5	0.46
		(b) *O. volvulus*	
4	5×10^{-5}	53.8	0.007
4	3.1×10^{-6}	65.3	0.003
14	5×10^{-6}	58.2	0.006
14	3.1×10^{-6}	39.6	0.09

[a] *O. gutturosa* culture system included LLCMK2 feeder cells. MTT assay carried out after 168 h. [b] *O. volvulus* culture system excluded LLCMK2 feeder cells. MTT assay carried out after 96 h.

Motility levels of male *O. gutturosa* were noted every 30 min for the first 4.5 h, then at 1, 2, 3, 4, and 7 days. Motility was scored on a scale of 0 (nonmotile) to 10 (maximum normal control activity under optimum conditions).[13]

The MTT/formazan colorimetry assay was described previously for worm viability.[14,15] Briefly, single intact male worms were placed in 0.5 mL of medium containing 0.5 mg/mL of MTT [3-(4,5-diethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma) and incubated at 36.5 °C (±0.5 °C) for 30 min. At the end of the MTT incubations worms were removed and carefully transferred to a separate well of a microtiter plate containing 200 μL of DMSO and allowed to stand at room temperature for 1 h to solubilize. Following gentle agitation to disperse the color evenly, the absorbance of the resulting formazan solution was determined at 490 nm in a multiwell scanning spectrophotometer relative to a DMSO blank.

Male *O. volvulus* were dissected from nodules within 24 h of removal from human patients in Guatamala, during which time they were maintained in medium on ice (0 °C). Drug tests were carried out in culture using medium including 10% serum but in the absence of LLCMK2 cells. Evaluation of drug effects was essentially the same as for *O. gutturosa*.

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