Table I—Urinary Excretion and Whole Blood Concentrations of Cimetidine with Related Parameters in 12 Human Subjects following Administration of Two Identical Single Oral Doses of 200 mg of Cimetidine

Parameter	Dose I	Dose II	Δ Dose II[a]
Average whole blood concentration and average intrasubject change, Dose I → Dose II, μg/ml, at:			
0.5 hr	0.65	0.71	0.19
1.0 hr	1.02	0.98	0.16
1.5 hr	0.86	0.85	0.12
2.0 hr	0.72	0.71	0.13
3.0 hr	0.59	0.58	0.15
4.0 hr	0.37	0.40	0.09
6.0 hr	0.16	0.17	0.04
8.0 hr	0.09	0.08	0.03
Average of individual peak whole blood concentrations, μg/ml	1.07	1.01	
Average peak time, hr	1.13	1.25	
Average area under whole blood concentration curves, 0 → 8 hr, μg/ml × hr	3.52	3.59	
Mean elimination half-life, hr	1.90	1.72	
Mean urinary excretion of cimetidine and mean intrasubject change, Dose I → Dose II, as % of dose, in intervals of:			
0–3 hr	32.4	33.0	5.6
3–12 hr	23.4	23.2	7.7
12–24 hr	1.9	1.6	1.1

[a] Δ Dose II is the absolute difference, regardless of the direction of change.

drug from the 16 spiked samples was 99.2 ± 1.1%. None of the control urines tested contained material that interfered with the analysis of cimetidine.

Mean blood levels and urinary excretion of drug for the 12 human subjects in the clinical study are shown in Table I. Also presented are parameters related to blood concentration data. Elimination half-lives of the drug in blood were obtained by fitting concentration data from the 4-, 6-, and 8-hr blood samples, using the method of least squares. Areas under blood level curves were computed with a trapezoidal program. No statistically significant differences were found in mean blood levels or urinary excretion of cimetidine, or in any related parameters, when Doses I and II were compared.

Metiamide, also an H_2-receptor antagonist, was employed in earlier studies of the effects of this class of compounds on gastric acid secretion. Obviously, since the method employs metiamide as the internal standard in the analysis of cimetidine, the reverse situation may be applied to analyze for metiamide. This analysis was successful on a number of samples. The only changes were that a detector wavelength of 235 nm was used and that concentrations of cimetidine in internal standard solutions were 0.5 (blood) and 5 (urine) mg/ml.

The described method is satisfactory for analysis of cimetidine and metiamide, both in its reproducibility and sensitivity. By application of the analytical procedure, it was determined that cimetidine is well absorbed following an oral dose, as indicated by the urinary excretion and blood levels of the drug. In clinical trials, the blood levels of cimetidine were measured and correlated with the inhibition of gastric acid secretion (4–6). These correlations, obtained with different doses of cimetidine, have been helpful in assessing the effect of circulating drug on gastric acid secretion.

REFERENCES

(1) A. S. F. Ash and H. O. Schild, *Br. J. Pharmacol. Chemother.*, **49**, 427 (1966).

(2) J. W. Black, W. A. M. Duncan, G. J. Durant, C. R. Ganellin, and M. E. Parsons, *Nature*, **236**, 385 (1972).

(3) R. W. Brimblecombe, W. A. M. Duncan, G. J. Durant, J. C. Emmett, C. R. Ganellin, and M. E. Parsons, *J. Int. Med. Res.*, **3**, 86 (1975).

(4) R. M. Henn, J. I. Isenberg, N. Maxwell, and R. A. L. Sturdevant, *N. Engl. J. Med.*, **293**, 371 (1975).

(5) R. E. Pounder, J. G. Williams, J. J. Misiewicz, and G. J. Milton-Thompson, *Proc. Br. Soc. Gastroenterol.*, **1975**, 32 (Abstract).

(6) G. F. Longstreth, J. R. Malagelada, and V. L. W. Go, *Gastroenterology*, **68**, A-85/942 (Abstract) (1975).

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[x] To whom inquiries should be directed.

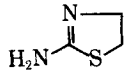
Anthelmintic 2-Arylhydrazino- and 2-Arylazo-2-thiazolines

M. T. WU, F. S. WAKSMUNSKI, D. R. HOFF, M. H. FISHER[x], J. R. EGERTON, and A. A. PATCHETT

Abstract □ Some 2-arylhydrazino- and 2-arylazo-2-thiazolines were synthesized for anthelmintic testing. The most potent compound, 2-(*o*-tolylazo)-2-thiazoline, was orally effective in sheep against a broad range of helminths.

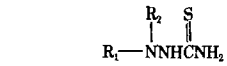
Keyphrases □ Thiazolines, substituted—synthesized, evaluated for anthelmintic activity in sheep □ Anthelmintic activity—evaluated in various substituted thiazolines in sheep □ Structure–activity relationships—various substituted thiazolines evaluated for anthelmintic activity in sheep

In a search for anthelmintic activity in novel structures, the known neuromuscular blocking agent 2-amino-2-thiazoline (I) (1) had a paralytic effect on third-stage larvae of *Haemonchus contortus in vitro*, similar to that of the anthelmintic drugs tetramisole and pyrantel. No activity could be demonstrated in *in vivo* assays. However, with this *in vitro* activity as a starting point, a series of related compounds was synthesized and tested for *in vivo* anthelmintic activity.



I

Table I—Physical Properties of 1-Substituted 3-Thiosemicarbazides[a]



Compound	R_1	R_2	Yield, %	Crystallization Solvent	Melting Point	Molecular Formula	Analysis, %	Calc.	Found
II	2-$CH_3C_6H_4$	H	85	Ethanol	158–160°	$C_8H_{11}N_3S$	C	53.03	52.89
							H	6.12	6.26
							N	23.19	23.47
III	3-$CH_3C_6H_4$	H	54	Ethanol–water	146–147°	$C_8H_{11}N_3S$	C	53.03	52.98
							H	6.12	6.04
							N	23.19	23.16
IV	4-$CH_3C_6H_4$	H	58	Ethanol	176–177.5°	$C_8H_{11}N_3S$	C	53.03	52.67
							H	6.12	6.05
							N	23.19	23.10
V	2-$C_2H_5C_6H_4$	H	69	Ethanol	182–183°	$C_9H_{13}N_3S$	C	55.36	55.55
							H	6.71	6.68
							N	21.52	21.56
VI	2-$CH_3OC_6H_4$	H	42	Ethanol	190–191°	$C_8H_{11}N_3OS$	C	48.73	49.10
							H	5.62	5.47
							N	21.31	21.29
VII[b]	4-$CH_3OC_6H_4$	H	22	Ethanol	212–213°	$C_8H_{11}N_3OS$	C	48.73	49.02
							H	5.62	5.71
							N	21.31	20.98
VIII[c]	2-ClC_6H_4	H	46	Ethanol	187–188°	$C_7H_8ClN_3S$	C	41.69	41.77
							H	4.00	4.17
							N	20.84	20.56
IX[d]	4-ClC_6H_4	H	11	Ethanol	200–201°	$C_7H_8ClN_3S$	C	41.69	41.67
							H	4.00	4.06
							N	20.84	20.80
X	4-$NO_2C_6H_4$	H	90	Ethanol–water	187–189°	$C_7H_8N_4O_2S$	C	39.63	39.77
							H	3.80	3.64
							N	26.41	26.27
XI	2,6-$(CH_3)_2C_6H_3$	H	46	Ethanol	204–205°	$C_9H_{13}N_3S$	C	55.35	55.26
							H	6.71	6.92
							N	21.52	21.34
XII	2,6-$(Cl)_2C_6H_3$	H	99	Ethanol–water	232–233.5°	$C_7H_7Cl_2N_3S$	C	35.60	35.61
							H	2.97	3.02
							N	17.77	17.83
XIII	C_6H_5	CH_3	46	Ethanol	191–192°	$C_8H_{11}N_3S$	C	53.03	52.69
							H	6.12	6.12
							N	23.19	22.81
XIV	C_6H_5	C_6H_5	53	Ethanol	213–215°	$C_{13}H_{13}N_3S$	C	64.19	64.32
							H	5.39	5.15
							N	17.27	17.36

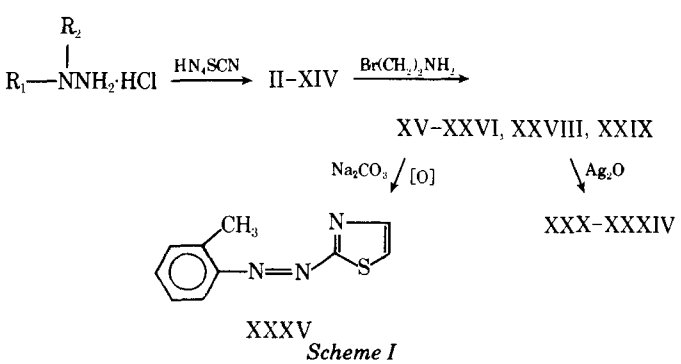
[a] Method A was used; see *Experimental.* [b] T. Pyl, K. H. Scheel, and H. Beyer, *J. Prakt. Chem.*, 20 (5–6), 255 (1963). [c] P. M. Parish and C. V. Delwali, *Indian J. Chem.*, 3, 45 (1965). [d] J. V. Mandlik and V. A. J. Patwardhan, *Univ. Poona Sci. Technol., No. 1*, 20, 45 (1961); through *Chem. Abstr.*, 55, 27274e (1961).

RESULTS AND DISCUSSION

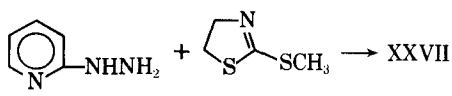
Chemistry—The compounds prepared are listed in Tables I–IV. Synthetic procedures, generalized where possible, are described under *Experimental.*

1-Aryl-3-thiosemicarbazides (Table I, II–XIV) were prepared from the corresponding arylhydrazine hydrochlorides by treatment with ammonium thiocyanate. Arylhydrazine hydrochlorides not commercially available were prepared by a described procedure (2). 2-Arylhydrazino-2-thiazolines (Table II, XV–XXVI, XXVIII, and XXIX) were synthesized by reaction of 1-aryl-3-thiosemicarbazides with 2-bromoethylamine hydrobromide in refluxing 2-propanol (3). 2-Arylazo-2-thiazolines (Table III, XXX–XXXIV) were prepared by oxidation of the corresponding 2-arylhydrazino-2-thiazolines with silver oxide in ethyl acetate (Scheme I). 2-(2-Pyridylhydrazino)-2-thiazoline (XXVII) was synthesized by direct reaction of 2-hydrazinopyridine with 2-methylmercapto-2-thiazoline (4) (Scheme II).

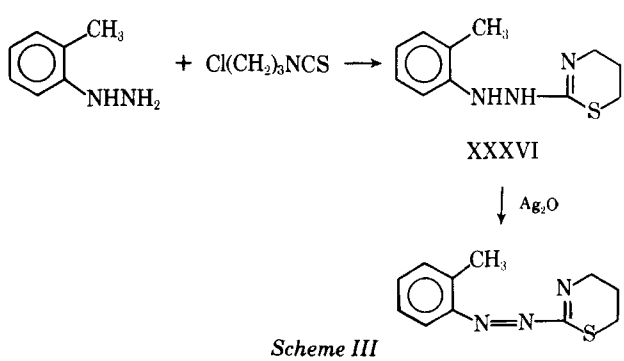
Air oxidation of 2-(*o*-tolylhydrazino)-2-thiazoline (XVI) mixed with sodium carbonate in refluxing ethanol gave 2-(*o*-tolylazo)thiazole (XXXV) in good yield (Scheme I). The dihydrothiazine (XXXVI) was obtained from the reaction of *o*-tolylhydrazine hydrochloride with 3-chloropropyl isothiocyanate (5) in the presence of triethylamine. Oxidation to the azo derivative (XXXVII) was achieved with silver oxide (Scheme III).



Scheme I

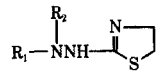


Scheme II



Scheme III

Table II—Physical Properties of 2-Arylhydrazino-2-thiazolines[a]



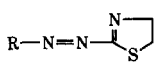
Compound	R_1	R_2	Yield, %	Crystallization Solvent	Melting Point	Molecular Formula	Analysis, %	Calc.	Found
XV	C_6H_5	H	24	Ethanol–water	135–136°	$C_9H_{11}N_3S$	C	55.95	55.87
							H	5.74	5.63
							N	21.75	21.37
XVI	2-$CH_3C_6H_4$	H	43	Methanol–water	126–127°	$C_{10}H_{13}N_3S$	C	57.96	57.62
							H	6.32	6.35
							N	20.28	20.34
XVII	3-$CH_3C_6H_4$	H	75	Ethyl acetate–hexane	95–97°	$C_{10}H_{13}N_3S$	C	57.96	57.72
							H	6.32	6.17
							N	20.28	20.15
XVIII	4-$CH_3C_6H_4$	H	70	Ethanol–water	136–138°	$C_{10}H_{13}N_3S$	C	57.96	57.70
							H	6.32	6.33
							N	20.28	20.23
XIX	2-$C_2H_5C_6H_4$	H	86	Ether	101–103°	$C_{11}H_{15}N_3S$	C	59.70	59.94
							H	6.83	6.77
							N	18.99	18.90
XX	2-$CH_3OC_6H_4$	H	48	Ethanol	141–142°	$C_{10}H_{13}N_3OS$	C	53.81	53.73
							H	5.87	5.82
							N	18.82	18.68
XXI	4-$CH_3OC_6H_4$	H	51	Methanol	124–126°	$C_{10}H_{13}N_3OS$	C	53.81	53.91
							H	5.87	5.76
							N	18.82	18.90
XXII	2-ClC_6H_4	H	71	Ethanol	98–100°	$C_9H_{10}ClN_3S$	C	47.50	47.68
							H	4.42	4.61
							N	18.45	17.94
XXIII	4-ClC_6H_4	H	85	Ethanol–water	146–149°	$C_9H_{10}ClN_3S$	C	47.50	47.37
							H	4.42	4.09
							N	18.45	18.44
XXIV	4-$NO_2C_6H_4$	H	37	Ethanol	203–205°	$C_9H_{10}N_4O_2S$	C	45.38	45.46
							H	4.23	4.36
							N	23.52	23.26
XXV	2,6-$(CH_3)_2C_6H_3$	H	63	Ethanol	159–161°	$C_{11}H_{15}N_3S$	C	59.70	59.30
							H	6.83	7.09
							N	18.99	18.83
XXVI	2,6-$(Cl)_2C_6H_3$	H	8	Ethanol–water	140–142°	$C_9H_9Cl_2N_3S$	C	41.20	41.01
							H	3.43	3.53
							N	16.04	16.30
XXVII	2-Pyridyl	H	40	Ethanol	248–250°	$C_8H_{10}N_4S$	C	49.46	49.75
							H	5.19	4.96
							N	28.84	29.14
XXVIII	C_6H_5	CH_3	25	Ethanol	113–114°	$C_{10}H_{13}N_3S$	C	57.96	58.07
							H	6.32	6.36
							N	20.28	20.15
XXIX	C_6H_5	C_6H_5	29	Ethanol	183–184°	$C_{15}H_{15}N_3S$	C	66.90	66.83
							H	5.61	5.40
							N	15.60	15.61

[a] Method B was used except for XXVII; see *Experimental.*

Biology—Biological results are shown in Table V. Anthelmintic activity was determined by a modification of the assay of Lynch and Nelson (6).

None of the intermediate thiosemicarbazides in Table I had anthelmintic activity. Of the compounds in Table V, the highest activity was found with the 2-arylhydrazino-2-thiazolines and their oxidized derivatives. Within these groups, structural requirements for activity were quite narrow. 2-Phenylhydrazino-2-thiazoline (XV) was slightly active

Table III—Physical Properties of 2-Arylazo-2-thiazolines[a]



Compound	R	Yield, %	Crystallization Solvent	Melting Point	Molecular Formula	Analysis, %	Calc.	Found
XXX	C_6H_5	79	Ethanol–water	73–75°	$C_9H_9N_3S$	C	56.54	56.74
						H	4.74	4.46
						N	21.98	22.04
XXXI	2-$CH_3C_6H_4$	85	Ethanol–water	79–80°	$C_{10}H_{11}N_3S$	C	58.53	58.61
						H	5.40	5.60
						N	20.48	20.18
XXXII	2-$CH_3C_6H_4$	25	Ethanol	88–90°	$C_{10}H_{11}N_3S$	C	58.53	58.50
						H	5.40	5.34
						N	20.48	20.50
XXXIII	2-$C_2H_5C_6H_4$·HCl	92	Methanol–ether	133° dec.	$C_{11}H_{13}N_3S$·HCl	C	51.66	51.28
						H	5.52	5.56
						N	16.43	16.22
XXXIV	2-$CH_3OC_6H_4$	63	Ethyl acetate–hexane	67–68°	$C_{10}H_{11}N_3OS$	C	54.30	54.18
						H	5.01	4.99
						N	18.99	18.84

[a] Method C was used; see *Experimental.*

Table IV—Physical Properties of Several Miscellaneous Analogs [a]

Compound	Yield, %	Crystallization Solvent	Melting Point	Molecular Formula	Analysis, % Calc.	Found
XXXV	66	Ethanol–water	48–49°	$C_{10}H_9N_3S$	C 59.11	58.98
					H 4.46	4.57
					N 20.68	20.63
XXXVI	44	Ethanol	116–117°	$C_{11}H_{15}N_3S$	C 59.71	60.05
					H 6.83	6.93
					N 18.99	19.03
XXXVII	73	Methanol–ether	160–161°	$C_{11}H_{13}N_3S \cdot HCl$	C 51.65	51.41
					H 5.52	5.24
					N 16.44	16.29

[a] For XXXVII, Method C was used; see *Experimental.*

at 200 mg/kg; insertion of a 2-methyl substituent gave XVI, which was fully active at 25 mg/kg. Analogs containing chloro, methoxy, and ethyl substituents in the *ortho*-position were not active at 200 mg/kg, nor did movement of the methyl group to the 3- or 4-position yield active compounds.

Surprisingly, the 2,6-dimethyl analog (XXV) was completely inactive. Oxidation of the hydrazine (XVI) to the azo derivative (XXXI) gave the most potent compound in the series, active at 12.5 mg/kg. Replacement of the thiazoline ring by thiazole (XXXV) substantially reduced activity, whereas expansion of the ring to the thiazines (XXXVI and XXXVII) abolished activity at the screening level.

It was considered that perhaps all activity could be accounted for by reductive cleavage to 2-amino-2-thiazoline *in situ.* Such conversion would constitute a delivery system for the established neuromuscular blocking agent. *In vitro* evaluation of I, XVI, and XXXI against third-stage larvae of *H. contortus* showed all three compounds to have a similar paralytic effect, but XXXI was the most potent. Furthermore, examination of the urine of sheep dosed orally with XVI or XXXI revealed only the presence of the azo compound, XXXI. These data suggest that although all three compounds have intrinsic activity *in vitro*, XXXI is mainly responsible for the *in vivo* activity.

2-(*o*-Tolylazo)-2-thiazoline (XXXI) was tested in sheep at a single oral dose of 76 mg/kg and was effective against *Haemonchus, Ostertagia, Trichostrongylus, Cooperia, Nematodirus, Oesophagostomum,* and *Dictyocaulus* species.

EXPERIMENTAL[1]

Method A: 1-(*o*-Tolyl)-3-thiosemicarbazide (II)—A mixture of *o*-tolylhydrazine hydrochloride (15.9 g, 0.1 mole), ammonium thiocyanate (15.2 g, 0.2 mole), 30 ml of water, and 300 ml of ethanol was heated under reflux for 24 hr. The solvent was evaporated, and water was added. The solid residue was crystallized from aqueous ethanol to obtain 15 g (85%) of II, mp 158–160°.

Method B: 2-(*o*-Tolylhydrazino)-2-thiazoline (XVI)—A stirred mixture of II (27.2 g, 0.15 mole), 2-bromoethylamine hydrobromide (30.8 g, 0.15 mole), and 2-propanol (800 ml) was heated under reflux for 16 hr. The resulting mixture was filtered hot to remove ammonium bromide, and the filtrate was allowed to cool slowly. The precipitated solid (XVI hydrobromide) was collected, dissolved in hot water, and neutralized with dilute sodium bicarbonate solution. The precipitated title compound was filtered, washed well with water, and recrystallized from methanol to yield 13.2 g (43%) of product, mp 126–127°.

Method C: 2-(*o*-Tolylazo)-2-thiazoline (XXXI)—To a stirred solution of XVI (8 g, 0.0386 mole) in 400 ml of ethyl acetate was added 4 g of silver oxide. After stirring for 18 hr at room temperature, silver oxide was filtered off. The residue obtained upon evaporation was crystallized from aqueous ethanol to give 6.7 g (85%) of XXXI as orange needles, mp 79–80°.

2-(*o*-Tolylazo)thiazole (XXXVII)—A mixture of XVI (0.5 g, 0.0024 mole), sodium carbonate (1 g), and ethanol (50 ml) was refluxed for 3 days. The sodium carbonate was filtered, the solvent was removed *in vacuo*, and the residue was crystallized from aqueous ethanol to give 0.32 g (66%) of orange prisms, mp 48–49°.

5,6-Dihydro-2-(*o*-tolylhydrazino)-1,3(4*H*)-thiazine Hydrochloride (XXXVI)—To a stirred solution of 3-chloropropyl isothiocyanate (5) (15.6 g, 0.115 mole) and *o*-tolylhydrazine hydrochloride (18.3 g, 0.115 mole) in 200 ml of ethanol, triethylamine (11.7 g, 0.115 mole) was added. The reaction mixture was refluxed for 16 hr. The separated solids were filtered hot, washed with cold ethanol, and dried, yielding 8.9 g (30%), mp 255–256°.

A solution of XXXVI hydrochloride in water was neutralized with sodium bicarbonate. The solid residue was recrystallized twice from ethanol to give its free base as white platelets, mp 116–117°.

2-(2-Pyridylhydrazino)-2-thiazoline (XXVII)—2-Methylmercapto-2-thiazoline hydroiodide (4) (26.1 g, 0.1 mole) and 2-hydrazinopyridine (10.9 g, 0.1 mole) in methanol (100 ml) were heated together under reflux for 5 hr. The reaction mixture was diluted with methanol (1.5 liters) and passed through a resin[2] column (500 ml) in the hydroxyl form with methanol. The column was eluted with methanol, and the eluate was evaporated *in vacuo.* The residue was triturated with 2-propanol and recrystallized from ethanol, yielding 7.8 g (40%), mp 248–250°.

Table V—Anthelmintic Activity of 2-Arylhydrazino- and 2-Arylazo-2-thiazolines and Analogs

Compound	Activity[a]
XV	SA 200
XVI	A 25
XVII	I 200
XVIII	I 200
XIX	I 200
XX	I 200
XXI	I 200
XXII	SA 200
XXIII	I 200
XXIV	I 200
XXV	I 200
XXVI	I 200
XXVII	I 200
XXVIII	SA 200
XXIX	I 200
XXX	SA 100
XXXI	A 12.5
XXXII	I 200
XXXIII	SA 100
XXXIV	I 200
XXXV	SA 200
XXXVI	I 200
XXXVII	I 200
Thiabendazole	A 50

[a] Activity expressed as A (active), SA (slightly active), or I (inactive) at the test level shown in milligrams per kilogram given orally.

REFERENCES

(1) H. Ozawa and K. Kubota, *Yakugaku Zasshi,* **89,** 1 (1969).

(2) G. H. Coleman, *Org. Synth.,* I, 442 (1941).

(3) T. P. Johnson, C. R. Stringfellow, Jr., and A. Gallagher, *J. Org. Chem.,* **30,** 2073 (1965).

(4) A. F. McKay, D. J. Whittingham, and M. E. Kreling, *J. Am. Chem. Soc.,* **80,** 3339 (1958).

(5) P. Friis, *Acta Chem. Scand.,* **19,** 766 (1965).

(6) J. E. Lynch and B. Nelson, *J. Parasitol.,* **45,** 659 (1959).

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Microanalyses were determined by Mr. R. N. Boos and Mr. J. P. Gilbert and their associates.

[x] To whom inquiries should be directed.

[1] Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. All intermediates and final products were checked by IR and NMR spectroscopy, and their spectra were in accord with the structures assigned. IR spectra were obtained as mineral oil mulls using a Perkin-Elmer model 137 Infracord spectrophotometer. NMR spectra were obtained in deuterochloroform using a Varian T-60A spectrometer and tetramethylsilane as an internal standard.

[2] IRA-401.