radiation. The crystal showed no deterioration during data collection. The data were corrected for Lorentz and polarization effects, but not for extinction or absorption effects. A total of 5440 unique reflections were collected to $2\theta = 130°$. The structure was solved by using the direct methods programs MULTAN[13] and NQEST.[14] Non-hydrogen atom thermal parameters were made anisotropic and refined by full-matrix least-squares techniques. Hydrogen atoms were located in Fourier difference maps and were given isotropic thermal parameters one unit greater than the heavy atoms to which they were bound; their thermal parameters were held constant during refinement. The structure converged to a final R index of 0.059 with 3335 ($I \geq 3\sigma(I)$) intensities.

X-ray Studies on 4-[*N*-[(2,4-Diamino-6-pteridinyl)methyl]amino]benzoic Acid. Crystals of PMAB (Sigma) were grown from an ethanol solution containing trace amounts of HCl. The crystal used for data collection was rectangular with dimensions 0.08 × 0.12 × 0.20 mm. The cell parameters for the monoclinic $P2_1/c$ lattice are as follows: $a = 9.182$ (2) Å, $b = 8.771$ (2) Å, $c = 16.561$ (3) Å, $\beta = 96.52$ (2)°, $Z = 4$, $V = 1325.2$ Å^3, $D_{calcd} = 1.560$ g/cm^{-1}. Intensities for 3086 independent reflections were measured on a Nicolet P3 diffractometer, using Mo Kα ($\lambda = 0.7069$) radiation. All non-hydrogen atoms were located by direct methods using MULTAN[13] and NQEST.[14] Acceptable atomic positions for all hydrogen atoms were located from Fourier difference maps. The structure was refined by full-matrix least-squares techniques. Hydrogen positional parameters were refined, while their isotropic thermal parameters were held constant at values one unit greater than the heavy atom to which they were bound. The structure converged to a final R index of 0.063 with 1787 ($I \geq 3\sigma(I)$ and $2\theta < 50°$) intensities.

Structural Studies. The data for structural comparisons were taken from the CCD.[11] Individual crystal structures were obtained by using the connectivity of **6** with the following restrictions: ring members were either carbon or nitrogen, except for positions 6, 7, 11, and 16, which were restricted to carbon; ring substitution was not restricted except for positions 7 and 16, which required a hydrogen; substitution on N(10) was restricted to alkyl substituents; position 9 was kept as a $-CH_2-$. A total of 11 independent conformations from nine crystal structures was found in the search.

Geometric and Conformational Analysis. The molecular geometry and rotational energies were evaluated on the National Institutes of Health PROPHET[15] system. Rotational energy barriers were calculated by using the program CAMSEQ.[12] The data from CAMSEQ were processed with the program CAMMAP and plots were generated with the program CONTOUR. This version of CAMSEQ is only dimensioned for structures the size of fragment **6**; therefore, calculations were performed on molecular fragments derived from the X-ray coordinates of PMAB, QU, MTX, and TMQ, respectively.[16] All hydrogen atoms on carbons were placed in calculated positions. The energy surfaces were calculated at 10° intervals over the full 360° range. No energy minimization was performed.

Acknowledgment. This work was supported in part by grants from NCI-CA-34714, FRA-287 (V.C.) from the American Cancer Society Faculty Research Award, and the Buffalo Foundation.

Supplementary Material Available: Tables of hydrogen-bonding geometries, anisotropic thermal parameters, hydrogen positional parameters, and torsional angle listing and references for fragment **6**, and a figure of bond lengths and angles (6 pages). Ordering information is given on any current masthead page.

Isothiourea Derivatives of 6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole with Broad-Spectrum Anthelmintic Activity

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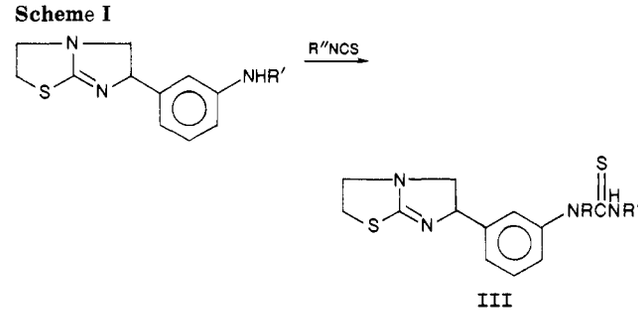
A series of isothiourea derivatives of 6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (tetramisole) is described. The compounds are prepared by the S-alkylation of the thioureas that were obtained either by the reaction of an amine with 6-(3-isothiocyanatophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole or by the reaction of an isothiocyanate with 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole. These derivatives have an improved spectrum of activity over tetramisole and are active against nematodes, cestodes, and trematodes. The structure–activity relationships are discussed.

Since the discovery of the broad-spectrum nematode anthelmintic 6-phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]-thiazole (tetramisole) (I) in 1966[1] many groups have prepared analogues with the aim of finding derivatives with improved activity and spectrum. While tetramisole is effective against a wide range of nematodes, it shows no activity against other important parasite species such as cestodes and trematodes.[2] Of the analogues of tetramisole reported in the literature, the 6-(3-aminophenyl) derivative (II) and its acyl derivatives[3] constitute an important group with improved activity against certain nematodes but no reported activity against either cestodes or trematodes.

Scheme I



III

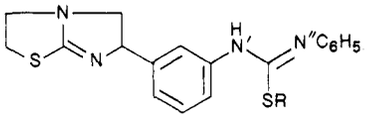
R' = H or CH_3

We now report on the synthesis and anthelmintic activity of a series of 3′-isothioureido-substituted tetramisole

(1) Raemaekers, A. H. M.; Allewijn, F. T. N.; Vandenberk, J.; Demoen, P. J. A.; van Offenwert, T. T. T.; Janssen, P. A. J. *J. Med. Chem.* **1966**, *26*, 714.
(2) Thienpont, J.; Vanparijs, O. F. J.; Raemaekers, A. H. M.; Vandenberk, J.; Demoen, P. J. A.; Allewijn, F. T. N.; Marsboom, R. P. H.; Niemegeers, C. J. E.; Schellekens, K. H. L.; Janssen, P. A. J. *Nature (London)* **1966**, *209*, 1084.
(3) Spicer, L. D.; Hand, J. J. U.S. Patent 3989835, 1976.

0022-2623/87/1830-1848$01.50/0

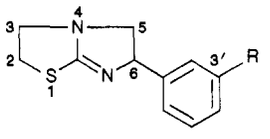
Table I. Activity of S-Substituted Isothiourea Derivatives of 6-(3-Aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole



no.	R	mp, °C	molecular formula	anal. (HRMS)[a]	% act. in mouse,[b,c] 200 mg/kg
1[d]	CH_3	oil	$C_{19}H_{20}N_4S_2$	$[M]^+$	99
2	C_2H_5	oil	$C_{20}H_{22}N_4S_2$	$[M]^+$	92
3	$(CH_2)_2CH_3$	52–55	$C_{21}H_{24}N_4S_2$	$[M - HSC_3H_7]^+$	64
4	$CH(CH_3)_2$	oil	$C_{21}H_{24}N_4S_2$	$[M]^+$	0
5	$(CH_2)_3CH_3$	57–60	$C_{22}H_{26}N_4S_2$	$[M]^+$	0
6	$(CH_2)_7CH_3$	oil	$C_{26}H_{34}N_4S_2$	$[M]^+$	0
7	$CH_2C_6H_5$	44–47	$C_{25}H_{24}N_4S_2$	$[M]^+$	0
8	CH_3 (N′CH_3)	oil	$C_{20}H_{22}N_4S_2$	$[M]^+$	0
9	CH_3 (N″CH_3)	oil	$C_{20}H_{22}N_4S_2$	$[M]^+$	0

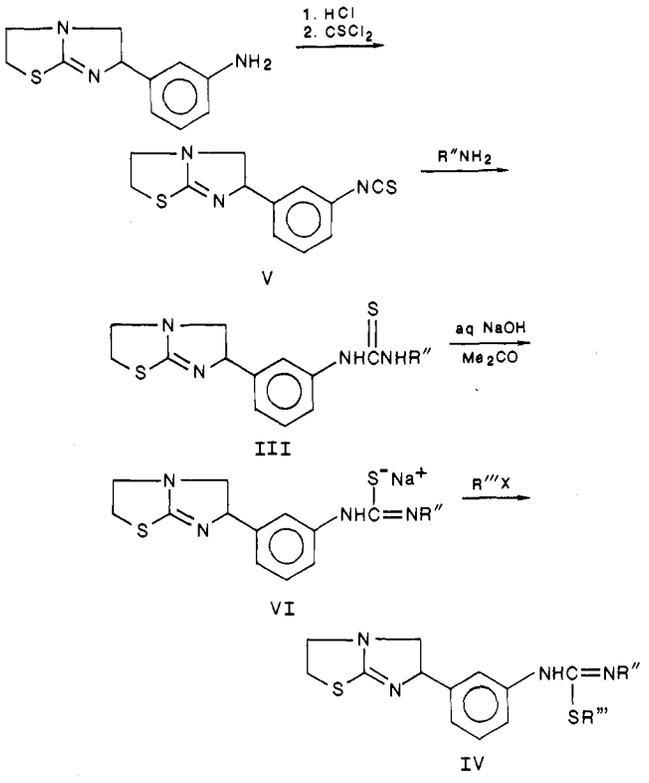
[a] High-resolution mass spectrum. Accurate mass measured. [b] Percentage reduction of *N. dubius* at necropsy. [c] Single oral dose. [d] Percent activity in the mouse at 100 mg/kg = 38 and at 50 mg/kg = 0.

derivatives (IV), which are active against nematodes, trematodes, and cestodes.



I R = H
II R = NH_2
III R = NHCSNHR″
IV R = NHC(SR‴)=NR″
V R = NCS
VI R = NC(S^-Na^+)=NR″

Scheme II

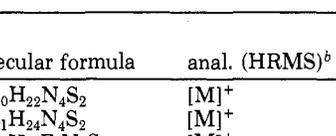


Chemistry

3′-Aminotetramisole (II) was prepared according to the method of Spicer and Hand[3] and used in the preparation of the thiourea derivatives.

An acetone solution of 3′-aminotetramisole (II) was treated with 1 equiv of a suitable isothiocyanate, and this

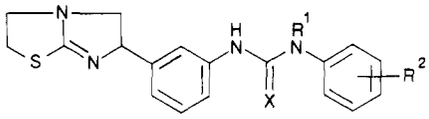
Table II. Activity of *S*-Methylisothiourea Derivatives of 6-(3-Aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole



no.	R[a]	mp, °C	molecular formula	anal. (HRMS)[b]	% act. in mouse[c,d] 200 mg/kg[d]	100 mg/kg	50 mg/kg
10	4-$CH_3C_6H_4$	oil	$C_{20}H_{22}N_4S_2$	$[M]^+$	0		
11	3,4-$(CH_3)_2C_6H_3$	59–61	$C_{21}H_{24}N_4S_2$	$[M]^+$	0		
12	4-$CF_3C_6H_4$	58–62	$C_{20}H_{19}F_3N_4S_2$	$[M]^+$	74		
13	4-$C_2H_5O_2CC_6H_4$	41–44	$C_{22}H_{24}N_4O_2S_2$	$[M]^+$	74		
14	4-$CH_3COC_6H_4$	50–53	$C_{21}H_{22}N_4OS_2$	$[M - HSCH_3]^+$	100	93	
15	2-CNC_6H_4	78–80	$C_{20}H_{19}N_5S_2$	$[M - H]^+$	0		
16	3-CNC_6H_4	55–59	$C_{20}H_{19}N_5S_2$	$[M - HSCH_3]^+$	100	54	
17	4-CNC_6H_4	61–63	$C_{20}H_{19}N_5S_2$	$[M - HSCH_3]^+$	100	99	74
18	(6*S*)-4-CNC_6H_4[e-g]	oil	$C_{20}H_{19}N_5S_2$	$[M - HSCH_3]^+$	100	100	100
19	3-$NO_2C_6H_4$	64–66	$C_{19}H_{19}N_5O_2S_2$	$[M - HSCH_3]^+$	95	95	
20	4-$NO_2C_6H_4$	73–75	$C_{19}H_{19}N_5O_2S_2$	$[M - HSCH_3]^+$	100	100	95
21	4-$CH_3OC_6H_4$	66–68	$C_{20}H_{22}N_4OS_2$	$[M]^+$	0		
22	4-$CH_3SC_6H_4$	oil	$C_{20}H_{22}N_4S_3$	$[M]^+$	95	30	
23	4-$CH_3SOC_6H_4$	75–80	$C_{20}H_{22}N_4OS_3$	$[M - HSCH_3]^+$	97	33	
24	4-$CH_3SO_2C_6H_4$	55	$C_{20}H_{22}N_4O_2S_3$	$[M]^+$	100	94	41
25	3-FC_6H_4	oil	$C_{19}H_{19}FN_4S_2$	$[M]^+$	100	100	64
26	4-FC_6H_4	oil	$C_{19}H_{19}FN_4S_2$	$[M]^+$	100	0	
27	2-ClC_6H_4	70–75	$C_{19}H_{19}ClN_4S_2$	$[M]^+$	23		
28	3-ClC_6H_4	51–52	$C_{19}H_{19}ClN_4S_2$	$[M]^+$	99	87	
29	4-ClC_6H_4	80–82	$C_{19}H_{19}ClN_4S_2$	$[M]^+$	88	0	
30	3,4-$Cl_2C_6H_3$	oil	$C_{19}H_{18}Cl_2N_4S_2$	$[M - HSCH_3]^+$	0	0	
31	3-BrC_6H_4	48–52	$C_{19}H_{19}BrN_4S_2$	$[M - HSCH_3]^+$	79	56	
32	4-BrC_6H_4	58–60	$C_{19}H_{19}BrN_4S_2$	$[M - HSCH_3]^+$	73	0	
33	$(CH_2)_5CH_3$	oil	$C_{19}H_{28}N_4S_2$	$[M]^+$	52		
34	c-C_6H_{11}	oil	$C_{19}H_{26}N_4S_2$	$[M]^+$	15		

[a] All compounds are racemic mixtures unless otherwise stated. [b] High-resolution mass spectrometry. Accurate mass measured. [c] Percentage reduction of *N. dubius* at necropsy. [d] Single oral dose. [e] Percent activity in the mouse at 25 mg/kg = 69. [f] Prepared from (6*S*)-3′-aminotetramisole. [g] $[\alpha]^{22}_D$ −77° (MeOH).

Table III. Thiourea and Urea Derivatives of 6-(3-Aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole



no.	X	R^1	R^2	mp, °C	molecular formula	anal.
35	S	H	H	133–134	$C_{18}H_{18}N_4S_2$	C, H, N
36	S	H	4-CH_3	158–160	$C_{19}H_{20}N_4S_2$	C, H, N
37	S	H	3,4-$(CH_3)_2$	152–153	$C_{20}H_{22}N_4S_2$	C, H, N
38	S	H	4-CF_3	159–161	$C_{19}H_{17}F_3N_4S_2$	C, H, N, S
39	S	H	4-CH_3CO	133–135	$C_{20}H_{20}N_4OS_2$	C, H, N, S
40	S	H	3-CN	148–150	$C_{19}H_{17}N_5S_2$	C, H, N
41	S	H	4-CN	162–164	$C_{19}H_{17}N_5S_2$	C, H, N, S
42	S	H	4-NO_2	124–126	$C_{18}H_{17}N_5O_2S_2$	C, H, N, S
43	S	H	4-CH_3O	153–154	$C_{19}H_{20}N_4OS_2$	C, H, N
44	S	H	4-CH_3S	110–115	$C_{19}H_{20}N_4S_3$	C, H, N, S
45	S	H	4-CH_3SO	117–120	$C_{19}H_{20}N_4OS_3$	C, H, S; N[a]
46	S	H	3-F	108–110	$C_{18}H_{17}FN_4S_2$	C, H, N, S
47	S	H	4-F	134–135	$C_{18}H_{17}FN_4S_2$	C, H, N, S
48	S	H	2-Cl	118–121	$C_{18}H_{17}ClN_4S_2$	C, H, N, S, Cl
49	S	H	3-Cl	165–167	$C_{18}H_{17}ClN_4S_2$	C, H, N, S, Cl
50	S	H	4-Cl	149–151	$C_{18}H_{17}ClN_4S_2$	C, H, N, S, Cl
51	S	H	3,4-Cl_2	163–165	$C_{18}H_{16}Cl_2N_4S_2$	C, H, N, Br
52	S	H	3-Br	172–174	$C_{18}H_{17}BrN_4S_2$	C, H, N, Br
53	S	H	4-Br	152–155	$C_{18}H_{17}BrN_4S_2$	C, H, N
54	S	CH_3	H	oil	$C_{19}H_{20}N_4S_2$	C, H, S; N[b]
55	O	H	H	102–103	$C_{18}H_{18}N_4OS$	C, H, N

[a] N: calculated, 13.46; found, 12.94. [b] N: calculated, 15.20; found, 14.70.

mixture, on standing, gave a precipitate of the thiourea III (Scheme I). Alternatively the 3′-isothiocyanato derivative of tetramisole (V) was treated with a suitable amine in acetone solution and the precipitated thiourea recovered by filtration (Scheme II).

The isothiocyanate V[3] was prepared by refluxing a solution of the amine II in dilute hydrochloric acid with thiophosgene according to the general method of Elderfield and Short.[4] This method was used to prepare all the isothiocyanates used in this work that were not commercially available.

Treatment of a suspension of the thiourea III in either acetone or acetonitrile with 1 equiv of aqueous 2 M sodium hydroxide solution gave a solution of the sodium derivative of the sulfur anion VI, which, on reaction with an alkyl halide, gave the isothiourea IV.

Biological Results and Discussion

Following our discovery that the phenylisothiourea **1** (Tables I and II) possessed nematocidal activity in the mouse, we investigated the urea derivative **55** (Table III) since we considered that this would be a likely metabolite of the isothiourea. The urea, however, proved to be inactive at comparable doses in our mouse nematode screen, indicating that the phenylisothiourea **1** was probably active per se.

It also became apparent that the thiourea derivatives III possessed some nematocidal activity, but, on testing for anthelmintic activity in the sheep, generally only the isothiourea derivatives possessed good activity against the liver fluke *Fasciola hepatica* (Table IV).

The nematocidal activity of the phenylisothiourea **1** was considerably less than that of tetramisole and 3-aminotetramisole (Table VII); however, the isothiourea **1** was active against liver flukes whereas neither tetramisole[2] nor 3-aminotetramisole displayed any activity against this parasite (Table VIII).

In order to improve the nematocidal activity of the isothioureas an investigation of the structure–activity relationships for these derivatives was initiated with a mouse roundworm screen being used to determine the relative

Table IV. Activity of *S*-Methylisothioureas against Adult Liver Fluke (*Fasciola hepatica*) in Controlled Sheep Tests[a]

	% activity at dose (mg/kg) po		
no.	30	20	15
1	94 (2)[d]		
2	97	I	
3	I[c]		
4	I		
5	I		
6	I		
7	I		
8	I		
9	I		
10	91		
11	85		
12	100		100
13	97	I	
14	100		100
15	I		
16	100 (3)	100	63
17	97	89	83
18[b]			
19	91		100
20	94 (2)	85	I
21	I		
22	100 (2)	80	71
23	100	66	14
24	100 (2)	89 (2)	74
25	66		
26	60		
27	I		
28	91	I	
29	100		
30	63		
31	100	100	77
32	94	100	
33	I		
34	I		

[a] Single animals were used in all tests unless otherwise indicated. [b] Percent activity at 10 mg/kg = 100, at 7.5 mg/kg = 91, and at 5 mg/kg = 85. [c] I = no effect on sheep fecal fluke egg output. [d] Numbers in parentheses indicate the number of animals used.

(4) Elderfield, R. C.; Short, F. W. *J. Org. Chem.* **1953**, *18*, 1092.

Table V. Comparison of the Activity of 17 and Its 6S Isomer 18 against *N. dubius* in the Mouse

treatment	dose, mg/kg	activity, %
17[a]	100	100
	50	99
	25	68
	12.5	28
18[a]	100	100
	50	100
	25	99.9
	12.5	66

[a] Mice dosed orally with solution of drug in PEG 400.

nematocidal activity of the analogues.

The effect of different *S*-alkyl substituents was investigated (compounds **1–7**, Table I). This clearly indicated that, for optimal nematocidal activity, either an *S*-methyl (**1**) or an *S*-ethyl (**2**) group was necessary and any further increase in alkyl chain length resulted in a sharp reduction in activity.

The presence of a tertiary nitrogen in the isothiourea group (compounds **8** and **9**, Table I) gave compounds of considerably reduced activity. Substitution of an alkyl or cycloalkyl group for the phenyl group in the isothioureas also produced compounds of reduced activity (**33** and **34**, Table II).

The effect of different substituents in the phenyl ring was also investigated (Table II) and demonstrated a requirement for electron-withdrawing groups ($+\sigma$) that did not substantially alter the lipophilicity of the molecule. Thus, the introduction of electron-withdrawing groups such as 4-NO_2 (**20**) and 4-CN (**17**) improved the activity while electron-releasing substituents, for example 4-OCH_3 (**21**) and 4-CH_3 (**10**), generally decreased the activity.

Electron-withdrawing groups that substantially alter the lipophilicity of the molecule from that of the parent compound (**1**) also reduced the improvement in activity brought about by the $+\sigma$ effect. Thus, both the 4-CF_3 (**12**) and 4-CH_3SO_2 (**24**) derivatives were less active than either the 4-CN (**17**) or 4-NO_2 (**20**) derivative, with the large $+\pi$ effect of the 4-CF_3 group (**12**) giving an overall net reduction in activity when compared to the parent compound (**1**).

A very marked reduction in activity was noted with ortho substituents. Thus, the 2-CN derivative (**15**) was far less active than either the 3-CN (**16**) or the 4-CN (**17**) derivative and a similar trend was noted for the chloro derivatives (**27–29**).

The isothiourea derivatives we describe in this paper possess at least one asymmetric center and may, therefore, like tetramisole (I), be prepared in optically active forms. Since it is known[5] that the 6*S* isomer of tetramisole (I) is responsible for the activity of the racemate, we prepared a 6*S* isomer of one of the most active racemic isothioureas (**17**) by synthesis from pure 6(*S*)-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole. The 6*S* isomers would seem to be responsible for the activity of the racemates, since the 6*S* isomer (**18**) was twice as active (Table V) as the racemate (**17**).

The compounds that showed good activity in the mouse screen were further evaluated in the target species, namely sheep, dogs, and cats. The 4-cyano derivative (**17**) and its 6*S* isomer (**18**) were found to be very effective in these tests and were selected for further study. They were found to be active against all important ruminant nematodes and liver flukes and also against the roundworm *Toxascaris* and tapeworm *Taenia* in the dog and cat (Table VI).

Unfortunately, toxicity has proved to be a major obstacle in the search for a broad-spectrum anthelmintic based on the tetramisole nucleus, and the therapeutic ratio (maximum tolerated dose/minimum effective dose) of these compounds, while being adequate when dealing with many of the parasites in the above target species, was not sufficiently high in all cases to warrant further progression.

Experimental Section

Melting points were taken on a Büchi melting point apparatus and are uncorrected. All compounds were routinely examined by TLC using alumina plates eluted with chloroform. In addition, several compounds (**1**, **3**, **14**, **17**, **18**, **20**, **31**, **32**, **34**) were examined by HPLC using the following system: Ultrasphere cyano column (15 cm × 4.6 mm diameter) eluted with methanol/0.01 M NH_4HCO_3 (aq) 70:30 at 1.5 mL/min and detection by UV absorption at 254 nm. ^1H NMR spectra were recorded on a Perkin-Elmer Hitachi Model R24A 60-MHz spectrophotometer using Me_4Si as internal standard. ^{13}C NMR spectra were recorded on a Brücker WM-250 spectrophotometer using Me_4Si as internal standard. Elemental analyses are within ±0.4% of the calculated values except where noted. The elemental compositions of the isothioureas were established by high-resolution mass spectrometry (HRMS). All HRMS determinations were within 4 millimass units of the theoretical values.

***N*-(4-Methylphenyl)-*N'*-[3-(2,3,5,6-tetrahydroimidazo-[2,1-*b*]-thiazol-6-yl)phenyl]thiourea (36) via 4-Methylphenyl Isothiocyanate.** To a solution of 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (II) (4.4 g, 20 mmol) in acetone (60 mL) at room temperature was added a solution of 4-methylphenyl isothiocyanate (3 g, 20 mmol) in acetone (60 mL). The mixture was kept at room temperature overnight, the resulting precipitate was collected by filtration, washed with acetone (50 mL), and dried in vacuo at 60 °C to afford 5.26 g (72%) of **36**: mp 158–160 °C; ^1H NMR (Me_2SO-d_6) δ 2.7–3.7 (6 H, m), 3.7 (3 H, s, Ar CH_3), 5.3 (1 H, t, J = 9 Hz, NC*H*CH_2), 6.8–7.4 (9 H, m, aromatic), 9.7 (1 H, br s, NH). Anal. ($C_{19}H_{20}N_4S_2$) C, H, N.

Similarly prepared were the thioureas described in Table III.

***N*-Phenyl-*N'*-[3-(2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazol-6-yl)phenyl]urea (55).** By use of phenyl isocyanate in the above example, the urea **55** was obtained in 90% yield: mp 102–103 °C. Anal. ($C_{18}H_{18}N_4OS$) C, H, N.

6-(3-Isothiocyanatophenyl)-2,3,5,6-tetrahydroimidazo-[2,1-*b*]thiazole (V). To a solution of 6-(3-aminophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (II) (10.9 g, 50 mmol) in 2 N aqueous HCl (100 mL) at 50 °C was added thiophosgene (4 mL, 52 mmol). The temperature was maintained at 50–60 °C for 2 h, and the solution was cooled to room temperature, basified with 2 N aqueous NaOH, and extracted with $CHCl_3$ (3 × 75 mL). The extracts were dried ($MgSO_4$) and evaporated to yield 10 g of a friable solid, which was used without further purification.

***N*-(4-Methylphenyl)-*N'*-[3-(2,3,5,6-tetrahydroimidazo-[2,1-*b*]thiazol-6-yl)phenyl]thiourea (36) via *p*-Toluidine.** To a solution of 6-(3-isothiocyanatophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole (4 g, 15 mmol) in acetone (200 mL) was added *p*-toluidine (2 g, 19 mmol). The solution was kept at room temperature overnight, and the resulting precipitate was collected by filtration, washed with acetone, and dried in vacuo at 60 °C to afford 4.25 g (77%) of **36**, which showed identical physical and chemical properties to a sample prepared by the alternative method described above.

Similarly prepared was *N*-methyl-*N*-phenyl-*N'*-[3-(2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazol-6-yl)phenyl]thiourea (**54**) by the use of *N*-methylaniline. Anal. ($C_{19}H_{20}N_2S$) C, H, S; N: calculated, 15.20; found, 14.70.

***S*-Methyl-*N*-phenyl-*N'*-[3-(2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazol-6-yl)phenyl]isothiourea (1).** To *N*-phenyl-*N'*-[3-(2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazol-6-yl)phenyl]thiourea (**35**) (5 g, 14 mmol) suspended in acetone (50 mL) was added a solution of NaOH (565 mg, 14 mmol) in H_2O (5 mL). The mixture was stirred for 1 h at room temperature, resulting in a clear solution, which was cooled in an ice bath, and methyl iodide (0.93 mL, 15 mmol) was added. The solution was stirred for 2 h, the acetone

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Table VI. Activity of **17** in Target Species

species	dose, mg/kg[b]	route	activity[a] abomasum *T.a.*[c]	*Os.*[d] spp.	*H.c.*[e]	small intestine *Tr.* spp.[f]	*C.c*[g]	*Ne.* spp.[h]	*S.p.*[i]	*B.t.*[j]	*Ca.* spp.[k]	large bowel *C.o.*[l]	*O.c.*[m]	*T.o.*[n]	*D.f.*[o]	*F.h.*[p]
sheep	30	po	100	100	100	100	100	73	100	100	-[q]	100	93	100	100	-
	30	sc	100	100	-	100	91	100	100	100	-	96		100	100	100
	15	po	92	95	-	100	99	100	100	100	0	99	100	100	100	100

species	dose, mg/kg	route	*Toxascaris*	tapeworm (*Taenia*)
dog	30	po	worms voided and *Toxascaris* egg count reduced to zero for the duration of the test	
cat	30	po		worms expelled and no further segments appeared in feces for the duration of the test

[a] Activity is expressed as the percentage of worms eliminated in feces. [b] Single animals at each dose. [c] *Trichostrongylus axei.* [d] *Ostertagia* spp. [e] *Haemonchus contortus.* [f] *Trichostrongylus* spp. [g] *Cooperia curticei.* [h] *Nematodirus* spp. [i] *Strongyloides papillosus.* [j] *Bunostomum trigonocephalum.* [k] *Capillaria* spp. [l] *Chabertia ovina.* [m] *Oesophagostomum columbianum.* [n] *Trichuris ovis.* [o] Lungworm *Dictyocaulus filaria.* [p] Liver fluke *Fasciola hepatica.* [q] (-) Test animal was not infected with this species of parasite.

Table VII. Comparison of Nematocidal Activity of **17** with Tetramisole and 3′-Aminotetramisole

compound	activity against *N. dubius* in the mouse at the indicated dose, mg/kg po 100	50	25	5	1	0.5
3′-aminotetramisole		*a*		100	99	43
tetramisole	100	100	98	57	43	
17	100	99	68			

[a] All animals died at this dose.

Table VIII. Comparison of Toxicity and Activity against Liver Fluke in the Sheep between **17** and 3′-Aminotetramisole

compound	toxicity and % activity against liver fluke in the sheep at indicated dose, mg/kg po 15	30	300
17	83	97	no toxic symptoms observed
3′-aminotetramisole	0	lethal	

removed in vacuo, and the residue taken up in $CHCl_3$ (50 mL). This solution was washed with H_2O (30 mL), dried ($MgSO_4$), and concentrated in vacuo to afford 5 g (96%) of **1** as an oil: ^1H NMR ($CDCl_3$) δ 2.35 (3 H, s, SCH_3), 2.7–4.0 (6 H, m), 5.4 (1 H, t, J = 9 Hz, NC*H*CH_2), 6.1 (1 H, br s, NH), 6.9–7.4 (9 H, m, aromatic); ^{13}C NMR (Me_2SO-d_6) δ 14.7 (SCH_3), 34.2 (2-C), 48.4 (3-C), 57.4 (6-C), 75.3 (7-C), 118.5–143.2 (aromatic), 149.7 (N=*C*SCH_3), 179.2 (7a-C). Calcd for $C_{19}H_{20}N_4S_2$: m/e 368.1130. Found: m/e 368.1132 $[M]^+$.

By use of the appropriate thiourea, the isothioureas **10–34** described in Table II were similarly prepared. These were purified, where necessary, by column chromatography on alumina and elution with chloroform. All the isothioureas were pure by TLC and exhibited a singlet at δ 2.35 in their ^1H NMR spectrum ($CDCl_3$).

By use of other alkyl iodides in place of methyl iodide, the *S*-alkylisothioureas **2–7** described in Table I were prepared.

Biological Methods. Preliminary investigation of anthelmintic activity and elucidation of structure–activity relationships were carried out in experimentally infected laboratory mice. Further evaluation of the spectrum of activity against roundworms, tapeworms, and flukes was performed in sheep, cats, and dogs.

Mouse Tests. Seven-week-old female LACA mice were infected orally with 100 *Nematospiroides dubius* L_3 larvae. When the infection was 10 days old, a single dose of the test compound was administered by gavage as an aqueous suspension to a group of four mice. Animals were necropsied 4 days later, and the percentage activity was determined from a comparison of the worm burdens in treated and untreated groups.

Sheep Tests. Critical tests were performed in young sheep experimentally infected with 100 *Fasciola hepatica* metacercariae (liver fluke) and naturally infested with lungworms and many species of gastrointestinal roundworms. Drugs were administered orally in gelatin capsules or subcutaneously as a solution in polyethylene glycol 400. Total daily fecal output was collected from the sheep for 4 days after treatment and examined for expelled worms. Seven days after dosing, animals were necropsied and their lungs, liver, and gastrointestinal tract were removed and any worms present were identified. Activity was calculated from a comparison of worms passed out in feces with those remaining in the host at autopsy.

In controlled tests against liver fluke (*Fasciola hepatica*), postmortem worm burdens in treated animals were compared with those in untreated control sheep. Animals were only necropsied if a reduction in fecal egg count was observed. The mean number of flukes from 10 control animals was 33.3.

Dog Tests. Dogs experimentally infected with *Toxascaris leonina* were dosed orally with the test compound in a gelatin capsule. The numbers of worm eggs voided in feces were monitored daily for at least 3 days before and 7 days after treatment. Total daily fecal output was examined for expelled worms during the 4 days after dosing. Activity was registered by a depression of worm egg output.

Cat Tests. Cats with confirmed experimental infections of *Taenia taeniaeformis* were dosed orally with the test compound in a gelatin capsule. Posttreatment examination of total daily fecal output was carried out for up to 9 days. Treatment was considered to be successful if the expulsion of tapeworm segments ceased. This may be accompanied by the expulsion of entire or semidigested worms.

Registry No. (±)-**1**, 109335-13-9; (±)-**2**, 109335-14-0; (±)-**3**, 109335-15-1; (±)-**4**, 109335-16-2; (±)-**5**, 109335-17-3; (±)-6, 109335-18-4; (±)-**7**, 109363-35-1; (±)-**8**, 109335-19-5; (±)-**9**, 109335-20-8; (±)-**10**, 109335-21-9; (±)-**11**, 109335-22-0; (±)-**12**, 109335-23-1; (±)-**13**, 109335-24-2; (±)-**14**, 109335-25-3; (±)-**15**, 109335-26-4; (±)-**16**, 109335-27-5; (±)-**17**, 109335-28-6; (*S*)-**18**, 109430-20-8; (±)-**19**, 109335-29-7; (±)-**20**, 109335-30-0; (±)-**21**, 109335-31-1; (±)-**22**, 109335-32-2; (±)-**23**, 109335-33-3; (±)-**24**, 109335-34-4; (±)-**25**, 109335-35-5; (±)-**26**, 109335-36-6; (±)-**27**, 109335-37-7; (±)-**28**, 109335-38-8; (±)-**29**, 109335-39-9; (±)-**30**, 109335-40-2; (±)-**31**, 109335-41-3; (±)-**32**, 109335-42-4; (±)-**33**, 109335-43-5; (±)-**34**, 109335-44-6; (±)-**35**, 109335-45-7; (±)-**36**, 109335-46-8; (±)-**37**, 109335-47-9; (±)-**38**, 109335-48-0; (±)-**39**, 109335-49-1; (±)-**40**, 109335-50-4; (±)-**41**, 109335-51-5; (±)-**42**, 109335-52-6; (±)-**43**, 109335-53-7; (±)-44, 109335-54-8; (±)-**45**, 109335-55-9; (±)-**46**, 109335-56-0; (±)-**47**, 109335-57-1; (±)-**48**, 109335-58-2; (±)-**49**, 109335-59-3; (±)-**50**, 109335-60-6; (±)-**51**, 109335-61-7; (±)-**52**, 109335-62-8; (±)-**53**, 109335-63-9; (±)-**54**, 109335-64-0; (±)-**55**, 109335-65-1; (±)-II, 60348-13-2; (*S*)-II, 41774-03-2; (±)-III (R″ = 4-$SO_2C_6H_4Me$), 109335-69-5; (±)-III (R″ = 3-$C_6H_4NO_2$, 109335-68-4; (±)-III (R″ = 2-C_6H_4CN), 109335-67-3; (±)-III (R″ = 4-$C_6H_4CO_2C_2H_5$), 109335-66-2; (±)-V, 109335-71-9;

MeI, 74-88-4; C_2H_5I, 75-03-6; $Me(CH_2)_2I$, 107-08-4; Me_2CHI, 75-30-9; $Me(CH_2)_3I$, 542-69-8; $Me(CH_2)_7I$, 629-27-6; $C_6H_5CH_2I$, 620-05-3; C_6H_5NCS, 103-72-0; 4-MeC_6H_4NCS, 622-59-3; 3,4-$Me_2C_6H_3NCS$, 19241-17-9; 4-$F_3CC_6H_4NCS$, 1645-65-4; 4-$MeCOC_6H_4NCS$, 2131-57-9; 3-NCC_6H_4NCS, 3125-78-8; 4-NCC_6H_4NCS, 2719-32-6; 4-$O_2NC_6H_4NCS$, 2131-61-5; 4-$MeOC_6H_4NCS$, 2284-20-0; 4-$MeSC_6H_4NCS$, 15863-41-9; 4-MeS(O)C_6H_4NCS, 109335-70-8; 3-FC_6H_4NCS, 404-72-8; 4-FC_6H_4NCS, 1544-68-9; 2-ClC_6H_4NCS, 2740-81-0; 3-ClC_6H_4NCS, 2392-68-9; 4-ClC_6H_4NCS, 2131-55-7; 3,4-$Cl_2C_6H_3NCS$, 6590-94-9; 3-BrC_6H_4NCS, 2131-59-1; 4-BrC_6H_4NCS, 1985-12-2; C_6H_5NCO, 103-71-9; C_6H_5NHMe, 100-61-8; 4-$MeC_6H_4NH_2$, 106-49-0.

Renin Inhibitors. Statine-Containing Tetrapeptides with Varied Hydrophobic Carboxy Termini

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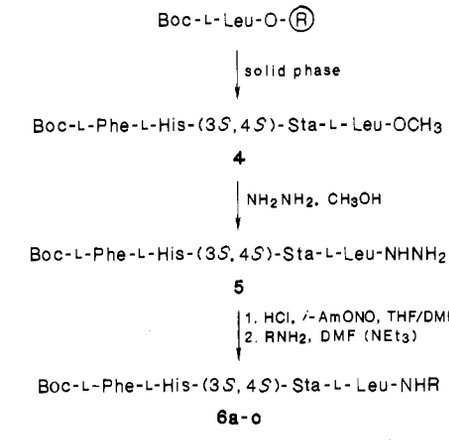
A series of statine-containing tetrapeptides, systematically modified at the carboxy terminus with various hydrophobic aromatic groups, is described. These compounds were tested in vitro for their ability to inhibit porcine, human plasma, and purified human kidney renins. These analogues help to define optimal binding aspects in a region of the enzyme that appears to be specific for spatial arrangement of aromatic groups. Replacement of the metabolically labile Phe amide with nonpeptidal groups proved possible while achieving inhibitory potency in the nanomolar range vs. porcine kidney renin. For the compounds **6i**, **6m**, and **6o**, a large discrepancy in potency between the human plasma and the purified human kidney renin assays was observed. This disparity does not appear to be a consequence of a previously proposed plasma binding component.

In two recent reports[1,2] we described the synthesis and renin-inhibitory activity of modified tetrapeptides. The common features of these potent renin inhibitors are twofold. First, they contain the unusual amino acid statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, which has been postulated to serve as a $Leu^{10}Leu^{11}$ dipeptide surrogate in renin inhibitors designed to resemble angiotensinogen[3,4] (e.g. compound **1**, Table II) and, by extrapolation, to mimic the tetrahedral intermediate for the renin–angiotensinogen reaction. Secondly, these renin inhibitors are smaller peptides than most of the previously reported renin inhibitors of equal potency and contain nonpeptidal fragments. Considering the high substrate specificity exhibited by renin, for which the minimum kinetically competent substrate is an octapeptide around the cleavage site,[5] and the demonstrated rate-enhancing effects of peptide substituents distal to the cleavage site,[5,6] decreasing peptide chain length and making fundamental alterations of the substrate peptide might have been expected to result in less potent renin inhibitors. However, our preliminary studies[1,2] showed that a reduction of peptide chain length was possible without suffering unacceptable losses in inhibitor potency and further, that alterations at the carboxy terminus of the known substrate analogue inhibitors Boc-Phe-His-Sta-Leu-Phe-NH_2 (**2**) and Boc-Phe-Phe-Sta-Leu-Phe-NH_2 (**3**) were allowed and in some instances highly favorable.

The purpose of this study was to identify suitable amino acid replacements for the Phe residue and to determine if they could be incorporated at the C-terminus in the renin inhibitor sequence Boc-L-Phe-L-His-(3*S*,4*S*)-Sta-L-Leu-L-Phe-NH_2 (**2**) with positive effect. As the dominant influence in this position appears to be hydrophobic in nature, these substitutions were chosen to probe varying placements of aryl and substituted aryl groups. An additional aim of this work was to further define a previously described phenomenon in which weak inhibition was obtained in the human plasma assay (I_{50}) for a compound(s) determined to be a good inhibitor(s) in the purified human renin assay (K_i).[2,7]

Scheme I



Results

Chemistry. The tetrapeptide **4** (Scheme I) was synthesized according to standard solid-phase methodology and elaborated to the amides **6a–o** via the intermediacy of acyl hydrazide **5**, followed by azide coupling with the

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