5'-Deoxy-5'-(3-methyl-3-nitrosoureido)adenosine (16a). To a solution of 5'-deoxy-5'-(3-methylureido)adenosine (**15a**; 180 mg, 0.56 mmol) in H_2O (5 mL) containing HOAc (0.5 mL) at 0–5 °C was added sodium nitrite (306 mg, 5.6 mmol). The solid that formed was removed by filtration, washed with H_2O, and dried: yield 142 mg (72%); UV λ_{max} ($\epsilon \times 10^{-3}$) 226 nm at pH 1 and 7 (17.4), 258 at pH 13 (14.7); ^1H NMR δ 3.1 (s, CH_3), 3.7 (m, 2 $H_{5'}$), 4.2 (m, $H_{3'}$ and $H_{4'}$), 4.75 (t, $H_{2'}$), 5.9 (d, $J_{1',2'}$ = 5 Hz, $H_{1'}$), 7.3 (s, NH_2), 8.15 and 8.35 (2 s, H_2 and H_8), 9.0 (t, NH). Anal. ($C_{12}H_{16}N_8O_5 \cdot 0.25H_2O$) C, H, N.

A solution of **16a** (50 mg) in H_2O was refluxed for 1 h before cooling. The precipitate that formed (**17**) was collected and dried: yield 30 mg; MS (FD) 581 [$(M + Na)^+$], 559 [$(M + 1)^+$]; ^1H NMR δ 3.4 (m, CH_2), 3.9 (m, $H_{4'}$), 4.1 (m, $H_{3'}$), 4.7 (m, $H_{2'}$), 5.23 (d, $C_{3'}$ OH), 5.45 (d, $C_{2'}$ OH), 5.9 (d, $J_{1',2'}$ = 6 Hz, $H_{1'}$), 6.2 (t, NH), 7.3 (s, NH_2), 8.2 and 8.35 (H_2 and H_8).

5'-Deoxy-5'-(3-methyl-3-nitrosoureido)uridine (16b). To a solution of 5'-deoxy-5'-(3-methylureido)uridine (**15b**; 300 mg, 1 mmol) in H_2O (5 mL) containing HOAc (0.6 mL) at 0–5 °C was added slowly $NaNO_2$ (345 mg, 5 mmol). The product was isolated by chromatography on a cellulose column (BuOH–H_2O, 6:1) and crystallized from H_2O: yield 50 mg (15%); mp 231 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) 257 nm at pH 1 and 7 (13.3), 262 at pH 13 (7.83). Anal. ($C_{11}H_{15}N_5O_7$) C, H, N.

5'-Deoxy-5'-(3-methyl-3-nitrosoureido)cytidine (16c). N_2O_3 was bubbled in short bursts into a solution of 5'-deoxy-5'-(3-methylureido)cytidine (**15c**; 100 mg, 0.34 mmol) in H_2O (2 mL) at 5–10 °C. The addition of EtOH (10 mL) and ether (30 mL) to the cold solution caused precipitation of a hygroscopic white solid (10 mg), shown by TLC (char, Greiss test) and high-pressure LC to be pure **16c**: MS (FD) 339 [$(M + 1)^+$]; ^1H NMR δ 3.1 (s, CH_3), 3.6 (m, 2 $H_{5'}$), 3.9 (m, $H_{4'}$), 4.15 (m, $H_{2'}$, $H_{3'}$), 5.7 (d, $J_{1',2'}$ = 3 Hz, $H_{1'}$), 6.05 (d, $J_{5,6}$ = 7 Hz, H_5), 8.05 (d, $J_{5,6}$ = 7 Hz, H_6), 8.9 (m, NH).

This compound (**16c**) on warming in MeOH decomposed to a new compound that traveled slower on TLC and high-pressure LC, failed to give a Greiss test or ninhydrin test, and gave a molecular ion of 300 (FD), indicating that it is 5'-deoxy-5'-[(methoxycarbonyl)amino]cytidine.

Acknowledgment. This investigation was supported by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare (Grant RO1 CA-23173 and Contract NO1-CM-43762). The authors are indebted to Dr. W. C. Coburn, Jr., and Mrs. M. C. Thorpe, who interpreted NMR data, and to other members of the Molecular Spectroscopy Section of Southern Research Institute, who performed most of the microanalytical and spectral determinations reported.

References and Notes

(1) Huber, G. *Angew. Chem.* **1956**, *68*, 706.
(2) Johnston, T. P.; Fikes, A. L.; Montgomery, J. A. *J. Org. Chem.* **1962**, *27*, 973.
(3) Johnston, T. P.; Holum, L. B.; Montgomery, J. A. *J. Am. Chem. Soc.* **1958**, *80*, 6265.
(4) Balsiger, R. W.; Fikes, A. L.; Johnston, T. P.; Montgomery, J. A. *J. Org. Chem.* **1961**, *26*, 3446.
(5) Skipper, H. E.; Schabel, F. M., Jr.; Trader, M. W.; Thomson, J. R. *Cancer Res.* **1961**, *21*, 1154.
(6) Johnston, T. P.; McCaleb, G. S.; Montgomery, J. A. *J. Med. Chem.* **1963**, *6*, 669.
(7) Wheeler, G. P. *ACS Symp. Ser.* **1976**, *no. 30*, 87.
(8) Babson, J. R.; Reed, D. J.; Sinkey, M. A. *Biochemistry* **1977**, *16*, 1584.
(9) Recently, the synthesis and biological activity of some 2-chloroethyl- and methylnitrosoureido analogues of thymidine were reported.[10]
(10) Lin, T.-S.; Fischer, P. H.; Shiau, G. T.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 130.
(11) Reed, D. J.; May, H. E.; Boose, R. B.; Gregory, K. M.; Beilstein, M. A. *Cancer Res.* **1975**, *35*, 568.
(12) Ludlum, D. B. "Cancer, A Comprehensive Treatise", F. F. Becker, Ed.; Plenum Press: New York, 1976; p 285.
(13) Montgomery, J. A. *Cancer Treat. Rep.* **1976**, *60*, 651.
(14) Agarwal, R. P.; Parks, R. E., Jr. *Biochem. Pharmacol.* **1977**, *26*, 663.
(15) Cotton, F. A.; Day, V. W.; Hazen, E. E., Jr.; Larsen, S. *J. Am. Chem. Soc.* **1973**, *95*, 4834.
(16) Fujiwara, A. N.; Acton, E. M.; Goodman, L. *J. Heterocycl. Chem.* **1970**, *7*, 891.
(17) Fujiwara, A. N.; Acton, E. M.; Henry, D. W. *J. Med. Chem.* **1974**, 17, 392.
(18) Karplus, M.; *J. Chem. Phys.*, **1959**, *30*, 11.
(19) Montgomery, J. A.; Thomas, H. J. *Carbohydr., Nucleosides, Nucleotides.* **1975**, *2*, 91.
(20) Vince, R.; Almquist, R. G. *Carbohydr. Res.* **1974**, *36*, 214.
(21) Jahn, W. *Chem. Ber.* **1965**, *98*, 1705.
(22) Horowitz, J. P.; Tomson, A. J.; Urbanski, J. A.; Chua, J. *J. Org. Chem.* **1962**, *27*, 3045.
(23) Kissman, H. M.; Weiss, M. J. *J. Am. Chem. Soc.* **1958**, *80*, 2575.
(24) Bennett, L. L., Jr.; Vail, M. H.; Chumley, S.; Montgomery, J. A. *Biochem. Pharmacol.* **1966**, *15*, 1719.
(25) Geran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 7.
(26) Lin, T.-S.; Prusoff, W. H. *J. Med. Chem.* **1978**, *21*, 109.
(27) Montgomery, J. A.; James, R.; McCaleb, G. S.; Johnston, T. P. *J. Med. Chem.* **1967**, *10*, 668.

Antiparasitic Agents. 3.[1] Synthesis and Anthelmintic Activities of Novel 2-Pyridinyl-5-isothiocyanatobenzimidazoles

R. D. Haugwitz,* B. V. Maurer, G. A. Jacobs, V. L. Narayanan,

The Squibb Institute for Medical Research, Princeton, New Jersey 08540

L. Cruthers, and J. Szanto

Squibb Agricultural Research Center, Three Bridges, New Jersey. *Received November 30, 1978*

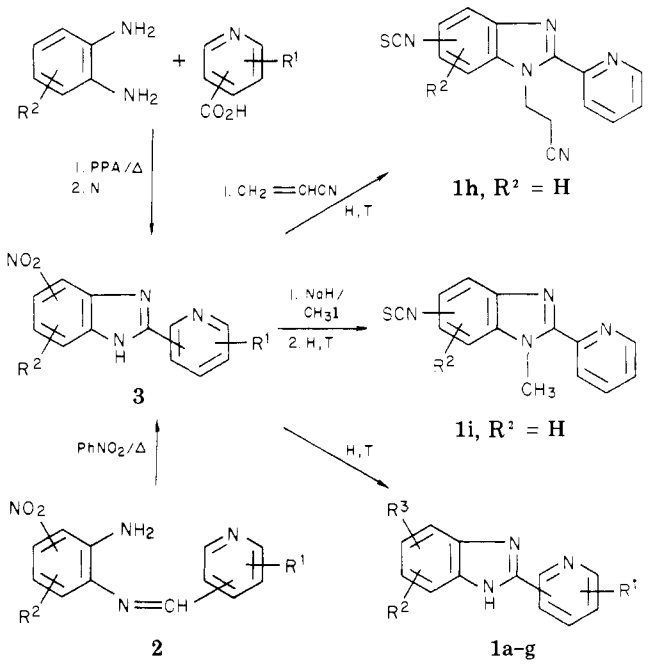
The preparation and anthelmintic activities of a series of 2-pyridinyl-5-isothiocyanatobenzimidazoles are described. In the primary oral mouse screen, six derivatives showed 100% taeniacidal activity at 0.2% in diet. The most active member in this series, **1c**, is potentially an effective gastrointestinal nematocide in sheep at 50 mg/kg po.

In connection with an ongoing program to synthesize novel anthelmintic agents, the preparation of several 2-pyridinyl-5-isothiocyanatobenzimidazoles was undertaken. It was felt that these benzimidazoles should be capable of forming chelates similar to thiabendazole,[2] as well as be electrophilic enough to interact with sulfhydryl, hydroxy, and amino groups, and it was rationalized that this combination of properties might result in anthelmintic activity superior to that of thiabendazole.

Several investigators have reported antiparasitic properties for substituted phenyl isothiocyanates.[3] Recent studies have shown 1,4-diisothiocyanatobenzene to be an

0022-2623/79/1822-1113$01.00/0

Scheme I[a]



[a] N = nitration; H = catalytic hydrogenation; T = thiocarbonylation.

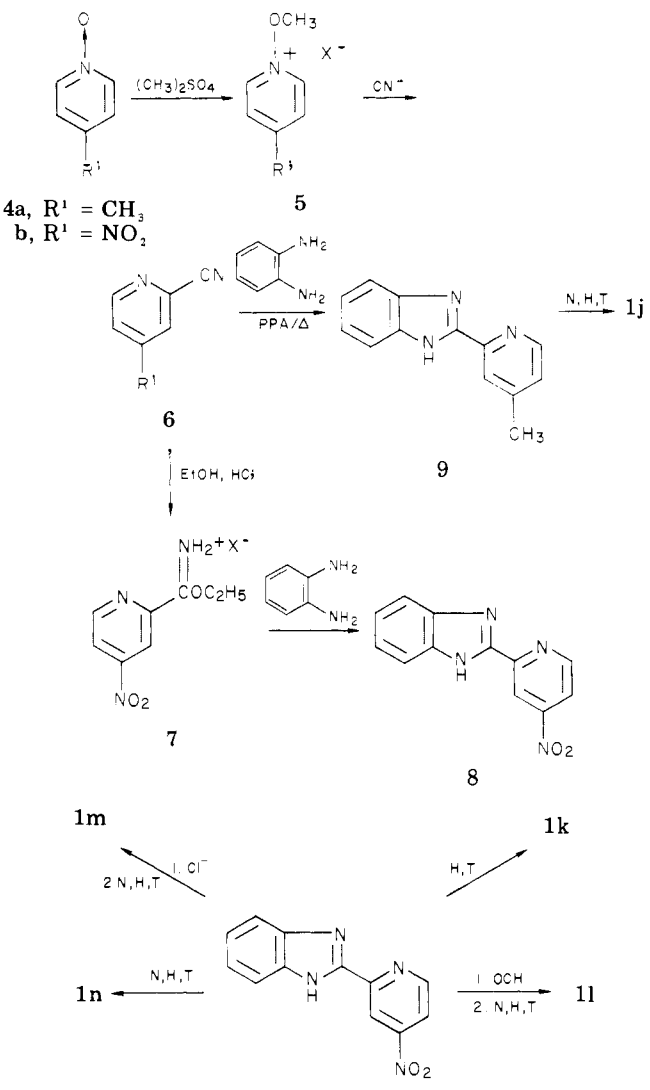
effective human anthelmintic.[4] The trematocidal and cestocidal activities of isothiocyanates have been compiled by Islip.[5] Noteworthy is the extensive work of Brenneisen, Margot, and co-workers in the area of isothiocyanates as antiparasitics.[6]

Chemistry. The synthesis of 2-pyridinyl-5-isothiocyanatobenzimidazoles proved to be straightforward, even though, initially, there was some concern over the potential intermolecular reaction between the NH- and the SCN-groups of **1**. Fortunately, none of the derivatives showed any tendency to polymerize. Two reliable routes for the syntheses of the required intermediate 2-pyridinyl-5-nitrobenzimidazoles **3** were developed: (a) oxidative ring closure of the Schiff bases **2**; (b) PPA-catalyzed cyclodehydration of *o*-phenylenediamines and pyridinecarboxylic acids, followed by nitration of the resulting benzimidazoles. Catalytic hydrogenation of **3** followed by thiocarbonylation using thiophosgene gave target compounds **1a–g**. Cyanoethylation of **3** in the presence of Triton B, followed by hydrogenation/thiocarbonylation, furnished **1h**. Methylation of the Na salt of **3** and hydrogenation/thiocarbonylation led to **1i** (Scheme I).

Substituted pyridines **1j–n** were conventiently prepared from the corresponding pyridine *N*-oxides **4**. PPA-catalyzed ring closure of the nitrile **6a**[7] with *o*-phenylenediamine furnished **9**, which on nitration/hydrogenation/thiocarbonylation gave **1j**. Conversion of **6b**[7] to the imino ether **7**, followed by cyclization with *o*-phenylenediamine, yielded the key intermediate **8** which on hydrogenation/thiocarbonylation furnished **1k**. Displacement of the nitro group of **8** with chloride or methoxide ion and subsequent nitration/hydrogenation/thiocarbonylation gave **1l** and **1m**, respectively. The diisothiocyanate **1n** was prepared by subjecting **8** to the standard nitration/hydrogenation/thiocarbonylation sequence (Scheme II).

The key compound **1c** was subjected to the following reactions. Treatment of the Na salt of **1c** with 2-chloroethyl isothiocyanate[8] cleanly furnished **1o**. The Na salt of **1c**, when stirred with BrCN, gave the anticipated *N*-nitrile, which could not be obtained analytically pure. Treatment of **1c** with 40% aqueous CH_2O gave **1p**.

Scheme II

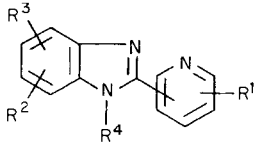


[a] N = nitration; H = catalytic hydrogenation; T = thiocarbonylation.

Heating **1c** with CH_3I at 100 °C (sealed tube) furnished **1q** after fractional crystallization. Oxidation of **1c** with MCPBA in analogy to the preparation of 2-pyridinylbenzimidazole *N*-oxides[9] furnished **1r** in 10% yield. Alternatively, **1r** was prepared from **3** ($R^1 = R^2 = H$) in a five-step sequence. The chelating properties of 2-pyridinylbenzimidazoles and related compounds have been reviewed.[10] It was not surprising then to find that **1c** readily chelates a variety of metal ions (i.e., Zn^{2+}, Sb^{3+}, Fe^{3+}, Hg^{2+}, Ag^{+}, Zn^{2+}, Sn^{2+}). The isolation of these chelates in a pure state offered considerable difficulties. However **1s**, **1t**, and **1u** were obtained as analytically pure 1:1 chelates (Scheme III). The chemical properties of compounds **1a–u** and summarized in Table I.

Anthelmintic Activity. The laboratory helminth systems that are used in screening chemicals are many and varied. Compounds **1a–u** were tested for anthelmintic activity in mice experimentally infected with *Nematospiroides dubius* (nematode) and *Hymenolepis nana* (tapeworm). Carworth CF1 mice (18–20 g weight) were each infected per os with 50 larvae of *N. dubius* and 500 embryonated *H. nana* eggs. Sixteen days after infection, they were fed Wayne mouse diet containing 0.2% of the text compound for 4 days. Using the press-plate technique, the small intestine was examined to determine the worm

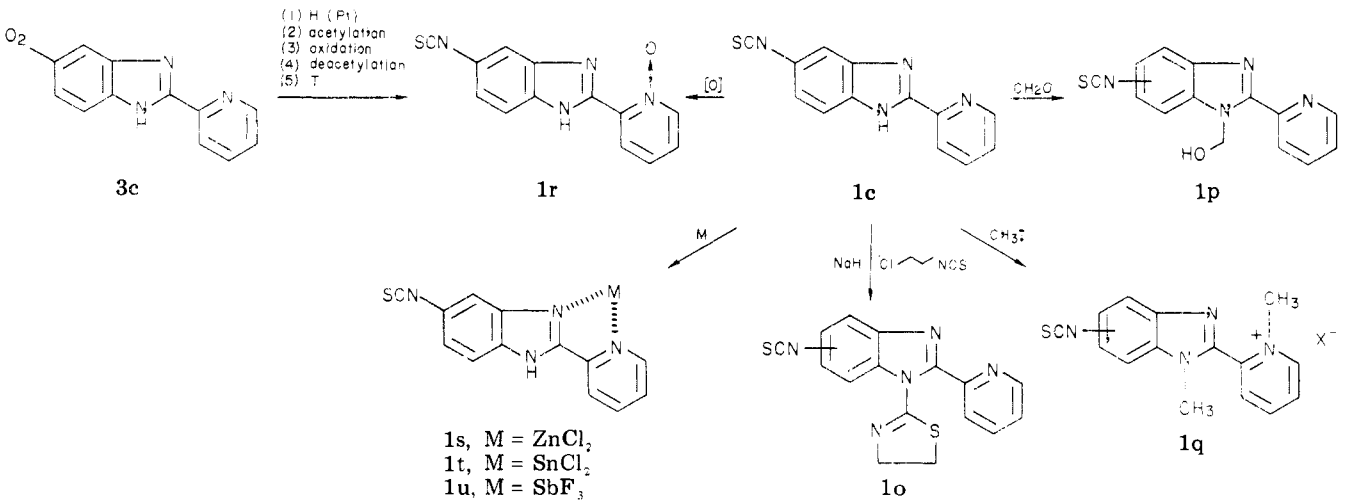
Table I. Properties of Isothiocyanato-2-pyridinylbenzimidazoles



no.	R^1	R^2	R^3	R^4	py attach	mp, °C	meth	yield,[a] %	crystn solv	formula	anal.
1a	H	H	5-NCS	H	4	236–238	B	15	MeCN	$C_{13}H_8N_4S$	C, H, N
1b	H	H	5-NCS	H	3	258–260	B	25	EtOAc	$C_{13}H_8N_4S$	C, H, N
1c	H	H	5-NCS	H	2	187–188	A, B	80	MeCN	$C_{13}H_8N_4S$	C, H, N
1d	6-CH_3	H	5-NCS	H	2	157–159	B	63	Et_2O	$C_{14}H_{10}N_4S$	C, H, N
1e	H	6-Cl	5-NCS	H	2	185–207	A	13	MeCN	$C_{13}H_7ClN_4S$	C, H, N
1f	H	5-CH_3	4- & 6-NCS	H	2	140–158	A	73	*b*	$C_{14}H_{10}N_4S$	C, H, N, S
1g	H	H	4-NCS	H	2	173–175	B	67	Et_2O	$C_{13}H_8N_4S$	C, H, N
1h	H	H	5- & 6-NCS	$-CH_2CH_2CN$	2	135–137		50	MeCN	$C_{16}H_{11}N_5S$	C, H, N
1i	H	H	5- & 6-NCS	$-CH_3$	2	129–141		56	$CHCl_3$	$C_{14}H_{10}N_4S$	C, H, N
1j	4-CH_3	H	5-NCS	H	2	174–177		68	MeCN	$C_{14}H_{10}N_4S$	C, H, N
1k	4-NCS	H	H	H	2	207–209	C	30	MeCN	$C_{13}H_8N_4S$	C, H, N
1l	4-OCH_3	H	5-NCS	H	2	180–181	C	56	MeCN	$C_{14}H_{10}N_4S$	C, H, N
1m	4-Cl	H	5-NCS	H	2	178–179	C	37	MeCN	$C_{13}H_7ClN_4S$	C, H, N
1n	4-NCS	H	5-NCS	H	2	205–207	C	40	MeCN	$C_{14}H_7N_5S_2$	C, H, N
1o	H	H	5- & 6-NCS	$-C(=NCH_2CH_2S)-$	2	140–150		45	Et_2O	$C_{16}H_{11}N_5S_2$	C, H, N
1p	H	H	5- & 6-NCS	$-CH_2OH$	2	184–184.5		71	MeCN	$C_{14}H_{10}N_4OS$	C, H, N
1q	1-CH_3I	H	5- & 6-NCS	$-CH_3$	2	225–227		11	MeOH–Et_2O or CH_3CN–Et_2O	$C_{15}H_{13}N_4S{\cdot}I$	C, H, N
1r	1-O	H	5-NCS	H	2	241–244		5	$CHCl_3$	$C_{13}H_8N_4OS$	C, H, N
1s	H	H	5- & 6-NCS	$ZnCl_2$	2	342–350		86	*c*	$C_{13}H_8N_4S{\cdot}ZnCl_2$	C, H, N
1t	H	H	5- & 6-NCS	$SnCl_2$	2	235–240		83	*c*	$C_{13}H_8N_4S{\cdot}SnCl_2$	N, S
1u	H	H	5- & 6-NCS	SbF_3	2	225–230		84	*c*	$C_{13}N_8N_4S{\cdot}SbF_3$	S

[a] Yield calculated on hydrogenation/thiocarbonylation only. [b] Chromatographed (PE–Et_2O, 1:1) to yield compound which crystallized spontaneously. [c] Precipitated from solution and washed with MeCN.

Scheme III



burden. Nontreated controls and a positive control using parbendazole as standard were run with each test. Parbendazole at 0.03% × 4 days generally gave complete clearance of *N. dubius* and *H. nana*. The results are summarized in Table II. None of the compounds demonstrated activity against *N. dubius*.

All evaluations in sheep were preliminary in that parasite egg counts per gram of feces were utilized to determine activity. Egg per gram of feces (EPG) counts were conducted 3 days prior to treatment in order to determine the degree of parasitism of the test animal. Generally, animals were used which had at least 10000 eggs per gram of feces, although, on occasion, lambs with 8000–9000 eggs per gram were used. An average pretreatment EPG was calculated for each test animal and medication was given according to individual body weights. Compounds were administered orally in gelatin capsules. EPG's were conducted daily during the 7 days the animal was on test and the final three EPG's were used to calculate an average posttreatment EPG.

The percent reduction in the EPG count for a given compound was calculated by the following equation:

$$\frac{\text{pretreatment egg count} - \text{posttreatment egg count}}{\text{pretreatment egg count}} \times 100$$

For comparative purposes the anthelmintic activities of 2-phenyl-1*H*-benzimidazole (**10a**), 2-(2-pyridinyl)-1*H*-benzimidazole (**10b**), 5-isothiocyanato-2-phenyl-1*H*-benzimidazole (**10c**),[11] and 5-isothiocyanato-2-(4-thiazolyl)-1*H*-benzimidazole (**10d**)[12] were also determined. The results are summarized in Table II.

The unsubstituted benzimidazole **10a** was marginally active in the in vivo mouse screen and **10b** was inactive. In sheep, **10b** showed little activity at 50 mg/kg. In critical tests, **10a** has demonstrated one-sixth the potency of thiabendazole.[13] In our screen, **10a** gave 17% reduction at 50 mg/kg. Introduction of the 5-isothiocyanato group resulted in enhanced activity: **10c** similar to **1c** was 100% active against *H. nana*. The lack of activity in sheep at 50 mg/kg was unexpected. Benzimidazole **10d** was the only derivative to show dual activity in the mouse screen.

Initial screening of **1a–c** using the in vivo mouse oral test indicated 100% taeniacidal activity for **1b** and **1c**, which in the case of **1c** was still observed at the 0.50% level. When **1c** was tested at 200 mg/kg in sheep naturally infected with gastrointestinal nematodes, 100% reduction of the fecal egg count was observed. This activity was still observed at 50 mg/kg (100%), but at 25 mg/kg **1c** was virtually inactive (11%). Compound **1c** was not active, however, against *Monezia* species (tapeworm) in sheep or against *Taenia pisiformis* (tapeworm) in dogs.

Conversion of **1c** into the hydrochloride salt (**1c**·HCl) resulted in a slightly less active derivative (75% clearance of *H. nana* in the mouse and a 95% reduction in the fecal egg count in sheep). Surprisingly, the toluenesulfonic acid salt of **1c** (**1c**·TsOH), while 100% effective against *H. nana* in mice, was inactive in sheep. The quaternization product **1q** was also inactive in sheep at 50 mg/kg.

Table II. Anthelmintic Activity of 2-Pyridinyl-5-isothiocyanatobenzimidazoles and Related Benzimidazoles

compd	Mouse oral:[a] *H. nana* % clearance	Sheep oral:[b] % reduct. in fecal egg count
1a	0	0
1b	100	71[c]
1c	100	100,[c] 100, 11[d]
1c·HCl	75	95
1c·TsOH	100	0
1d	75	52
1e	50	15
1f	0	0
1g	25	83
1h	50	nt[j]
1i	0	0
1j	25	0
1k	50	0
1l	75	0
1m	75	0
1n	0	0
1o	100	0
1p	0	0
1q	0	nt
1r	100	9[e]
1s	100	33
1t	0	42
1u	100	0
10a[f]	25	17
10b[g]	0	28
10c[h]	100	0
10d[i]	100[a]	nt

[a] None of the compounds demonstrated activity against the nematode *N. dubius*, except 10d which gave 90% clearance. [b] All sheep dosed at 50 mg/kg unless indicated otherwise. [c] Dosed at 200 mg/kg. [d] Dosed at 25 mg/kg. [e] Dosed at 43 mg/kg. [f] 2-Phenyl-1*H*-benzimidazole. [g] 2-(2-Pyridinyl)-1*H*-benzimidazole. [h] 5-Isothiocyanato-2-phenyl-1*H*-benzimidazole. [i] 5-Isothiocyanato-2-(4-thiazolyl)-1*H*-benzimidazole. [j] nt = not tested.

Isomer **1b** demonstrated a 71% reduction in the fecal egg count in sheep at 200 mg/kg. The anthelmintic activity (82% reduction at 50 mg/kg) for **1g** was somewhat surprising, since only minimal anthelmintic activity had been observed in the primary mouse screen.

Attempts to improve the anthelmintic activity of **1c** in sheep through molecular modification failed. (a) Addition of chlorine or a methyl group to the benzene ring of **1c** resulted in reduced activity (**1e** and **1f**). (b) Substitution of the pyridine ring with either methyl, methoxy, isothiocyanato, or chlorine diminished or abolished the anthelmintic activity (**1d** and **1j–n**). (c) Substitution on the imidazole NH destroyed the activity (**1h**, **1j**, **1o**, and **1p**). (d) Oxidation of the pyridine nitrogen rendered the molecule virtually inactive (**1r**). (e) Chelation of **1c** reduced activity (**1s–u**). Thus, substitution of the NH group or the benzene or pyridine rings of **1c** led to decreased activity. Maximum activity required attachment of the isothiocyanato group at the 5(6) position.

The lack of nematocidal (*N. dubius*) activity of this series in the primary mouse screen and surprising nematocidal activity in sheep of several derivatives again demonstrates the value of testing in the target species.

Experimental Section

Melting points were determined in capillary tubes on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. The NMR spectra were obtained in Me_2SO-d_6 with Me_4Si as internal standard. Neutral alumina (Woelm activity IV) was used for chromatography. Combustion analyses were within ±0.4% of the theoretical values. IR and ^1H NMR spectra were consistent with assigned structures for all compounds.

The isothiocyanato compounds **1a–g** were synthesized from the appropriate nitro compounds **3** using a general procedure for hydrogenation and thiocarbonylation. The synthesis of **1c** will serve as an example.

5-Nitro-2-(2-pyridinyl)-1*H*-benzimidazole (3c). Method A. 2-(2-Pyridinyl)-1*H*-benzimidazole was synthesized by PPA cyclization[14] of *o*-phenylenediamine and picolinic acid and then nitrated as follows.

Nitration. To a solution of 320 g (1.64 mol) of 2-(2-pyridinyl)-1*H*-benzimidazole in 750 mL of H_2SO_4 (concentrated) there was added 125 mL of HNO_3 (concentrated) dropwise between 0 and 10 °C. The mixture was stirred at room temperature for 2 h and then poured into ice–water. Cautious neutralization with 50% NaOH provided a solid, which was filtered off and crystallized from MeOH to yield 250 g of 5-nitro-2-(2-pyridinyl)-1*H*-benzimidazole (**3c**), mp 208–210 °C.

Method B. To a slurry of 153 g (1.0 mol) of 4-nitro-*o*-phenylenediamine in 800 mL of absolute EtOH there was added 107 g (1.0 mol) of 2-pyridinecarboxaldehyde, and the mixture was stirred at room temperature until the azomethine precipitated. Then there was added 125 mL of nitrobenzene and the mixture was heated to 210 °C after distilling off the EtOH. The mixture was cooled, and the resulting solid was filtered off, washed with Et_2O, and crystallized from absolute EtOH to yield 75 g of **3c**.

5-Isothiocyanato-2-(2-pyridinyl)-1*H*-benzimidazole (1c). Hydrogenation. A mixture of 12.0 g (0.05 mol) of **3c**, 1.2 g of PtO_2, and 200 mL of absolute EtOH was reduced on a Parr hydrogenator at 50 psi until the required amount of H_2 was absorbed. The mixture was filtered and the solvent was removed in vacuo. The residue was crystallized from EtOH to yield 8.4 g of 5-amino-2-(2-pyridinyl)-1*H*-benzimidazole, mp 216–218 °C.

Thiocarbonylation. To a solution of 8.4 g (0.04 mol) of 5-amino-2-(2-pyridinyl)-1*H*-benzimidazole in 120 mL of glyme and 80 mL of H_2O there was added 4.0 g (0.04 mol) of $CaCO_3$, and the mixture was cooled to 0 °C. Dropwise, 3.2 mL (0.04 mol) of thiophosgene was added and the mixture was stirred for 2 h. The solvent was removed in vacuo at room temperature and the residue was crystallized from MeCN to give 7.5 g of **1c**, mp 187–188 °C.

5- and 6-Isothiocyanato-2-(2-pyridinyl)-1*H*-benzimidazole-1-propanenitrile (1h). To a solution of 15.0 g of **3c** in 500 mL of dioxane, 20 drops of Triton B was added. Then there was added 150 mL of acrylonitrile with stirring, and the mixture was refluxed overnight. Evaporation of the solvent gave a solid, which was crystallized from MeCN to give 14.8 g of 5- and 6-nitro-2-(2-pyridinyl)-1*H*-benzimidazole-1-propanenitrile, mp 182–184 °C. This compound was subjected to hydrogenation/thiocarbonylation to yield **1h**, mp 135–137 °C.

5- and 6-Isothiocyanato-1-methyl-2-(2-pyridinyl)-1*H*-benzimidazole (1i). To a solution of 12.0 g (0.05 mol) of **3c** in 250 mL of dry glyme there was added 2.4 g (0.05 mol) of NaH (50% oil dispersion) and the mixture was stirred at room temperature for 4 h. Then there was added 7.1 g (0.05 mol) of MeI and the mixture was refluxed for 2 h. The solvent was evaporated in vacuo, a small amount of MeOH was added to destroy unreacted NaH, and H_2O was added to the residue. The resulting solid was filtered off and crystallized from $CHCl_3$ to yield 9.7 g of 5- and 6-nitro-1-methyl-2-(2-pyridinyl)-1*H*-benzimidazole, mp 185–200 °C. Hydrogenation/thiocarbonylation of this compound yielded **1i**, mp 129–141 °C.

Substituted Pyridinyl Compounds 1j–n. The substituted nitriles **6** were synthesized by the method of Okamato and Tani.[7]

5-Isothiocyanato-2-(4-methyl-2-pyridinyl)-1*H*-benzimidazole (1j). A mixture of 21.6 g (0.2 mol) of *o*-phenylenediamine and 23.6 g (0.2 mol) of 2-cyano-4-methylpyridine (**6a**) in 300 mL of PPA was heated to 195 °C under N_2 for 2 h. After cooling to 130 °C the mixture was poured cautiously into H_2O and neutralized with 50% NaOH. The solid was filtered off and washed with H_2O. Crystallization yielded 16.2 g of 2-(4-methyl-2-pyridinyl)-1*H*-benzimidazole (**9**), mp 219–222 °C (EtOH), which was further subjected to nitration/hydrogenation/thiocarbonylation to yield **1j**, mp 174–177 °C.

2-(4-Isothiocyanato-2-pyridinyl)-1*H*-benzimidazole (1k) and 5-Isothiocyanato-2-(4-isothiocyanato-2-pyridinyl)-1*H*-benzimidazole (1n). Method C. In a round-bottom flask fitted with a drying tube and a gas-inlet tube, there was placed a solution of 19.0 g (0.128 mol) of 2-cyano-4-nitropyridine (**6b**) in 60 mL of absolute EtOH. The solution was layered with PE (30–60 °C) and cooled to 20 °C. HCl was bubbled through the solution until it was saturated. Et_2O was added until no further precipitation occurred. The resulting solid was filtered off and dried to yield 10.5 g of the imidate **7**. To a solution of 4.3 g (0.04 mol) of *o*-phenylenediamine in 50 mL of absolute EtOH there was added 9.0 g (0.04 mol) of **7** with stirring. The reaction mixture became warm; on cooling, the product crystallized. The crystals were collected by filtration and washed with H_2O to yield 7.5 g of 2-(4-nitro-2-pyridinyl)-1*H*-benzimidazole (**8**), mp 223–225 °C. Hydrogenation/thiocarbonylation of **8** yielded **1k**, mp 207–209 °C. Nitration/hydrogenation/thiocarbonylation of **8** yielded **1n**, mp 205–207 °C.

5-Isothiocyanato-2-(4-methoxy-2-pyridinyl)-1*H*-benzimidazole (1l). A mixture of 8.4 g (0.035 mol) of **8** and 3.2 g (0.07 mol) of $NaOCH_3$ in 200 mL of MeOH was refluxed for 2 h. The solvent was removed in vacuo and the residue was washed with H_2O. Crystallization of the resulting solid from EtOH yielded 7.5 g of 2-(4-methoxy-2-pyridinyl)-1*H*-benzimidazole, mp 186–188 °C. Subsequent nitration/hydrogenation/thiocarbonylation yielded **1l**: yield 3.2 g; mp 180–181 °C.

2-(4-Chloro-2-pyridinyl)-5-isothiocyanato-1*H*-benzimidazole (1m). A mixture of 8.4 g (0.035 mol) of **8** and 25 mL of HCl (concentrated) was heated on the steam bath for 2 h. The reaction mixture was poured into H_2O and neutralized cautiously with 50% NaOH. Crystallization of the resulting solid from EtOH yielded 7.3 g of 2-(4-chloro-2-pyridinyl)-1*H*-benzimidazole, mp 191–193 °C. Nitration/hydrogenation/thiocarbonylation provided **1m**, mp 178–179 °C.

1-(4,5-Dihydro-2-thiazolyl)-5- and 1-(4,5-Dihydro-2-thiazolyl)-6-isothiocyanato-2-(2-pyridinyl)-1*H*-benzimidazole (1o). **1c** (5.0 g, 0.02 mol) was reacted according to published procedure[8] to yield after chromatography 3.0 g of **1o**, mp 140–150 °C.

5- and 6-Isothiocyanato-2-(2-pyridinyl)-1*H*-benzimidazolemethanol (1p). To a solution of 5.0 (0.02 mol) of **1c** in 200 mL of MeCN there was added 20 mL of 40% formaldehyde solution. The mixture was kept on a steam bath for 5 min. On cooling, the product precipitated as feathery needles. After standing for 3 h at room temperature, the crystallized mass was filtered off and washed with MeCN to yield 4.0 g of **1p**, mp

184–184.5 °C.

2-(5-Isothiocyanato-1-methyl-1*H*-benzimidazol-2-yl)-1-methylpyridinium Iodide (1q). A mixture of 4 g (0.016 mol) of 1c and 80 mL of MeI in a sealed tube was heated on the steam bath for 1 h. A solid separated on standing. The MeI was poured off and the solid was dissolved in the minimum amount of DMF. The solution was chilled to yield a solid. The above mixture was diluted with 3 mL of ice-cold MeCN, and the solid was filtered off. Recrystallization of the yellow-brown solid yielded 0.7 g of 1q, mp 225–227 °C.

5-Isothiocyanato-2-(2-pyridyl)-1*H*-benzimidazole *N*-Oxide (1r). Route A. To a solution of 5.0 g of 1c in $CHCl_3$ there was added 5.0 g of MCPBA, and the mixture was stirred at room temperature for 2 h. An additional 2.0 g of MCPBA was added, and the mixture was stirred for 2 h. The solvent was removed in vacuo and the residue was chromatographed. Elution with $CHCl_3$ furnished a solid, which was crystallized from $CHCl_3$ to give 0.4 g of 1r, mp 241–244 °C.

Route B. To a solution of 4.2 g of 5-amino-2-(2-pyridinyl)-1*H*-benzimidazole in 40 mL of pyridine there was added 2.3 g of Ac_2O. After standing for 1 h, the precipitated product was filtered off to yield 4.7 g. Crystallization from MeCN yielded 5-(acetylamino)-2-(2-pyridinyl)-1*H*-benzimidazole, mp 250–252 °C. This compound was oxidized[9] to the pyridine *N*-oxide. A mixture of 1.4 g of 5-(acetylamino)-2-(2-pyridinyl)-1*H*-benzimidazole *N*-oxide and 14 mL of concentrated HCl was refluxed for 10 min. The precipitated product was filtered off and washed with a small amount of cold EtOH and then Et_2O to furnish 5-amino-2-(2-pyridinyl)-1*H*-benzimidazole *N*-oxide, which was immediately subjected to thiocarbonylation using $CHCl_3$–H_2O as the solvent system. After 1 h, the mixture was filtered and the organic layer was separated, dried, and evaporated to yield 1.0 g, identical (IR, mixed mixture melting point, microanalysis) with 1r.

5-Isothiocyanato-2-(2-pyridinyl)-1*H*-benzimidazole–Zinc Chloride Complex (1:1; 1s). To a solution of 3 g of 1c in 250 mL of MeCN there was added a saturated solution of methanolic $ZnCl_2$. The resulting precipitate was filtered off and washed with MeCN to yield 4 g of 1s, mp 342–350 °C dec. 1t and 1u were prepared analogously.

Acknowledgment. We thank the Squibb Analytical Section under the direction of Dr. A. I. Cohen for microanalytical data and spectra.

References and Notes

(1) For previous papers in this series, see (a) R. D. Haugwitz, B. V. Maurer, and V. L. Narayanan, *J. Med. Chem.*, **15**, 856 (1972); (b) L. R. Cruthers, R. D. Haugwitz, M. Haslanger, B. V. Maurer, J. Watrous, and W. H. Linkenheimer, *Experientia*, 1574 (1978).
(2) C. Kowalk, K. S. Murray, J. M. Swan, and B. O. West, *Aust. J. Chem.*, **24**, 1369 (1971).
(3) J. C. Katiyar and A. B. Sen, *Indian J. Med. Res.*, **59**, 949 (1971); H. Teubner, W. Weuffen, and H. Hoeppe, *Arch. Exp. Veterinaermed.*, **29**, 621 (1975); S. Sharma, R. Bindra, and R. N. Iyer, *Indian J. Pharm.*, **35**, 13–17 (1973).
(4) S. H. Ahmed and S. Vaishnava, *Fortschr. Arzneimittelforsch.*, **19**, 2–5 and following papers through p 175 (1975).
(5) P. J. Islip, *Fortschr. Arzneimittelforsch.*, **17**, 241–319 (1973).
(6) Ger. Offen. 1935338; 1935381; 2053178; 2143838; 2225071; 2259220; U.S. Patent 3558784; 3586670; 3697555; 3755406.
(7) T. Okamoto and H. Tani, *Chem. Pharm. Bull.*, **7**, 925 (1959).
(8) R. D. Haugwitz, B. V. Maurer, A. J. Martinez, G. A. Jacobs, and V. L. Narayanan, *Synthesis*, 336 (1976).
(9) U.S. Patent 3864350.
(10) A. D. Garnovskii, D. A. Osipov, L. I. Kuznetsova, and N. N. Bogdashev, *Russ. Chem. Rev.*, **42**, 89 (1973).
(11) U.S. Patent 3822356.
(12) U.S. Patent 3743738.
(13) H. D. Brown, A. R. Matzuk, I. R. Ilves, L. H. Peterson, S. A. Harris, L. H. Sarett, J. R. Egerton, J. J. Yakstis, W. C. Campbell, and A. C. Cuckler, *J. Am. Chem. Soc.*, **83**, 1764 (1961); J. R. Egerton, W. H. Oh, and A. C. Cuckler, *Nature (London)*, **198**, 309 (1963).
(14) D. W. Hein, R. J. Alheim, and J. J. Leavitt, *J. Am. Chem. Soc.*, **79**, 427 (1957).

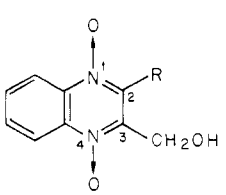
Synthesis and Antibacterial Activity of 1-Hydroxy-1-methyl-1,3-dihydrofuro[3,4-*b*]quinoxaline 4,9-Dioxide and Related Compounds

John P. Dirlam,* Leonard J. Czuba, Beryl W. Dominy, Richard B. James, Richard M. Pezzullo, Joseph E. Presslitz, and Wendell W. Windisch

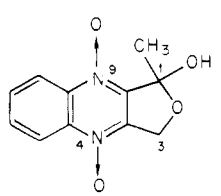
Pfizer Central Research, Groton, Connecticut 06340. *Received February 20, 1979*

A Free–Wilson analysis of the antibacterial activity found in a variety of quinoxaline 1,4-dioxides prepared and tested in these laboratories unexpectedly predicted that potent activity should be found in the case where the heterocyclic ring system was substituted with an acetyl group in the 2 position and a hydroxymethyl group in the 3 position (**2**). The synthesis and antibacterial activity of this compound, which was actually isolated in the hemiketal form (**3**), and of several of its derivatives are reported. 1-Hydroxy-1-methyl-1,3-dihydrofuro[3,4-*b*]quinoxaline 4,9-dioxide (**3**) possesses exceptional activity in vivo against *Escherichia coli*, *Salmonella choleraesuis*, and *Pasteurella multocida*.

Quinoxaline 1,4-dioxides (QNO's) are a well-known class of synthetic antibacterial agents.[1] A series of QNO's of medicinal interest includes analogues substituted with a hydroxymethyl group in the 3 position (**1**).[2] In the present work, a Free–Wilson analysis[3] was carried out from the antibacterial activity found in a variety of QNO's prepared and tested in these laboratories. Unexpectedly, this analysis predicted that potent activity should be found in the case where the heterocyclic ring system was substituted with an acetyl group in the 2 position and a hydroxymethyl group in the 3 position (**2**). The synthesis and antibacterial activity of this compound, which was actually isolated in the hemiketal form (**3**), are reported in this paper. Several additional derivatives of this 1,3-dihydrofuro[3,4-*b*]-quinoxaline ring system were also prepared.[4]



1, R = H, CH_3, or CH_2OH
2, R = $COCH_3$



3

Rationale for Drug Design. A Free–Wilson analysis of 78 QNO's previously prepared in these laboratories

0022-2623/79/1822-1118$01.00/0