Antiparasitic Agents. I. 2-(Nitro-heterocyclic) Benzimidazoles, Benzoxazoles, and Benzothiazoles

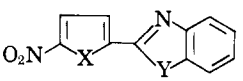
G. L. DUNN, P. ACTOR, AND V. J. DIPASQUO

Research and Development Division, Smith Kline and French Laboratories, Philadelphia, Pennsylvania

Received November 18, 1965

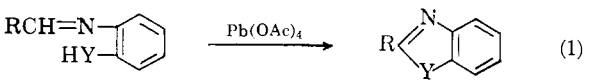
A series of benzimidazole, benzoxazole, and benzothiazole derivatives of nitrothiophene and nitropyrrole were synthesized and were shown to be potent inhibitors of *Trichomonas foetus in vitro*. However, only 2-(5-nitro-2-thienyl)benzimidazole showed significant *in vivo* activity in mice. Several compounds also had anthelmintic activity in a natural mouse pinworm infection.

A number of heterocyclic nitro compounds are effective inhibitors of a variety of parasites.[1] Compounds of this type are particularly active against *Trichomonas* species[2] and at present are the only synthetic compounds to show significant systemic activity in trichomonad infections. A recent report[3] by Bavin describing the systemic antitrichomonal activity of 2-(5-nitro-2-furyl)benzimidazoles prompted us to report our finding of antitrichomonal and anthelmintic activity with a group of similar condensed-ring nitro heterocycles (I).



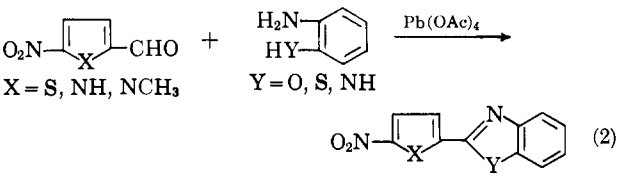
I

Numerous methods have been described for synthesis of benzimidazoles,[4] benzoxazoles,[5] and benzothiazoles.[6] One simple and convenient method, applicable[7] to the preparation of these heterocycles and reported to give good yields, utilizes an oxidative ring closure resulting from the action of lead tetraacetate on an appropriate Schiff base (see eq 1). Berger, *et al.*,[8] also used this procedure to prepare substituted nitrofuran derivatives.



(1)

Schiff bases[9] of 5-nitro-2-thiophenecarboxaldehyde,[10] 5-nitro-2-pyrrolecarboxaldehyde,[11] and N-methyl-5-nitro-2-pyrrolecarboxaldehyde[12] with *o*-phenylenediamine, *o*-aminophenol, and *o*-aminothiophenol were heated briefly with lead tetraacetate in glacial acetic acid to accomplish ring closure (see eq 2).

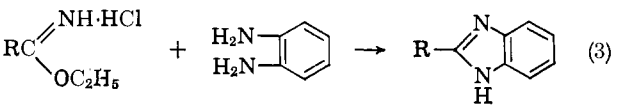


(2)

The crude reaction products, shown by thin layer chromatography to contain several impurities, were easily purified by alumina column chromatography. Though yields were low we obtained pure products without difficulty. The compounds listed in Table I were synthesized by this technique. Also a few known compounds were synthesized by reported methods for biological comparison.

The presence of condensed-ring structures in these compounds is supported by their characteristic ultraviolet absorption spectra (Table I).

The lead tetraacetate procedure proved impractical for preparing large quantities of 2-(5-nitro-2-thienyl) benzimidazole (**1**). However, the imido ester benzimidazole synthesis described by Bavin[3] and DeSelms[13] (see eq 3), using ethyl 5-nitro-2-thiophenecarboximidate hydrochloride[14] and *o*-phenylenediamine, gave a good yield of 1 and eliminated the need for column chromatography.



(3)

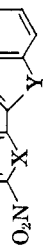
Biological Results.— In general, the nitro-heterocyclic compounds demonstrated significant activity against *Trichomonas foetus in vitro* (Table II). Minimum inhibitory concentrations of **1**, **5**, **8**, and **9** were comparable to or better than those of metronidazole,[15] which was used as a reference compound. Compounds **10–13** were much less active *in vitro* (MIC >100 μg/ml) than their nitro-heterocyclic analogs. Despite the good activity *in vitro* of several of these compounds, only **1** showed systemic activity in a lethal *Trichomonas foetus* infection of mice. It was slightly less active than metronidazole when given subcutaneously and one-eighth as active orally.

The structural similarity of these compounds to thiabendazole,[16] a broad-spectrum anthelmintic agent,[17]

(1) "Experimental Chemotherapy," Vol. I, R. J. Schnitzer and F. Hawking, Ed., Academic Press Inc., New York, N. Y., 1963.
(2) R. J. Schnitzer, ref 1, p 289.
(3) P. M. G. Bavin, *J. Med. Chem.*, **9**, 788 (1966).
(4) (a) K. Hofmann in "The Chemistry of Heterocyclic Compounds," Vol. 6, A. Weissberger, Ed., Interscience Publishers, Inc., New York, N. Y., 1953, p 247; (b) E. S. Schipper and A. R. Day in "Heterocyclic Compounds," Vol. 5, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1957, p 274.
(5) J. W. Cornforth, ref 4b, p 418.
(6) J. M. Sprague and A. H. Land, ref 4b, p 506.
(7) (a) F. F. Stephens and J. D. Bower, *J. Chem. Soc.*, 2971 (1949); (b) F. F. Stephens and J. D. Bower, *ibid.*, 1722 (1950).
(8) H. Berger, E. Haack, and W. Voemel, German Patent 1,138,058 (Oct. 18, 1962); *Chem. Abstr.*, **58**, 9088*f* (1963).
(9) Only Schiff bases of *o*-phenylenediamine were isolated before oxidation.
(10) J. Tirouflet and P. Fournari, *Compt. Rend.*, **243**, 61 (1956).
(11) J. Tirouflet and P. Fournari, *ibid.*, **246**, 2003 (1958).
(12) P. Fournari, *Bull. Soc. Chim. France*, 488 (1963).
(13) R. C. DeSelms, *J. Org. Chem.*, **27**, 2163 (1962).
(14) M. Bercot-Vatteroni, *Ann. Chim.*, **7**, 303 (1962).
(15) 1-(2-Hydroxyethyl)-2-methyl-5-nitroimidazole, Flagyl®.
(16) 2-(4-Thiazolyl)benzimidazole.
(17) 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).

Table

2-(Nitro-heterocyclic) Benzimidazoles, Benzoxazoles, and Benzothiazoles

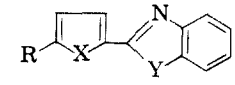


Compd	X	Y	Preparative procedure	Recrystn solvents	Mp, °C dec	Yield,[a] %	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Ultraviolet absorption, λ_{max}, mμ (ε)
1	S	NH	A	Isopropyl alcohol	230–232	19	$C_{11}H_7N_3O_2S$	53.87	2.88	17.13	53.62	2.81	17.13	252 (8000), 270 (7900), 390 (16,800)
2	NH	NH	A	Acetone–toluene	265–266.5[b]	36	$C_{11}H_8N_4O_2$	57.89	3.53	24.55	58.08	3.79	24.47	235 (12,150), 287 (8800), 382 (20,400)
3	NCH_3	NH	A	Acetone–toluene	242–243	16	$C_{12}H_{10}N_4O_2$	59.50	4.16	23.13	59.65	4.31	23.38	234 (11,400), 277 (8200), 376 (18,900)
4	S	O	B	Toluene	200.5–201.5	24	$C_{11}H_6N_2O_3S$	53.65	2.46	11.38	53.81	2.45	11.55	249 (9500), 262 (10,200), 369 (21,300)
5	NH	O	B	Acetonitrile	161–161.5	24	$C_{11}H_7N_3O_3$	57.64	3.08	18.34	57.55	3.04	18.46	247 (10,700), 264 (11,600), 368 (23,200)
6	NCH_3	O	B	Toluene	186.5–187.5	20	$C_{12}H_9N_3O_3$	59.26	3.73	17.28	59.53	3.90	17.38	Sh 248 (11,000), 263 (12,300), 368 (24,000)
7	S	S	B	Toluene	205–207	29	$C_{11}H_6N_2O_2S_2$	50.37	2.31	10.68	50.56	2.24	10.49	Sh 225 (16,200), 268 (9000), 378 (21,100)
8	NH	S	B	Acetonitrile	160–161	27	$C_{11}H_7N_4O_2S$	53.87	2.88	17.13	53.80	3.18	17.07	224 (18,900), 277 (9000), 378 (24,100)
9	NCH_3	S	B	Acetone–toluene	140–141	21	$C_{12}H_9N_4O_2S$	55.59	3.50	16.21	55.81	3.52	16.06	226 (19,600), 274 (9400), 374 (23,700)

[a] After recrystallization. [b] Heating at 100° *in vacuo* for 48 hr was necessary to remove final traces of solvent.

Table II

In Vitro Antitrichomonal Activity



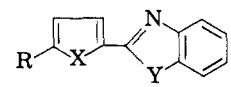
Compd	R	X	Y	MIC, μg/ml[a]
1	NO_2	S	NH	0.20
2	NO_2	NH	NH	2.30
3	NO_2	NCH_3	NH	0.59
4	NO_2	S	O	56.0
5	NO_2	NH	O	0.02
6	NO_2	NCH_3	O	0.78
7	NO_2	S	S	9.40
8	NO_2	NH	S	0.04
9	NO_2	NCH_3	S	0.20
10[b]	H	O	NH	>100
11[b]	H	S	NH	>100
12[c]	H	NH	NH	>100
13[d]	NO_2	HC=CH	NH	>100

[a] See Biological Methods section for a definition of MIC. Metronidazole (Flagyl®), used as reference compound, had a MIC of 0.20 μg/ml. [b] Prepared by the imido ester method described by R. C. DeSelms.[13] [c] Prepared by the Weidenhagen synthesis.[13] [d] See ref 7a.

led us to examine their anthelmintic activity (Table III). Against mouse pinworm, **1** and 2-(5-nitro-2-pyrryl)benzimidazole (**2**) were comparable to thiabendazole. Enhancement of activity by the nitro group is evident by comparison of compounds **1** with **11**, and **2**

Table III

Anthelmintic Activity against Mouse Pinworm



Compd	R	X	Y	Dose, mg/kg/day	% reduction of worm burden[a]
1	NO_2	S	NH	50	96
				250	96
				500	100
2	NO_2	NH	NH	50	92
				100	100
				200	100
3	NO_2	NCH_3	NH	50	49
				200	15
4	NO_2	S	O	50	55
				200	47
5	NO_2	NH	O	50	0
				200	0
6	NO_2	NCH_3	O	50	0
				200	0
7	NO_2	S	S	50	8
				200	53
8	NO_2	NH	S	50	0
				200	2
9	NO_2	NCH_3	S	50	0
				200	0
11	H	S	NH	50	8
				200	53
12	H	NH	NH	50	0
				200	Toxic
13	NO_2	CH=CH	NH	50	15
				200	0
14	NO_2	O	NH	50	0
				250	0
				500	0

[a] Thiabendazole gave 87% reduction at 50 mg/kg.

with **12**. The inactivity of 2-(5-nitro-2-furyl)benzimidazole[18] (**14**) was unexpected since 2-(2-furyl)benzimidazole is reported to have anthelmintic activity.[17]

Compounds **1** and **2** are being studied further in a variety of helminth infections.

Experimental Section[19]

Melting points were determined in open capillary tubes using a Thomas–Hoover Uni-Melt apparatus and are corrected. All final products were homogeneous as determined by thin layer chromatography. Column chromatography was carried out on Woelm neutral alumina, activity grade I. Ultraviolet spectra were determined in 95% ethanol using a Cary Model 14 recording spectrophotometer.

Syntheses of compounds listed in Table I were carried out by two general procedures each of which is illustrated by a detailed procedure. Commercial $Pb(OAc)_4$ was dried *in vacuo* over KOH pellets before use.

Procedure A. 2-(5-Nitro-2-thienyl)benzimidazole.—To a cold (−10°) stirred suspension of *o*-phenylenediamine (3.5 g, 0.033 mole) in 35 ml of absolute alcohol was added a solution of 5-nitro-2-thiophenecarboxaldehyde (5.0 g, 0.033 mole) in 50 ml of 2B alcohol. The resulting deep red mixture was allowed to warm to room temperature over a period of 1 hr, and the precipitated solid was collected and air dried to give 7.0 g (85%) of maroon Schiff base, mp 156–157°.

The Schiff base (6.9 g, 0.028 mole) was suspended in 60 ml of glacial acetic acid, and $Pb(OAc)_4$ (12.4 g, 0.028 mole) dissolved in 150 ml of warm glacial acetic acid was added in one portion. The dark mixture was stirred 15 min at 50–60°, cooled to 25°, and then diluted with 900 ml of water. The precipitate was collected, air dried, and chromatographed on alumina (150 g). Elution with 1:1 ethyl acetate–CH_2Cl_2 gave 1.5 g of material, mp 230–232° dec. An analytical specimen was prepared by recrystallization from isopropyl alcohol, mp 230–232° dec.

Procedure B. 2-(5-Nitro-2-thienyl)benzoxazole.—To a magnetically stirred solution of *o*-aminophenol (2.18 g, 0.02 mole) and 5-nitro-2-thiophenecarboxaldehyde (3.14 g, 0.02 mole) in 70 ml of warm glacial acetic acid was added in one portion a warm (70°) solution of $Pb(OAc)_4$ (8.8 g, 0.02 mole) in 80 ml of glacial acetic acid. The resulting dark solution was stirred at 80° for 5 min, then cooled to 20° and the precipitated brown solid was collected. Dilution of the filtrate with 700 ml of water gave another brown solid. Tlc comparison of the two solids showed similar complex composition. The combined solids (3.2 g) were chromatographed on alumina (100 g) in CH_2Cl_2 to give 1.5 g of lemon yellow solid, mp 200–202°. One recrystallization from toluene gave 1.2 g of product as lemon yellow plates, mp 200.5–201.5°.

2-(5-Nitro-2-thienyl)benzimidazole (1). Imido Ester Method.—To a solution of *o*-phenylenediamine (5.45 g, 0.05 mole) in 60 ml of warm absolute ethanol was added ethyl 5-nitro-2-thiophenecarboximidate hydrochloride[20] (12 g, 0.05 mole). The deep red solution was warmed on a steam bath for 20 min during which time a yellow precipitate formed. The mixture was cooled and diluted with water, and the solid was collected to give 13 g of yellow benzimidazole, mp 228–231°. One recrystallization from aqueous ethyl alcohol gave 11.3 g of 1, mp 230–232°, identical in every respect with that prepared by method A.

Biological Methods.—*In vitro* trials used *T. foetus* grown in Diamond's medium[21] as the test organism. The procedure was a standard twofold tube dilution assay in which the maximum concentration of test material in the culture medium was 100 μg/ml. Compound activity was described as the minimum inhibitory concentration (MIC), defined as that quantity of compound that completely inhibits growth of the organism after 48 hr of incubation at 37°.

In vivo antitrichomonal trials were carried out in 16–18-g male Charles River Farm mice infected intraperitoneally with 1×10^6 *T. foetus* organisms grown in STS medium.[22] The compounds were administered either subcutaneously or orally for three successive days starting on the day of infection. Percentage survival was the criterion used for evaluation.

The *in vivo* antipinworm evaluation was carried out in 18–20-g male CF_1 mice naturally infected with two species of pinworm (*Syphacia obvelata* and *Aspiculuris tetraptera*). The test compounds were suspended in 0.5% gum tragacanth and administered *per os* for 3 successive days. At 48 hr posttreatment the animals were sacrificed and appropriate helminth counts on pooled samples were made. The criterion for evaluation was the per cent reduction in worm burden as compared with the placebo-treated controls.

(18) We wish to thank Dr. P. M. G. Bavin of Smith Kline and French Laboratories, Ltd., Welwyn Garden City, Hertfordshire, England, for supplying this compound.

(19) We are grateful to Miss M. Carroll and her staff and to Dr. W. Thompson and his staff of our Analytical and Physical Chemistry Section for elemental analyses and ultraviolet spectra, respectively.

(20) We obtained mp 190–192° in contrast to 152° reported by Bercot-Vatteroni.[14]

(21) L. S. Diamond, *J. Parasitol.*, **46**, 484 (1960).

(22) A. B. Kupferberg, G. Johnson, and H. Sprince, *Proc. Soc. Exptl. Biol. Med.*, **67**, 304 (1948).

5,6-Dihydro-4H-1,3,4-oxadiazines. V. Base-Catalyzed Cyclodehydrohalogenation of 2-(β-Chloroalkyl)carboxylic Acid Hydrazides

DONALD L. TREPANIER,[1] VILMARS SPRANCMANIS, AND JOHN N. EBLE

Chemistry Research and Pharmacology Departments, Human Health Research and Development Center, The Dow Chemical Company, Indianapolis, Indiana

Received March 30, 1966

The scope of the base-catalyzed cyclodehydrohalogenation of 2-(β-chloroalkyl)carboxylic acid hydrazides into 5,6-dihydro-4H-1,3,4-oxadiazines is discussed. The pharmacological activity of a series of 5,6-dihydro-4H-1,3,4-oxadiazines is presented. Some compounds in this series were found to have anticonvulsant activity in mice.

During a search for new compounds possessing central nervous system activity *via* molecular modification of (−)-ephedrine, we observed that certain 2-(β-hydroxyalkyl)carboxylic acid hydrazides would undergo acid-catalyzed cyclodehydration to give substituted 5,6-dihydro-4H-1,3,4-oxadiazines.[2] Certain of these substituted 5,6-dihydro-4H-1,3,4-oxadiazines exhibited central nervous system activity as shown by their ability to greatly prolong hexobarbital sleep times in mice and to protect mice against maximal electroshock.[3]

(1) To whom correspondence should be sent.

(2) (a) D. L. Trepanier, V. Sprancmanis, and K. G. Wiggs, *J. Org. Chem.*, **29**, 668 (1964); (b) D. L. Trepanier and V. Sprancmanis, *ibid.*, **29**, 673 (1964); (c) *ibid.*, **29**, 2151 (1964); (d) D. L. Trepanier, V. Sprancmanis, D. S. Tharpe, and P. E. Krieger, *J. Heterocyclic Chem.*, **2**, 403 (1965).

(3) D. L. Trepanier, P. E. Krieger, and J. N. Eble, *J. Med. Chem.*, **8**, 802 (1965).